The Metabolism of Oxytocin during Lactation in the Rabbit

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1. It has been suggested that changes in the concentration of hypothalamic enzymes inactivating oxytocin might be taken as an index of hormone production (Hooper, 1966). The present work describes the changes in enzyme concentration shortly after parturition and during lactation. 2. Two groups of animals were used; one consisted of lactating animals from which litters had been removed shortly after parturition. 3. Two fractions obtained from homogenized hypothalamus contained enzyme activity. In animals which had been suckled for 3 days the enzyme activity in the supernatant fraction was three times as great as that observed during pregnancy. Between the third and fifth days the activity reverted to pregnancy levels, and remained fairly constant for as long as the animals were suckled. 4. In animals whose litters were removed shortly after birth, the enzyme activity reverted to pregnancy levels by about the third day *post partum*, and by the sixth day non-pregnancy levels were reached. 5. The enzymes of a particulate fraction behaved somewhat differently; 6 hr. after parturition the enzyme activity was similar to that found in pregnant animals and there was no detectable activity by the tenth day in suckled animals. In non-suckled animals the enzyme activity decreased more rapidly, and non-detectable levels were reached by the fourth day *post partum*.

The posterior pituitary hormone oxytocin is known to be released into the blood stream when lactating animals are suckled and also during parturition. However, very little is known about the effect of such stimuli on the production and metabolism of oxytocin. It is possible that the stimuli merely cause a release of hormone from the site of storage; on the other hand, release may be accompanied by changes in the rate of production. Oxytocin is considered to be stored in the posterior pituitary gland and synthesized in certain neurones of the hypothalamo-hypophysial tract. It was stated by Hooper (1962, 1963, 1964) that the hypothalamus contains certain enzymes capable of inactivating oxytocin, and evidence was given which suggested that the enzymes were concerned with the metabolism of oxytocin in the hypothalamus (Hooper, 1966). It was thought that an increase in the concentrations of the enzymes reflected an increase in the production of the hormone, and that changes in enzyme concentrations may be used as a means of studying variations in the rates of the production of oxytocin under various physiological stimuli. Since release of oxytocin is occasioned by suckling and parturition,

it was of interest to follow changes in the concentration of hypothalamic enzymes immediately after parturition, and to see if there was a correlation between the release of the hormone into the blood stream, and the rate of hormone turnover at the site of production. Two groups of animals were used for the work described here. The first group consisted of lactating animals with litters, and the second group consisted of animals whose litters were removed shortly after parturition. The significance of the difference in enzyme activity in the two groups of animals is discussed, and some conclusions are drawn concerning the metabolism of oxytocin during parturition and lactation.

METHODS

The New Zealand White strain of rabbit was used. Animals bought from outside sources were kept in isolation for approximately 1 month before mating to ensure that none was pregnant or pseudopregnant. After mating, the animals were housed in isolated breeding cages and were allowed to give birth to litters. The number in the litter was not kept to any fixed value and lactating does with young were disturbed as little as possible. In a second series of experiments, litters were removed from the does as soon as possible after parturition, and the does were kept in isolation

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until they were killed. Most of the litters were born during the night, consequently an error of ± 5 hr. is possible in the ages of these litters. The time of parturition, to within an hour, was known for approximately one in four of the experimental animals, and these animals were used when it was necessary to measure enzyme activities shortly after parturition.

Preparation of tissue. Animals were killed by a blow on the neck and bled out; the brain was removed rapidly and the hypothalamus dissected out after removal of pial vessels and corpora mammillaria. The weights of the hypothalami were similar to those recorded previously (Hooper, 1966). Hypothalami were homogenized in 9 vol. of 0.25 M-sucrose in a hand-operated homogenizer, and the homogenate was centrifuged at 2° successively for 15 min. at 600g, 60 min. at 7600g and 60 min. at 25000g to give nuclear, mitochondrial, microsomal and supernatant fractions respectively. The mitochondrial fractions were then centrifuged through a density gradient system prepared from 0.32, 0.8 and 1.2 M-sucrose solutions. The density gradient system was prepared by layering the sucrose solutions, and was used immediately after preparation. Three well-defined layers of particles were obtained after centrifuging in a Spinco model L preparative ultracentrifuge at approximately 7.5×10^6 g/min. (60 min. at 39000 rev./min.) in a SW 39 rotor. The layers were separated one from another by the test-tube-slicing technique, and the concentration of sucrose in each fraction was made approx. 0.25 M by the addition of water. The particles were then sedimented by centrifuging for 60 min. at 25000g at 2°. The pellets were suspended in water (about 0.5 ml.) and all fractions were dialysed against cold ($< 8^\circ$) water overnight. The volumes of the dialysed fractions were recorded, and the nitrogen content was measured by a micro-Kjeldahl method.

Measurement of enzyme activity. Enzyme activity is expressed as a function of the destruction of substrate (oxytocin) resulting from incubation with hypothalamic fractions under specified conditions. Tissue fractions, concentration 60 μ g. of N/ml., were incubated with oxytocin (0.5 i.u.) at 37° for 3 hr. at pH 7.3 (0.2 vol. of 0.4 M- Na_2HPO_4 - NaH_2PO_4 buffer), the total volume of incubation medium being made up to 1 ml. with water. Enzyme action was stopped by heating in a boiling-water bath for 15 min., and the residual concentration of oxytocin was measured on an isolated rat uterus by using a bracket-type assay (Hooper & Jessup, 1959).

The amount of hormone detected after incubation is expressed in terms of the amount of hormone recovered from a control medium: the control medium differed from that used in the definitive experiment in that it contained tissue which had been denatured by heating for 15 min. in a boiling-water bath before incubation with hormone. Residual hormone concentration is expressed as $\log A_0/A$ where A_0 and A are the amounts of hormone recovered from the control and definitive experiments respectively. The recovery of hormone from the control experiments was very similar to that obtained in earlier work (Hooper, 1966).

The pelts were removed from all animals, and the mammary glands were examined histologically by a method similar to the one described for rat mammary glands by Benson & Folley (1957).

Materials. The materials were as described by Hooper (1966).

RESULTS

Previous work has shown that the activity of certain enzymes in the rabbit hypothalamus increased during pregnancy, and remained fairly constant at this elevated level until late pregnancy (Hooper, 1966). Identical methods of tissue preparation and enzyme estimation have been employed in the work described here to facilitate the comparison of changes in enzyme activity during pregnancy and lactation. Two fractions, namely the supernatant and intermediate mitochondrial fraction (i.e. the middle band of the gradient) contained detectable amounts of enzyme activity when incubated at a concentration 60 μ g. of nitrogen/ml. Enzyme activities were measured in animals from 6 hr. to 27 days after birth of the young. The size of litters was not controlled, and Table 1 shows the number of live young shortly after delivery, and also the size of the litters at the time the enzyme activity was measured.

In a second series of experiments, litters were removed from animals shortly after parturition, and the does were then kept in isolation until required for estimation of hypothalamic enzymes. The enzymic activity of the supernatant fraction of these animals is shown in Fig. 1; to show the effect of the removal of litters, the enzyme activity of the soluble fraction of suckling animals is also given. The enzyme activity of the particulate fraction is shown in Fig. 2; here also, the activity detected in animals whose litters were removed is given and the corresponding activity in the suckled animals is also shown.

Table 1. *Number of young in litters at delivery and at end of the period of lactation*

Litter size at birth	Final litter size	Duration of lactation (days)	Condition of mammary glands
9	9	$\frac{1}{2}$	Normal lactating
7	6	$\frac{1}{2}$	Normal lactating
10	8	$2\frac{1}{2}$	Normal lactating
8	8	$4\frac{1}{2}$	Normal lactating
8	8	$4\frac{1}{2}$	Normal lactating
9	4	$5\frac{1}{2}$	Normal lactating
11	9	$6\frac{1}{2}$	Normal lactating
8	7	$9\frac{1}{2}$	Normal lactating
9	6	$10\frac{1}{2}$	Normal lactating
8	4	$17\frac{1}{2}$	Normal lactating
9	9	$19\frac{1}{2}$	Normal lactating
8	4	$21\frac{1}{2}$	Normal lactating
10	7	$21\frac{1}{2}$	Involuting
7	1	$22\frac{1}{2}$	Involuting
7	1	$24\frac{1}{2}$	Involuting
9	6	$26\frac{1}{2}$	Involuting

Fig. 1. Inactivation of oxytocin by supernatant fractions obtained from hypothalami of lactating rabbits and from rabbits which had litters removed shortly after parturition. Oxytocin was incubated with fractions for 3 hr. at 37° and residual hormone measured by the isolated rat uterus. ○, Lactating animal; ●, animals from which litters were removed. A_0 and A are control and residual polypeptide concentrations respectively (milliunit/ml.).

Fig. 2. Inactivation of oxytocin by particulate fraction obtained from hypothalami from lactating animals and animals from which litters were removed shortly after parturition: ○, Lactating animals; ●, litters removed shortly after parturition. A_0 and A as for Fig. 1.

DISCUSSION

A comparison of the enzyme activities of the supernatant fractions obtained from pregnant animals (Hooper, 1966) and lactating animals (Fig. 1) shows that there was approximately three times as much activity 6 hr. after parturition as there was during late pregnancy. The high activity noted after parturition was maintained until the third day *post partum*. Between the third and fifth days, however the activity dropped to levels

observed during the latter part of pregnancy. The stimulus of suckling was sufficient to maintain the activity of the enzyme at this elevated level even up to 26 days *post partum* (Fig. 1). The activity of this particular hypothalamic enzyme did not appear to be dependent on the size of the litter, for the latter was not constant for each animal and varied considerably, as is shown in Table 1. The activity of the enzyme dropped fairly rapidly in animals whose litters were removed shortly after parturition, and non-pregnant levels were reached by the seventh day. It seems certain therefore that the presence of a litter provided the stimulus which maintained the elevated activity of the enzyme. There appear to be three main stimuli associated with the presence of a litter. Firstly, and by far the most important as far as stimulation of oxytocin production is concerned, is the stimulus of suckling. There is the possibility, however, that visual and olfactory stimuli may be involved, although these factors would probably be of less significance than suckling. Suckling is known to cause a reflex release of pituitary oxytocin, and the hormone has been detected in jugular vein blood after suckling (Fitzpatrick, 1961). Oxytocin appears to be involved during parturition, and elevated levels of hormone in jugular vein blood have been noted during the second stage of labour (Fitzpatrick & Walmsley, 1962). The regions of the hypothalamus containing the supraoptic and paraventricular nuclei are considered to be in the pathway of nervous mechanisms which release oxytocin (Cross, 1961), and these nuclei are also considered to be responsible for the production of posterior pituitary hormones. Hooper (1966) stated that changes in the activities of hypothalamic enzymes inactivating oxytocin may be used as a measure of the rate of oxytocin production and turnover in the hypothalamus. The present work shows that there is agreement between the physiological requirements of oxytocin by the animal and the variations in the activities of the enzyme in the supernatant fraction of the hypothalamus. It seems likely therefore that stimuli which cause a release of oxytocin from the pituitary also cause an increase in the turnover of hormone in the hypothalamus.

The variation in the activities of the particulate bound enzyme did not follow the same pattern as those of the soluble enzyme. The activity of this enzyme did not appear to increase during parturition. However, if an increase did occur it must have been transitory, for no apparent change was noted 6 hr. after delivery. The stimulus of suckling was not sufficiently strong to maintain constant enzyme levels in lactating animals, for there was a gradual decrease in activity, and non-detectable levels were reached 10 days after delivery. However, suckling did provide some stimulus for enzyme production for in instances where litters were

removed after parturition, enzyme activities reverted to non-detectable levels by the fourth day *post partum* (Fig. 2). The variation in activity of the particulate bound enzyme cannot be readily correlated with the production of oxytocin in response to stimuli known to release the hormone. In this respect the enzymes of the supernatant and particulate fractions differ. During pregnancy the activities of both groups of enzymes increased over the same period of time, showing a similar pattern throughout pregnancy (Hooper, 1966). It seems possible therefore that the particulate bound enzyme is concerned with some hypothalamic function, the manifestations of which are of greater importance during pregnancy than they are during parturition and lactation.

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REFERENCES

- Benson, G. K. & Folley, S. J. (1957). *J. Endocrin.* **16**, 189.
Cross, B. A. (1961). In *Oxytocin*, pp. 24–46. Ed. by Caldeyro-Barcia, R. & Heller, H. Oxford: Pergamon Press Ltd.
Fitzpatrick, R. J. (1961). In *Oxytocin*, pp. 358–377. Ed. by Caldeyro-Barcia, R. & Heller, H. Oxford: Pergamon Press Ltd.
Fitzpatrick, R. J. & Walmsley, C. F. (1962). *J. Physiol.* **163**, 13.
Hooper, K. C. (1962). *Biochem. J.* **83**, 511.
Hooper, K. C. (1963). *Biochem. J.* **88**, 398.
Hooper, K. C. (1964). *Biochem. J.* **90**, 584.
Hooper, K. C. (1966). *Biochem. J.* **99**, 128.
Hooper, K. C. & Jessup, D. C. (1959). *J. Physiol.* **146**, 539.