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RELEASE OF OXYTOCIN IN UNANAESTHETIZED LACTATING RATS

BY

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The action of certain substances on the release of oxytocin has been studied in unanaesthetized lactating rats. The release of oxytocin has been assessed by the increase in weight after suckling of rats separated overnight from the mother. A large variety of substances appear to block the release of oxytocin. These include pentolinium, atropine and chlorpromazine. Adrenaline and noradrenaline also cause a block in the release of oxytocin, and it is suggested that adrenaline blocks the central release of oxytocin as well as blocking its peripheral action. Reserpine and oestradiol in the doses administered did not block the release of oxytocin. Block also occurred after the ingestion of water, ethanol and normal saline, an effect which may be produced indirectly by impulses from the gastro-intestinal tract.

It has been observed that any stimulus to the neurohypophysis releases both oxytocin and vasopressin. This has been shown in rabbits after electrical stimulation of the supraopticohypophysial tract (Harris, 1955), in dogs after injections of hypertonic saline (Abrahams & Pickford, 1954) and in rats after injections of nicotine (Walker, 1957). Very little is known about substances which block the release of the neurohypophysial hormones or whether the release of one of these hormones can be blocked independently of the other. The effect of various substances on the release of oxytocin in unanaesthetized lactating rats after suckling has been studied. The substances investigated were water and ethanol, which are known to inhibit the release of vasopressin ; the tranquillizers reserpine and chlorpromazine, which are thought to act at the level of the hypothalamus ; and oestrogen in doses which block the release of the anterior pituitary hormones. The interesting suggestion has been made by Pickford (1947) that the hypothalamic nuclei may be analogous to a peripheral autonomic ganglia in that they possess a synapse at which acetylcholine may be the chemical transmitter of afferent nerve impulses while the supraopticohypophysial tract may constitute the efferent path for the release of the neurohypophysial hormones (Walker, 1957). The action of a ganglion-blocking drug, pentolinium, and an anticholinergic drug, atropine, on the release of oxytocin has been studied by administering these substances to the lactating mother just before suckling. The mechanism of action of adrenaline and noradrenaline in blocking the milk-ejecting reflex has also been investigated.

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METHODS

Lactating rats (200 to 250 g in weight) with a litter of 4 to 5 pups were used for studying the release of oxytocin 10 to 12 days after birth of the litter. The release of oxytocin was assessed by the increase in weight after suckling of the young rats separated overnight from the mother. The pups were removed from the mother overnight and weighed next morning before suckling. They were returned to the mother for 15 min at the end of which period they were weighed again. When the weight gain of the litter was constant on several mornings the substance to be tested was administered to the mother just before suckling. If there was no increase in the weight of the litter after 25 min of feeding, then oxytocin was administered intraperitoneally to the mother in doses varying from 10 to 100 m-u. and the litter placed with the mother again. If at the end of this second period of feeding the litter increased in weight as on the other days, it was concluded that the substance administered had specifically blocked the release of oxytocin. The block has been classified as complete block, partial block or no block in the release of oxytocin. Litters were sometimes used for a second experiment, after an interval of 5 days and when the rate of increase of weight was again constant.

The drugs used were: adrenaline hydrochloride, noradrenaline hydrochloride, chlorpromazine hydrochloride (Largactil), reserpine (Serpasil), oestradiol dipropionate, atropine sulphate, pentolinium (Ansolysen), oxytocin (Syntocinon). All these drugs were administered to the mother intraperitoneally. Water and ethanol and saline were administered by mouth by means of a soft rubber catheter 15 to 35 min before suckling.

RESULTS

Water. Tap water (3.5% to 7% of body weight) when administered to the lactating mothers completely blocked milk ejection in 10 out of 12 experiments. When oxytocin in doses of 25 to 100 m-u. was administered intraperitoneally to the mother just after the first suckling the litter gained their normal increase in weight after suckling in 8 experiments and had a partial increase in weight in 2 experiments. Table 1 shows the results of 4 typical experiments when the release of oxytocin was completely blocked by the administered water, the block being overcome by subsequent administration of oxytocin showing that exogenous oxytocin was still effective.

TABLE 1

ACTION OF WATER ON THE MILK-EJECTION REFLEX

Litter	Weight of pups before feeding (in g)	% body weight of water administered to mother orally	Weight of pups after 15 min suckling	Oxytocin injected in m-u. to mother	Weight of pups after suckling again for 15 min
T	96·0	4·4%	96·0	50	99
S	96·5	4·2%	96·5	25	99·5
V	75·0	3·5%	75·0	25	78·5
C	134·5	5·5%	134·0	100	136·5

Ethanol. When 5 ml./100 g of body weight of 7.5% to 10% (v/v) ethanol was administered to the mother 15 to 30 min before feeding in 8 experiments there was no increase in the weight gain of the litter in 6 experiments and partial increase in weight in 2 experiments. Oxytocin administered in doses of 50 to 100 m-u. allowed the litter to gain their normal increase in weight after feeding in 5 experiments and allowed a partial increase in weight in one experiment. Fig. 1 depicts the results of 2 experiments indicating a block in the release of oxytocin in 2 rats

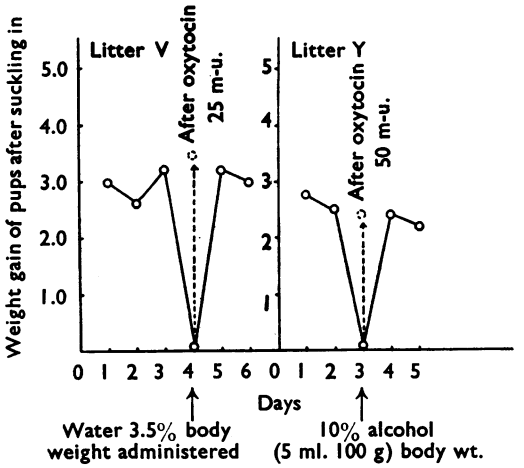

Fig. 1. Action of water and alcohol on the milk-ejection reflex.

treated with water or ethanol. The block was overcome in both cases by an intraperitoneal injection of oxytocin.

Saline. When 5 ml./100 g 0.9% sodium chloride solution was given by stomach tube, there was a block of the reflex similar to that caused by water and ethanol. When the litter was weighed after suckling at 30 min intervals, it was found that the block lasted for about 1.5 hr, after which time the pups were able to obtain about 70% of the normal amount of milk.

Adrenaline. Adrenaline when administered intraperitoneally to the lactating mother in doses of 1.0 μg and above caused a complete block in the milk-ejection reflex in 8 out of 10 experiments and a partial block in 2 experiments. The results are shown in Table 2. Subsequent administration of oxytocin (50 m-u.) overcame the block completely in 3 experiments and caused up to 70% reversal of the block in the remaining 5 experiments. In 5 experiments oxytocin (50 m-u.) was administered to the mother at the same time as adrenaline but at a different site. In 4 of these experiments milk ejection occurred (Table 2). Oxytocin when administered in doses of 10 m-u. at the same time as the adrenaline injection did not prevent the block caused by adrenaline. In 7 experiments adrenaline (1 μg) was administered 30 min after an injection of phenoxybenzamine (50 μg/kg). Adrenaline caused a complete block in the release of oxytocin in 5 of these experiments, partial block in one and no block in another experiment. In another 5 experiments acetylcholine administered in doses of 5.0 μg 2 min before the administration of 1.0 μg did not prevent the block caused by adrenaline (Table 2). When adrenaline was administered in doses lower than 0.5 μg it did not always block the lactating reflex.

Noradrenaline. In 5 experiments noradrenaline in doses of 1.0 μg administered to the lactating mother just before feeding completely blocked milk ejection in 4 experiments and caused a partial block in one experiment. After the administration of 50 m-u. oxytocin the litter gained their normal weight after suckling in 2 experiments and gained partial weight in 3 experiments (70%, 66%, 50%). The results are summarized in Table 2.

TABLE 2
ACTION OF DRUGS ON MILK-EJECTION REFLEX IN RATS

Substances administered	No. of experiments in which block occurred		
	Total block	Partial block	No block
Adrenaline 1 μg and above	8	2	0
Adrenaline 1 μg+oxytocin 50 m-u.	0	1	4
Adrenaline 1 μg after phenoxybenzamine 50 μg/kg	5	1	1
Adrenaline 1 μg after acetylcholine 5 μg	5	0	0
Noradrenaline 1 μg	4	1	0
Chlorpromazine 5 mg/kg	4	2	0
Reserpine 2·5–4·0 mg/kg	0	0	5
Pentolinium 0·1 μg	6	2	0
Pentolinium 10–50 μg+oxytocin 50 m-u.	0	0	4
Pentolinium 1 μg after acetylcholine 10 μg	0	5	4
Atropine 1 μg and above	9	1	0
Atropine 1 μg after acetylcholine 2–5 μg	7	3	1
Atropine 1 μg after acetylcholine 10 μg	2	5	3
Atropine 1 μg+oxytocin 50 m-u.	0	1	4
Oestradiol 200 μg	0	0	5

Chlorpromazine. Chlorpromazine was administered in doses of 5.0 mg/kg to 6 lactating mothers before feeding. It caused a complete block in milk ejection in 4 experiments and a partial block in 2 experiments. The block was completely overcome by subsequent administration of oxytocin to the mother in 5 out of 6 experiments. Chlorpromazine when administered in a dose of 1, 2 and 3 mg/kg did not always block the reflex.

Reserpine. Reserpine when administered to the lactating mother 30 to 60 min before feeding in doses varying from 2.5 mg/kg to 4.0 mg/kg in 5 experiments caused no block of milk ejection. Reserpine could not be administered in higher doses as it was toxic to the animals.

Pentolinium. Pentolinium when administered to the lactating mother in doses of 1.0 μg and above just before feeding always caused a complete block of milk ejection. In the experiments in which pentolinium was administered in doses of 0.1 μg it caused a complete block of milk ejection in 6 experiments and a partial block in 2 experiments. When oxytocin in doses of 50 m-u. was administered at the same time as pentolinium (10 to 50 μg) the block caused by pentolinium did not occur in any of the 4 experiments (Table 2). In 9 experiments acetylcholine (10 μg) was administered to the mother 2 min before administration of 1.0 μg of pentolinium. The acetylcholine completely prevented the block in 4 experiments and partially prevented it in 5 experiments. Fig. 2 demonstrates 2 experiments on the same litter showing pentolinium (1.0 μg) causing a complete block in the weight gain of the litter on one day and only partial block in the weight gain on the litter on another day, when acetylcholine (10.0 μg) had been administered to the mother 2 min before the administration of the same dose of pentolinium. Acetylcholine when administered in doses less than 10.0 μg did not prevent the block caused by pentolinium.

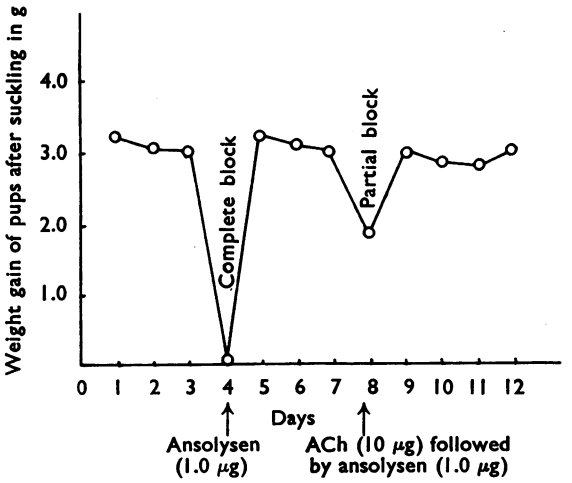

Fig. 2. Action of ansolysen on the milk-ejection reflex.

Atropine. Atropine in doses of 1.0 μg and above was administered to the lactating mother before feeding in 10 experiments. In 9 experiments milk ejection was completely blocked while in the other a partial block occurred (Table 2). Oxytocin in doses of 50 m-u. allowed the litter to gain this normal weight after suckling in all experiments. Oxytocin (50 m-u.) when administered at the same time as atropine completely prevented the block caused by atropine in 4 out of 5 experiments. Acetylcholine was administered to the mother in doses of 2 to 5 μg and 10 μg just prior to administration of atropine in 11 experiments and 10 experiments respectively. The results can be seen in Table 2.

Oestradiol. The intraperitoneal injection of oestradiol 1.5 to 3 hr before suckling failed to affect the reflex in 5 experiments (Table 2).

DISCUSSION

The simple method of measuring the release of oxytocin which has been followed in this investigation has the advantage that a physiological release of oxytocin is being measured in unanaesthetized animals. The action of ethanol and water in blocking the release of oxytocin in unanaesthetized lactating rats confirms earlier results in another species which demonstrated that the administration of water completely blocked the lactating reflex in unanaesthetized lactating rabbits (Chaudhury, 1960). Peeters, Stormorken & Vanschoubroek (1960) have recently shown that in lactating cows the effect of certain stimuli which normally caused milk ejection was inhibited by "conditions of hydration." These authors stress, however, that "conditions of hydration" indicated a complex of experimental details and procedures including the presence of a bladder catheter which may have constituted the inhibiting factor rather than hydration *per se*. The results presented above in this paper indicate that the administration of water just before suckling blocks the milk-ejection reflex. Cross (1951) in rabbits and Pickford (1960) in female dogs found that a water load did not inhibit the lactating reflex. It is possible that the time interval between hydration and the suckling may be responsible for the

apparently conflicting results. Cross had a time interval of 90 min and Pickford an interval of 2 hr between the first hydrating dose of water and the initiation of suckling. In the experiments described above only one water load was given and milk ejection assessed 15 to 30 min after the administration of water. Since it was possible that filling up of the stomach was responsible for the discrepant results, the action of normal saline was investigated. It was found that saline also blocked the reflex, and it is therefore likely that the block caused by substances given by mouth was, at least in part, due to distension of the stomach. It is interesting to note that substances which are known to inhibit the release of vasopressin, like water and alcohol, also block the release of oxytocin. It has previously been shown that both oxytocin and vasopressin are always released simultaneously in response to any stimuli, and these results indicate that a differential blocking of one of these hormones has not been possible. Dicker (1958) has shown that in rats methylpentynol, which belongs to a group of alcohols, also inhibits the release of the neurohypophysial hormones following osmotic stimulation. In women in the first stage of labour or two days after puerperium Dicker (1959) has recently demonstrated that contractions of the uterus were very little affected by ingestion of 1 l. of water or 20 to 30 ml. ethanol in 200 ml. water. It is, however, possible that the amount of water ingested was not adequate to suppress the release of oxytocin. It may also be stressed that uterine contractions are not a very reliable index of the release of oxytocin. Theobald (1959) has, however, got similar results using the suckling reflex as an index of oxytocin release. He has mentioned the importance of the time interval between hydration and the onset of suckling when antidiuresis is being studied in suckling.

The action of adrenaline inhibiting the milk-ejection reflex has been worked out in detail on lactating rabbits by Cross (1953, 1954). The present view appears to be that while there is evidence that adrenaline can block the peripheral effect of oxytocin on the mammary gland there is no evidence that adrenaline blocks the central release of oxytocin (Cross, 1957). The results on suckling rats presented in this paper indicate that adrenaline in addition to blocking the peripheral effects of oxytocin on the mammary gland also prevents the endogenous release of oxytocin in response to the stimulus of suckling.

Chlorpromazine appears to block the release of oxytocin while reserpine in the doses administered did not prevent the release of oxytocin. These results with reserpine do not agree with the findings of Moon & Turner (1959), who have shown that reserpine blocks milk ejection in lactating rats. Gaunt, Renzi, Antonchak, Miller & Gilman (1954) have also shown that reserpine does not inhibit the release of the neurohypophysial antidiuretic hormone in rats. Oestrogen, when administered to the lactating mothers in doses of 200 μg 1.5 to 3 hr prior to suckling, did not block the milk-ejection reflex in any of the 5 experiments.

The fact that the ganglion blocking drug pentolinium in as low doses as 0.1 μg completely blocked milk ejection is very interesting in view of the hypothesis (Pickford, 1947) that the hypothalamic nuclei may be analogous to a peripheral autonomic ganglion in that they possess a synapse at which acetylcholine may be the chemical transmitter. It is also intriguing to note that when acetylcholine was

administered to the lactating rat in doses of 10 μg, pentolinium 1 μg did not completely block milk ejection in any experiment. Pentolinium administered in doses of 1.0 μg to a rat without prior injection of acetylcholine caused a complete block of milk ejection in all experiments. Acetylcholine when injected in doses lower than 10 μg did not, however, prevent the block of milk ejection caused by pentolinium, and it is possible that acetylcholine when administered in doses of 10 μg may have had a milk-ejecting action of its own or caused endogenous release of oxytocin itself.

Bisset & Walker (1957), studying the action of the ganglion blocking drug, hexamethonium, on the release of oxytocin and vasopressin, found that it did not block the release of these hormones after stimulation to the neurohypophysis by nicotine. However, as the authors point out, hexamethonium itself had an antidiuretic effect, and Walker (1957) has suggested the possibility that hexamethonium may have stimulated the neurohypophysis indirectly by this fall in blood pressure. It should also be kept in mind that the stimulus in one instance was pharmacological and in the other physiological, and the responses may not be the same for these two different types of stimuli to the neurohypophysis. Much more work needs to be done investigating the action of ganglion blocking drugs on the release of the neurohypophysial hormones. The dose of pentolinium which blocked the response is very small, and it is doubtful if in doses of 0.1 μg pentolinium would block the autonomic effects in a rat of 200 to 250 g.

The results presented indicating that atropine blocks the milk-ejecting reflex confirm the earlier work by Grosvenor & Turner (1957). Acetylcholine in doses of 10 μg administered just prior to the administration of atropine prevented a complete block of milk ejection in 8 out of 10 experiments. Pickford has found that acetylcholine injected into the supraoptic nucleus produced antidiuresis in the presence of atropine. The results presented above indicate that atropine blocks the milk-ejection reflex, but it is not suggested that atropine acts by virtue of its anticholinergic action. Further work has to be done elucidating how atropine blocks milk ejection. Acetylcholine in doses of 10 μg administered just prior to the administration of atropine prevented a complete block of milk ejection in 8 out of 10 experiments. This could have been due to a direct action of acetylcholine on the myoepithelium or due to acetylcholine itself releasing oxytocin.

It is interesting that such a variety of pharmacological agents block the lactating reflex. That this block was not due merely to the injection is shown by the findings that the milk-ejection reflex was not blocked when adrenaline and noradrenaline were administered intraperitoneally at doses below 0.1 μg and chlorpromazine at doses below 0.5 mg/kg. As the doses administered were raised partial block in milk ejection occurred such as when adrenaline was administered at doses of 0.1 to 0.4 μg or when chlorpromazine was administered at 1, 2 and 3 mg/kg. When the doses were further raised there was a complete block in milk ejection in nearly all experiments. The stress of the injection therefore did not block milk ejection as otherwise it would have blocked the milk ejection in those experiments also where low doses of the drugs were administered.

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