

EFFECTS OF CHOLINOCEPTOR ANTAGONISTS ON THE SUCKLING-INDUCED AND EXPERIMENTALLY EVOKED RELEASE OF OXYTOCIN

G. CLARKE, CAROLINE H.D. FALL, D.W. LINCOLN
& LYNDIA P. MERRICK

Department of Anatomy, University of Bristol Medical School, University Walk, Bristol, BS8 1TD

- 1 In the anaesthetized lactating rat, the suckling of the young causes the regular release (about every 7 min) of brief pulses of oxytocin (0.5 to 1.0 mu), which each produce a single transient increase in intramammary pressure.
- 2 The effects of several cholinceptor antagonists were studied in relation to this natural reflex, and also the release of oxytocin evoked by the intraventricular injection of cholinomimetics.
- 3 Reflex milk ejection was blocked by the nicotinic antagonists mecamlamine and hexamethonium, and the inhibition was dose-dependent (ED_{50} of 1 mg/kg i.v. and 5 mg/kg i.v., respectively). Despite the use of high doses, the muscarinic antagonists atropine (200 mg/kg), hyoscine (90 mg/kg) and benzhexol (30 mg/kg) all failed to prevent the reflex release of oxytocin.
- 4 Acetylcholine (20 to 100 μ g), bethanechol (0.2 to 4.0 μ g) and carbachol (0.01 to 0.2 μ g) injected into the cerebral ventricles stimulated a sustained release of oxytocin, which produced multiple increases in intramammary pressure. Nicotine (200 μ g) was relatively ineffective by this route.
- 5 The release of oxytocin by intraventricular bethanechol or carbachol was abolished by atropine (0.1 to 1.0 mg/kg) but not by mecamlamine (5 mg/kg) or hexamethonium (5 mg/kg).
- 6 None of the antagonists used significantly affected either the release of oxytocin following electrical stimulation of the neurohypophysis or the mammary sensitivity to endogenous or exogenous oxytocin.
- 7 The results suggest that the neural pathway controlling the reflex release of oxytocin during suckling in the rat contains a cholinergic component, which acts through nicotinic receptors. A second cholinergic pathway, of the muscarinic type, may also exist. The role of these two pathways is discussed.

Introduction

Oxytocin, the main functions of which are to promote the contractions of the uterus during labour and of the myoepithelial cells in the mammary gland during milk ejection, is produced in the 'magnocellular neurones' of the paraventricular and supraoptic nuclei of the hypothalamus and, after axonal transport to terminals in the neurohypophysis, the hormone is released by a process dependent on action potentials. Elevated levels of spike activity have been observed in these neurones during the physiological release of oxytocin, elicited by the suckling of the nipples by the young (Wakerley & Lincoln, 1973; Lincoln & Wakerley, 1974; 1975), and the time and frequency characteristics of this neural activity have been found to correspond closely to those parameters which are optimal for the electrical stimulation of oxytocin

release (Cross & Harris, 1952; Harris, Manabe & Ruf, 1969; Lincoln, Clarke, Mason & Dreifuss, 1977).

Acetylcholine, applied either systemically in the intact animal (Abrahams & Pickford, 1954) or topically to the isolated hypothalamus (Bridges, Hillhouse & Jones, 1976), causes the release of oxytocin. Cholinomimetics such as nicotine injected systemically (Bisset & Walker, 1957) and carbachol injected into the cerebral ventricles (Kühn & McCann, 1970) are similarly effective. Moreover, acetylcholine excites the majority of magnocellular neurones when applied iontophoretically (Moss, Urban & Cross, 1972; Dreifuss & Kelly, 1972). However, such collective evidence provides no proof that acetylcholine operates as a neurotransmitter in the natural reflex release of oxytocin. A more positive approach, and the one adopted in the

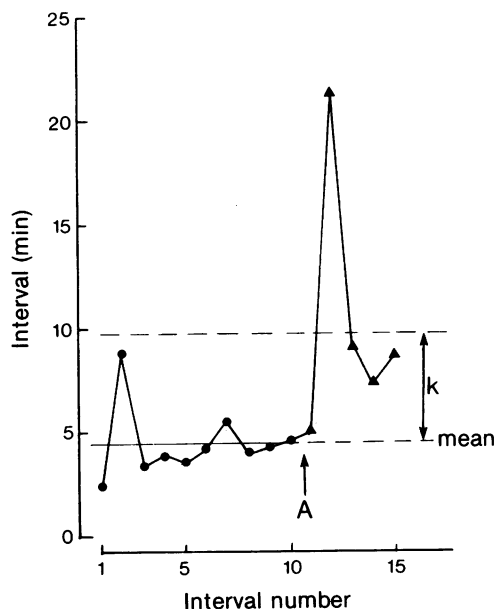


Figure 1 Statistical analysis used to assess the inhibition of the milk-ejection reflex by cholinergic antagonists. The intervals between the individual milk ejections are plotted sequentially, before (●) and after (▲) the injection of an antagonist, in this example mecamlamine, (1 mg/kg, i.v.). The lower horizontal line corresponds to the mean interval between milk ejections during the pre-treatment period. The upper dashed line represents a 99% probability estimate ($P=0.01$) based on the equation:

$$k = \text{s.d.} \times t \times \sqrt{[(n+1)/n]^*}$$

where s.d. is the standard deviation of the milk-ejection interval in the preinjection period, n , number of control intervals, t , t -value for $n-1$ degrees of freedom at $P=0.01$ and $*$ = Bessel's correction for small size of preinjection sample.

Milk ejection was judged to have been significantly inhibited if any milk ejection interval within 30 min of drug treatment exceeded the value calculated above. The duration of the longest interval after drug treatment divided by the mean interval before treatment is referred to as 'the milk-ejection ratio'.

present studies, has been to challenge the milk-ejection reflex with 'selective' cholinergic antagonists. Grosvenor & Turner (1957) found that atropine, a muscarinic-type antagonist, blocked oxytocin release when given as a large intravenous dose, as measured indirectly through the amount of milk obtained by the nursing young. Moos & Richard (1975) extended this approach and found that milk ejection was abolished by the intraventricular injection of both muscarinic- and nicotinic-type antagonists. In neither study

were the authors able to control or quantify the milk-ejection reflex that they were attempting to study. Results from iontophoretic studies are similarly confusing. Dreifuss & Kelly (1972) classified the excitation of the magnocellular neurones by iontophoretically applied acetylcholine as a nicotinic-type response, whilst Moss, *et al.* (1972) recognized both nicotinic- and muscarinic-type responses.

The problem of the cholinergic involvement in the milk-ejection reflex has been studied in the present experiments by the administration of cholinergic antagonists to lactating rats, anaesthetized and suckling a litter of hungry pups. In such anaesthetized rats reflex milk ejection occurs every 5 to 15 min, in response to the 'continuous' sucking of the nipples by the young, and by the measurements of the intramammary pressure from one of the unsucked nipples it is possible to obtain a semiquantitative measure of the amount of oxytocin released at every milk ejection (Lincoln, Hill & Wakerley, 1973). A similar, if not identical, pattern of milk ejection is found in the conscious animal.

Methods

All experiments were performed on lactating Wistar rats weighing 250 to 400 grams. Environmental conditions within the animal quarters were controlled for temperature (22°C) and photoperiod (14 h light: 10 h darkness), and food and water were provided *ad libitum*.

One evening, between Day 7–11 of lactation, the young were removed from their mothers and placed in adjacent cages. The following morning, between 09 h 00 min and 10 h 00 min, the mothers were anaesthetized with an intraperitoneal injection of ethyl carbamate at 1.25 g/kg (as a 25% w/v solution). A saphenous vein and two teat ducts were then cannulated for the administration of drugs and the measurement of intramammary pressure, respectively, and the hungry young were given a trial suckling of 10 min duration (Lincoln *et al.*, 1973).

Measurement of milk ejection

Three hours after anaesthesia, the young were applied to the uncannulated nipples (usually 10) for a second time, and left to feed for 3 h or more. Milk ejection commenced within 30 min in most animals and thereafter continued for the duration of the nursing period. Each milk ejection was characterized by an abrupt rise in intramammary pressure, and an associated vigorous increase in the sucking activity of the young (Lincoln *et al.*, 1973).

After at least six milk ejections had been observed, at intervals of 3–15 min, the drugs that were to be

tested were administered. Milk ejection was considered to have been inhibited if any of the intervals between milk ejections in the next 30 min period were significantly longer than the intervals observed in the pretreatment period (Figure 1). To quantify the degree of inhibition observed, the duration of the longest milk-ejection interval following drug treatment was divided by the mean interval before treatment ('milk-ejection ratio').

The sensitivity of the mammary glands to oxytocin was determined at various intervals before and after drug treatment by the intravenous injection of a synthetic hormone preparation.

Intraventricular injections

For experiments involving the injection of drugs into the lateral cerebral ventricles, the animals were subjected to a number of additional procedures. One common carotid artery was cannulated for the measurement of arterial blood pressure. The rats were then mounted in a stereotaxic frame, and small holes were drilled through the frontal bone of the skull at co-ordinates which permitted a 27 gauge needle, on the end of a 10 μ l syringe, to be lowered into one or other of the lateral cerebral ventricles. Drugs were injected intraventricularly using volumes no greater than 2 μ l. In the early experiments, the correct positioning of the microsyringe was checked at the end of each experiment by the injection of 1 μ l of cobaltous chloride (2 M solution). One minute later the animal was killed by an overdose of a barbiturate preparation, the brain was removed, rinsed in 0.9% w/v NaCl solution (saline) and then immersed for 15 min in ammonium sulphide (0.1% w/v). This precipitated the cobalt as cobalt sulphide, a black precipitate, which was visible throughout the ventricular system of the brain.

Drugs

Drugs were usually dissolved in freshly distilled water, and when necessary made isotonic with added sodium chloride. The following substances were used: acetylcholine chloride (BDH); atropine methyl nitrate (Sigma), atropine sulphate (BDH), benzhexol (Artane, Lederle), bethanechol chloride (Mecothane, Macarthys), carbamylcholine chloride (carbachol, BDH), ethyl carbamate (Urethane, BDH), hexamethonium bromide (Sigma), (-)-hyoscine hydrobromide (scopolamine, Sigma), mecamlamine hydrochloride (Sigma), nicotine hydrogen tartrate (BDH), propranolol hydrochloride (Inderal, ICI), sodium nitrite (BDH), oxytocin (Syntocinon, Sandoz) and vasopressin (Pitressin, Parke-Davis).

Drug doses are expressed in terms of the salt used.

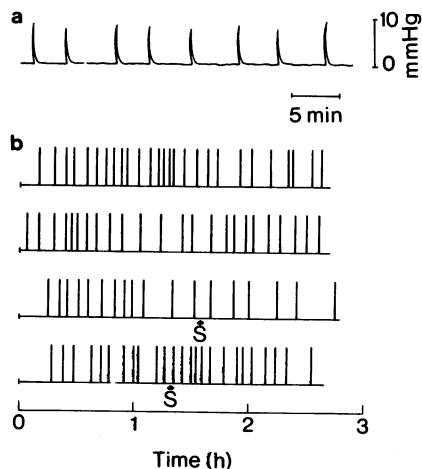


Figure 2 Reflex milk ejection in the urethane-anaesthetized rat during suckling of ten hungry young. (a) An intramammary pressure recording, to illustrate the abrupt but transient rises in pressure which characterize milk ejection in the rat. Note the uniformity of milk ejections and the regularity with which they recur. (b) Patterns of milk ejection in four control rats, two of which were injected at the arrows with saline (S) (1 ml/kg, i.v.). Each vertical event bar corresponds to an intramammary pressure response (i.e. an individual milk ejection), and these have been plotted against elapsed time. The pups were placed on the nipples and started sucking at time 0.

Results

Milk ejection in the anaesthetized rat

Sixty-eight percent of the anaesthetized rats used in this study, and prepared by the methods described, ejected milk in response to the sucking activities of 10 pups. Each milk ejection was a stereotyped event, and was characterized by an abrupt but transient (10 s) rise in intramammary pressure (10 mmHg) (Figure 2a). This rise in pressure was perceived by the young; they rose to their feet and with limbs outstretched sucked with considerable vigour. Both the pressure and behavioural responses were readily elicited by either electrical stimulation of the neurohypophysis (5 s at 60 Hz) or a bolus injection of oxytocin (0.5 to 1.0 mu, i.v.).

The delay to the first milk ejection was somewhat variable (5 to 60 min), but subsequent milk ejections occurred at regular intervals of about 7 min, though the milk-ejection interval for individual rats ranged from about 3 to 14 minutes. This period of regular milk ejection lasted in most animals for about 2 h, and thereafter the milk ejection intervals became pro-

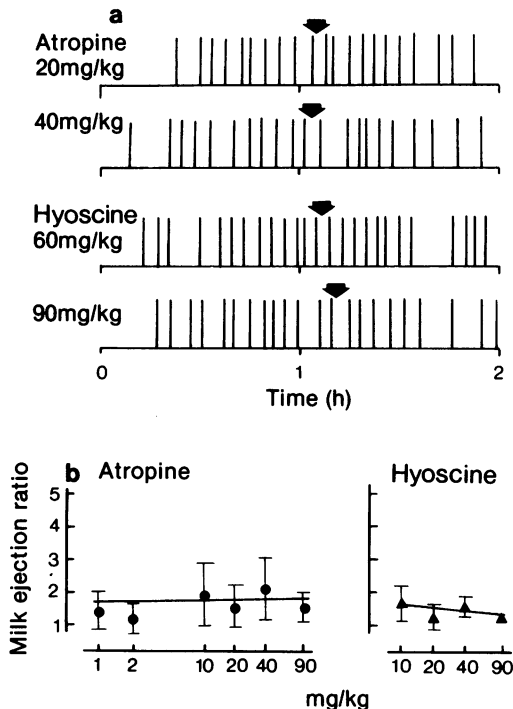


Figure 3 Lack of effect of the muscarinic antagonists atropine and hyoscyne upon the frequency of milk ejection. (a) Patterns of milk ejection in 4 rats during suckling of 10 pups for a period of 2 hours. Atropine or hyoscyne at the doses shown was injected intravenously (at the arrow) between 60–75 min after the onset of suckling (time zero). (b) Graphs for both atropine (●) and hyoscyne (▲) in which the milk-ejection ratio (see legend of Figure 1) at each dose has been plotted against the dose of antagonist administered. Where error bars are shown the points represent mean values and the bars show the standard deviation; the lines are regression lines.

gressively longer. Thus, a rat which received no drug treatment displayed 15 to 20 milk ejections during the 2 h period of regular suckling.

Effect of cholinceptor antagonists on reflex milk ejection

As a control to the administration of the various cholinceptor antagonists, 16 rats were given an intravenous injection of saline (1 ml/kg). Such treatment influenced neither the pattern nor the amplitude of the milk-ejection responses that were subsequently observed (Figure 2b).

All the antimuscarinic compounds tested failed to cause any marked inhibition of the suckling-induced milk-ejection reflex (Figure 3). Atropine was without

effect when given intravenously to 39/43 rats at doses up to 200 mg/kg; 4 rats exhibited a slight inhibition that was unrelated to the dose administered. Indeed, high doses of atropine improved the regularity and uniformity of the responses in rats that were otherwise ejecting milk erratically. Hyoscyne (2 to 90 mg/kg, i.v.) and benzhexol (10 to 30 mg/kg, i.v.) were ineffective when given to 11/11 and 7/7 milk-ejecting rats, respectively.

In contrast, intravenous injections of the nicotinic antagonists, mecamylamine and hexamethonium, blocked the suckling-induced milk ejection reflex in a reversible and dose-dependent manner (63 rats). Usually, inhibition was immediate and complete, there was no graded reduction in the size of the intramammary pressure responses. Likewise the recovery was abrupt, and the first milk ejection after a period of inhibition was as large (Figure 4a), in terms of the rise in intramammary pressure, or larger than the

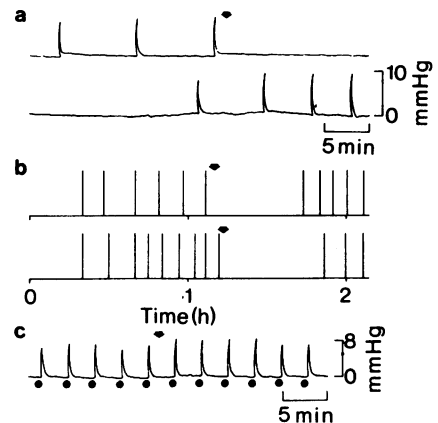


Figure 4 Effect of mecamylamine on the release of oxytocin evoked by the suckling stimulus and by electrical stimulation of the neurohypophysis. (a) Intramammary pressure record showing reflex milk ejection being blocked by the intravenous injection of mecamylamine 2 mg/kg (at arrow). The trace starts with the occurrence of the fourth intramammary response and the antagonist was injected after the sixth response, approximately 75 min after the onset of suckling. Milk ejection recommenced after a delay of 37 minutes. (b) Pattern of milk ejection in 2 rats injected with mecamylamine (2 mg/kg i.v., at arrow), after 75 min of suckling. (c) Changes in intramammary pressure elicited by releasing oxytocin from the neurohypophysis by electrical stimulation. A bipolar concentric electrode was placed stereotactically in the neurohypophysis using a dorsal approach. Stimuli were applied every 3 min (at dots) and consisted of 5s trains of biphasic square wave pulses at 150 μ A with a pulse width of 1 ms and frequency of 60 Hz. Mecamylamine (2 mg/kg) was injected at the black arrow and stimulation was continued.

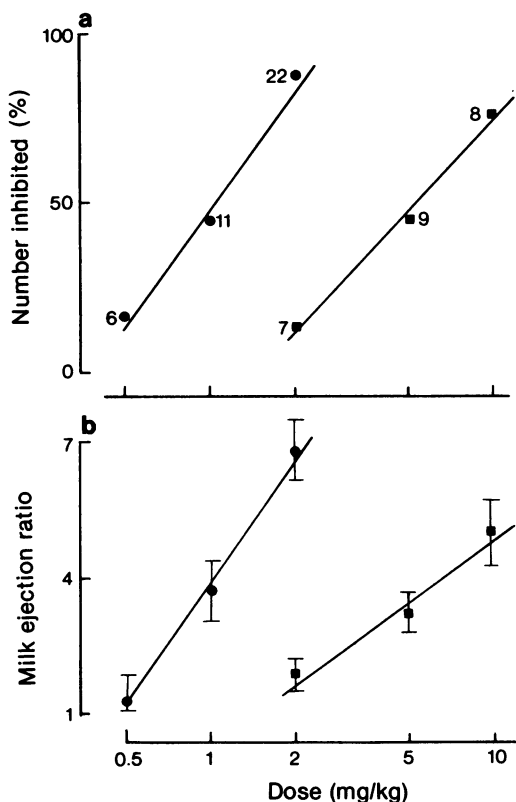


Figure 5 Dose-dependent effects of mecamlamine (●) and hexamethonium (■) upon milk ejection in rats. (a) Number of animals in which milk ejection was inhibited, expressed as a percentage of the total number used at each dose, plotted against the dose of drug used. The value beside each point gives the number of rats tested at each dose level. (b) The mean milk-ejection ratio, plotted against the dose of drug used. Each point is the mean value derived from all the rats tested at each dose (numbers as in a); the standard error of the mean is denoted by the vertical bar. [The number of milk-ejection responses predicted in the absence of the drug but blocked by the presence of the drug can be obtained from the expression, ('milk-ejection ratio') - 1.]

responses which had been observed before drug treatment. The period of inhibition created by these drugs was proportional to the logarithm of the dose (Figure 5). The doses required to create a significant inhibition ($P \leq 0.01$) in half the animals tested (ED_{50}) were 1 mg/kg, intravenously and 5 mg/kg, intravenously for mecamlamine and hexamethonium, respectively.

Neither mecamlamine nor hexamethonium reduced the sensitivity of the mammary glands to oxytocin, and neither influenced the releasability of oxytocin from the neurohypophysis, for the intra-

mammary pressure responses to exogenous oxytocin and electrical stimulation of the neurohypophysis were unchanged by the antagonists (Figure 4c). Twelve of the 63 rats studied in this section were given a pretreatment with propranolol (1 mg/kg, i.v.) to improve the sensitivity of the mammary glands to oxytocin; such treatment had no effect on the inhibition created by the nicotinic antagonists. Both mecamlamine and hexamethonium produced a large fall in blood pressure (20 to 40% for up to 1 h), but this response did not appear to be related to the inhibition of the milk-ejection reflex. Sodium nitrite (10 mg/kg, i.v.) produced a similar fall in blood pressure without inhibiting the milk-ejection reflex. Likewise, high doses of atropine and propranolol, which fail to inhibit milk ejection, frequently lowered blood pressure.

Release of neurohypophysial hormones by cholinomimetics

Acetylcholine was injected into the lateral cerebral ventricles in 7 rats, and at a dose of 20 to 100 μ g produced a repeatable increase in intramammary pressure in 5 animals. In contrast, an intravenous dose of 5 μ g produced a similar mammary contraction, presumably by a direct action on the mammary gland. Thus, the mammary response to centrally-administered acetylcholine may have been the result of an overflow of the drug into the peripheral circulation. However, atropine methyl nitrate (1 mg/kg i.v.), which antagonizes the direct action of acetylcholine on the mammary gland but does not gain access to the central nervous system, only partially reduced the mammary response to intraventricular acetylcholine, whilst eliminating the response to systemically-administered acetylcholine.

Synthetic cholinesterase-resistant cholinomimetics were administered into the lateral ventricles in place of acetylcholine. Both carbachol (0.01–0.2 μ g) and bethanechol (0.2–4.0 μ g) were most effective, producing multiple mammary contractions in 47/53 and 13/17 lactating rats, respectively. These responses were both large and prolonged (Figure 6), and as such they differed substantially from the mammary contractions evoked during suckling. Intravenously-administered carbachol (0.6 μ g) and bethanechol (6 μ g) caused no contraction of the mammary myoepithelium, though both produced a marked fall in blood pressure.

The injection of nicotine into the lateral cerebral ventricles produced a mammary contraction in only 4/17 rats, and doses up to 200 μ g/rat were tested. In three animals from which the blood pressure was recorded, intraventricular nicotine caused a rise in arterial pressure at a dose at which there was no rise in intramammary pressure. Nicotine was more effective at releasing neurohypophysial hormones when in-

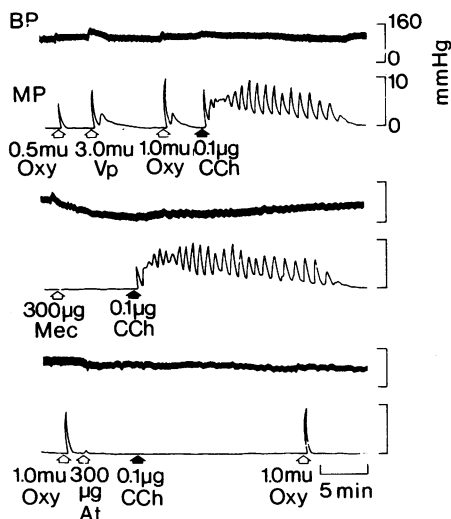


Figure 6 Combined blood pressure and mammary pressure records. Oxytocin (Oxy) injected intravenously (open arrows) gave a dose-dependent increase in intramammary pressure but little blood pressure change. Vasopressin (Vp) produced both an increase in mammary pressure and a sustained rise in blood pressure. Intracerebroventricular injection (black arrow) of carbachol (CCh) produced a prolonged multiple contraction of the mammary gland but little alteration in blood pressure. Mecamylamine (Mec, 1 mg/kg, i.v.) caused a fall in blood pressure (ganglion blocking dose) but had no effect upon the action of carbachol. The release of oxytocin in response to carbachol was prevented by the intravenous injection of atropine (1 mg/kg), but mammary gland sensitivity to oxytocin was unaltered.

jected intravenously and 5/7 rats displayed a mammary contraction to a dose of 0.5–1.0 mg, intravenously. In several other animals this dose proved fatal. In 2 animals the external carotid was cannulated, and in both a rise in blood pressure accompanied the mammary contraction induced by intravenous nicotine. In 3 rats given nicotine intravenously the release of oxytocin was blocked by hexamethonium (5 mg/kg i.v.).

To determine, albeit very indirectly, the identity of the hormone released by the intraventricular injection of cholinomimetics, further experiments were conducted in which both blood pressure and intramammary pressure were recorded. Both oxytocin and vasopressin will cause a contraction of the mammary gland, though oxytocin is five-times more effective, whereas only vasopressin creates a prolonged pressor action on the vascular system. Of 13 rats which received carbachol (0.1 to 0.2 μ g), only 4 displayed an elevation in blood pressure, whilst all displayed a prolonged

rise in intramammary pressure. Of 4 rats given bethanechol (2 to 4 μ g) 3 displayed a substantial rise in blood pressure. In all these animals oxytocin (0.5 to 1.0 mu, i.v.) caused a large mammary contraction and a change in blood pressure, if it occurred, was only transitory. Vasopressin, at a dose which caused a similar mammary contraction (2 to 5 mu, i.v.), produced a large and prolonged rise in blood pressure (Figure 6). In every experiment the change in blood pressure in response to vasopressin was much larger than that observed after the intraventricular injection of carbachol or bethanechol. In contrast, carbachol (0.6 μ g) and bethanechol (6 μ g) injected intravenously caused a pronounced fall in blood pressure. Thus, it seems that the two cholinomimetics, when injected into the cerebral ventricles, acted centrally to release oxytocin, and little, if any, vasopressin.

Effect of antagonists on the intraventricular injection of cholinomimetics

Atropine (1 mg/kg, i.v.) completely blocked the action of both carbachol (Figure 6) and bethanechol in all 11 rats examined; in some animals 0.1 mg/kg of atropine was effective. In contrast, mecamylamine and hexamethonium (5 mg/kg, i.v.) failed to abolish the carbachol-evoked release of hormone in 8/8 rats. Sympathetic ganglionic transmission was blocked, as indicated by the fall in arterial pressure, by both mecamylamine and hexamethonium at only 1 mg/kg.

Discussion

It has been observed that neurohypophysial hormones are released when acetylcholine or cholinomimetics are injected by various routes (Pickford, 1947; Abrahams & Pickford, 1954; Bisset & Walker, 1957; Kühn & McCann, 1970; Milton & Paterson, 1974). The present experiments, like those of Kühn & McCann (1971), indicate that the observed increase in intramammary pressure which occurs following the intraventricular injection of cholinomimetics is primarily due to the release of oxytocin. The cholinomimetics, carbachol and bethanechol, undoubtedly acted at a site within the central nervous system, for very much larger doses were required systemically to cause a contraction of the mammary myoepithelium. Although acetylcholine was only effective when high concentrations were placed in the ventricular system, and overflow into the systemic blood circulation may have occurred, a mammary contraction was still induced when peripheral cholinoceptors were blocked. The thousand-fold increase in sensitivity observed when acetylcholine was replaced by carbachol probably reflects the difference in the availability of the two compounds, acetylcholine being rapidly inacti-

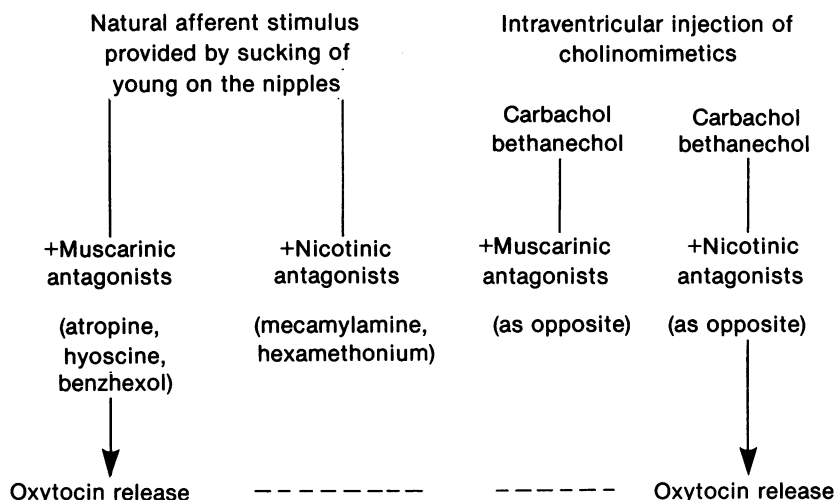


Figure 7 Summary of the effect of cholinergic antagonists on the release of oxytocin evoked by the suckling stimulus and by the intraventricular injection of carbachol and bethanechol. A dotted line indicates the failure to release oxytocin.

vated by enzymatic degradation. Likewise, the prolonged availability of carbachol probably accounts for the prolonged release of oxytocin that was observed.

Whilst carbachol can act at both muscarinic and nicotinic sites, its stimulatory action on oxytocin release would appear to have been mediated solely by muscarinic receptors. Not only was the muscarinic agent, bethanechol, very effective in the release of oxytocin, but also the muscarinic antagonist atropine blocked the actions of both carbachol and bethanechol. Further it has been shown previously that the release of oxytocin evoked by the intraventricular injection of carbachol can be blocked by atropine injected intraventricularly (Kühn & McCann, 1971). In contrast, nicotine was weak and variable as a stimulant of oxytocin release, and the nicotinic antagonist mecamylamine was without effect on the carbachol-evoked release (Figure 7).

It would appear, despite the fact that cholinomimetics evoke the release of oxytocin by a muscarinic action, that a cholinergic nicotinic mechanism is involved in the release of oxytocin in response to the natural stimulus of suckling. Atropine, and the related drug hyoscine, both failed to abolish the suckling-induced milk ejection at high doses (200 mg/kg, i.v.). Further, benzhexol a cholinergic antagonist of the anti-muscarinic type, which has a preferential action within the central nervous system (Farquharson & Johnston, 1959) was also without effect. Various authors have claimed that atropine blocks the milk-ejection reflex (Grosvenor & Turner, 1957; Ōba, Ōta & Yokoyama, 1971; Moos & Richard, 1975), but in none of these previous studies were the authors able

to control the suckling stimulus or measure effectively the release of oxytocin. Atropine is known to have a depressant action on some central neurones (Curtis & Phillis, 1960; Clarke & Davies, 1973), and thus the inhibition observed in some previous studies on conscious animals may have related to an indirect action, perhaps removing the desire of the mothers to nurse their young. The inhibition observed with the nicotinic antagonists mecamylamine and hexamethonium provides strong support for the opposing view, that the milk-ejection reflex involves a cholinergic nicotinic relay. These drugs would appear to have had a specific action which was within the central nervous system, for the inhibition created was dose-related and neither drug appeared to influence the release of oxytocin on electrical stimulation of the neurohypophysis or the response of the mammary gland to injections of oxytocin. Similarly, Moos & Richard (1975) showed that intraventricular hexamethonium, in the conscious rat, blocked milk ejection (as measured by the weight gain of the young). Mecamylamine was, as expected, the more effective drug for being a secondary amine and lipid soluble it readily gains access to the brain (Harrington, Kincaid-Smith & Milne, 1958), whereas the access of the more highly ionized drug hexamethonium (Dollery, 1964) would be more restricted. We see no evidence to implicate the fall in blood pressure produced by these nicotinic antagonists with the inhibition of the milk-ejection reflex, for no inhibition was observed with a variety of other vasodepressor substances. Likewise, the response appears to be independent of the central β -adrenoceptor mechanism which has

been shown to inhibit oxytocin release, as pretreatment with propranolol was ineffective (Tribollet, Clarke, Dreifuss & Lincoln, 1978).

Nicotinic synapses are also involved in the release of the other neurohypophysial hormone, vasopressin. Nicotine stimulates the release of vasopressin (Burn, Truelove & Burn, 1945; Bisset & Walker, 1957; Milton & Patterson, 1974), and the release of the hormone in response to cholinomimetics (Bridges & Thorn, 1970; Milton & Patterson, 1974), and injections of hypertonic saline (Bridges & Thorn, 1970) is blocked by nicotinic antagonists. Similarly, hexamethonium blocks acetylcholine-evoked release of vasopressin from cultured supraoptic neurones (Sladek & Knigge, 1977). It is surprising, in view of the apparent involvement of a nicotinic synapse in the milk-ejection reflex, that nicotine injected into the cerebral ventricles was relatively ineffective in the release of oxytocin and that the action of the intraventricular carbachol was *entirely* muscarinic. Certainly, one would expect substances injected into the lateral ventricles to reach the paraventricular nuclei, and probably the supraoptic nuclei of the hypothalamus. Evidence from iontophoretic experiments performed on the supraoptic nucleus of the rat indicate the presence there of excitatory nicotinic receptors (Dreifuss & Kelly, 1972), whilst within the paraventricular nucleus of the rabbit, both nicotinic and muscarinic receptors have been found which are excitatory (Moss *et al.*, 1972). Unfortunately no distinction was made in these studies between oxytocin and vasopressin containing neurones. More recently, it has been suggested that an area on the ventral surface of the medulla may be far more sensitive than the hypothalamus to nicotine-evoked release of vasopressin (Bisset, Feldberg, Guertzenstein & Rocha e Silva, 1975; Castro de Souza & Rocha e Silva, 1977).

Thus, it is quite possible that the 'nicotinic' synapses involved in the milk-ejection reflex are also remote from the hypothalamus. However, if a remote site is involved it is unlikely to be on the ventral surface of the medulla, at least in the cat, for selective release of vasopressin alone follows stimulation of that area (Bisset *et al.*, 1975). An alternative explanation is that 'nicotinic' synapses have no direct role in the reflex release of oxytocin. They could function external to the reflex pathway and simply interact with the pathway in a permissive role, either by disinhibition or facilitation. Thus, nicotinic antagonists would block the reflex, whilst stimulation with nicotine would not necessarily release oxytocin. In all these considerations, however, it should be remembered that nicotine is a poor agonist for characterizing nicotinic receptors (see Brimblecombe, 1974).

The presence of muscarinic receptors, which stimulate oxytocin release but which are not involved in reflex milk ejection, poses the question of what functional role, if any, they serve. Cholinergic mechanisms might be involved in the release of oxytocin in response to exteroceptive cues, such as the crying of the baby or the clatter of the milking machine. Presumably such stimuli are mediated via the cerebral cortex, and it is noteworthy that cholinergic mechanisms in the cerebral cortex are exclusively muscarinic (Krnjević & Phillis, 1963). Indeed, release of oxytocin in response to the intraventricular injection of carbachol and bethanechol may have involved an action on some part of the higher nervous system. Further studies are in progress to investigate this 'muscarinic phenomenon'.

This work was supported in part by the Medical Research Council.

References

- ABRAHAM, V.C. & PICKFORD, M. (1954). Simultaneous observations on the rate of urine flow and spontaneous uterine movements in the dog, and their relationship to posterior lobe activity. *J. Physiol.*, **126**, 329–346.
- BISSET, G.W., FELDBERG, W., GUERTZENSTEIN, P.G. & ROCHA E SILVA, M. Jr. (1975). Vasopressin release by nicotine: the site of action. *Br. J. Pharmacol.*, **54**, 463–474.
- BISSET, G.W. & WALKER, J.M. (1957). The effects of nicotine, hexamethonium and ethanol on the secretion of the antidiuretic and oxytocic hormones of the rat. *Br. J. Pharmacol. Chemother.*, **12**, 461–467.
- BRIDGES, T.E., HILLHOUSE, E.W. & JONES, M.T. (1976). The effect of dopamine on neurohypophysial hormone release *in vivo* and from the rat neural lobe and hypothalamus *in vitro*. *J. Physiol.*, **260**, 647–666.
- BRIDGES, T.E. & THORN, N.A. (1970). The effect of autonomic blocking agents on vasopressin release *in vivo* in release *in vivo* and from the rat neural lobe and hypothalamus *in vitro*. *J. Physiol.*, **260**, 647–666.
- BRIMBLECOMBE, R.W. (1974). Drug actions at central nicotinic sites. In *Drug Actions on Cholinergic Systems*. pp. 191–214. London: Macmillan Press.
- BURN, J.H., TRUELOVE, L.H. & BURN, I. (1945). The antidiuretic action of nicotine and of smoking. *Br. med. J.*, **1**, 403–406.
- CASTRO DE SOUZA, E.M. & ROCHA E SILVA, M. Jr. (1977). The release of vasopressin by nicotine: further studies on its site of action. *J. Physiol.*, **265**, 297–311.
- CLARKE, G. & DAVIES, J. (1973). The effects of anti-Parkinson drugs on cortical neurones. *Br. J. Pharmacol.*, **47**, 465–475.
- CROSS, B.A. & HARRIS, G.W. (1952). The role of the neurohypophysis in the milk-ejection reflex. *J. Endocr.*, **8**, 148–161.
- deduced by osmoreceptor stimulation. *J. Endocr.*, **48**, 265–276.

- CURTIS, D.R. & PHILLIS, J.W. (1960). The action of procaine and atropine on spinal neurones. *J. Physiol.*, **153**, 17–34.
- DOLLERY, C.T. (1964). Absorption, distribution and excretion of drugs used to treat hypertension. *Chemical Abstracts*, **61**, No. 12. (Absorption Distrib. Drugs Symp., Lond., pp. 157–164, 1963).
- DREIFUSS, J.J. & KELLY, J.S. (1972). The activity of identified supraoptic neurones and their response to acetylcholine applied by iontophoresis. *J. Physiol.*, **220**, 105–118.
- FARQUHARSON, M.E. & JOHNSTON, R.G. (1959). Antagonism of the effects of tremorine by tropine derivatives. *Br. J. Pharmac. Chemother.*, **14**, 559–566.
- GROSVENOR, C.E. & TURNER, C.W. (1957). Evidence for adrenergic and cholinergic components in milk let-down reflex in lactating rat. *Proc. Soc. exp. Biol. Med.*, **95**, 719–722.
- HARRINGTON, M., KINCAID-SMITH, P. & MILNE, M.D. (1958). Pharmacology and clinical use of pempidine in the treatment of hypertension. *Lancet*, **ii**, 6–11.
- HARRIS, G.W., MANABE, Y. & RUF, K.B. (1969). A study of the parameters of electrical stimulation of unmyelinated fibres in the pituitary stalk. *J. Physiol.*, **203**, 67–81.
- KRNJEVIĆ, K. & PHILLIS, J.W. (1963). Pharmacological properties of acetylcholine-sensitive cells in the cerebral cortex. *J. Physiol.*, **166**, 328–350.
- KÜHN, E.R. & McCANN, S.M. (1970). Injections of carbachol into the third ventricle and release of oxytocin and vasopressin in the lactating rat. *Arch. int. Pharmacodyn.*, **186**, 192–193.
- KÜHN, E.R. & McCANN, S.M. (1971). Release of oxytocin and vasopressin in lactating rats after injection of carbachol into the third ventricle. *Neuroendocrinology*, **8**, 48–58.
- LINCOLN, D.W., CLARKE, G., MASON, C.A. & DREIFUSS, J.J. (1977). Physiological mechanisms determining the release of oxytocin in milk ejection and labour. In *The Neurohypophysis*. pp. 101–109. ed. Moses, A.M. Basel: S. Karger.
- LINCOLN, D.W., HILL, A. & WAKERLEY, J.B. (1973). The milk-ejection reflex of the rat: an intermittent function not abolished by surgical levels of anaesthesia. *J. Endocr.*, **57**, 459–476.
- LINCOLN, D.W. & WAKERLEY, J.B. (1974). Electrophysiological evidence for the activation of supraoptic neurones during the release of oxytocin. *J. Physiol.*, **242**, 533–554.
- LINCOLN, D.W. & WAKERLEY, J.B. (1975). Factors governing the periodic activation of supraoptic and paraventricular neurosecretory cells during suckling in the rat. *J. Physiol.*, **250**, 443–461.
- MILTON, A.S. & PATERSON, A.R. (1974). A microinjection study of the control of antidiuretic hormone release by the supraoptic nucleus of the hypothalamus in the cat. *J. Physiol.*, **241**, 607–628.
- MOOS, F. & RICHARD, P. (1975). Adrenergic and cholinergic control of oxytocin release evoked by vaginal, vagal and mammary stimulation in lactating rats. *J. Physiol. (Paris)*, **70**, 315–322.
- MOSS, R.L., URBAN, I. & CROSS, B.A. (1972). Microelectrophoresis of cholinergic and aminergic drugs on paraventricular neurons. *Am. J. Physiol.*, **223**, 310–318.
- ÔBA, T., ÔTA, K. & YOKOYAMA, A. (1971). Inhibition of milk-ejection reflex in lactating rats by systemic administration and intracerebral implantation of atropine. *Neuroendocrinology*, **7**, 116–126.
- PICKFORD, M. (1947). The action of acetylcholine in the supraoptic nucleus of the chloralosed dog. *J. Physiol.*, **106**, 264–270.
- SLADEK, C.D. & KNIGGE, K.M. (1977). Cholinergic stimulation of vasopressin release from the rat hypothalamo-neurohypophyseal system in organ culture. *Endocrinology*, **101**, 411–420.
- TRIBOLLET, E., CLARKE, G., DREIFUSS, J.J. & LINCOLN, D.W. (1978). The role of central adrenergic receptors in the reflex release of oxytocin. *Brain Res.*, (in press).
- WAKERLEY, J.B. & LINCOLN, D.W. (1973). The milk-ejection reflex of the rat: a 20- to 40-fold acceleration in the firing of paraventricular neurones during oxytocin release. *J. Endocr.*, **57**, 477–493.

(Received December 1, 1977.
Revised January 20, 1978.)