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A comparison of analgesia and suppression of oxytocin release by opiates

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1 The potency of opiates for suppressing oxytocin release relative to their potency as analgesics was tested in lactating rats.

2 Oxytoxin release was evoked by the sucking of the young in urethane–anaesthetized and unanaesthetized rats, and was detected by the characteristic behaviour of the young and milk yield respectively.

3 The tail-flick test, using noxious radiant heat, was used to assess analgesia.

4 Intraperitoneal injection of morphine (1 mg kg^{-1} and 5 mg kg^{-1}) significantly reduced milk yield in unanaesthetized rats.

5 Urethane-anaesthetized rats displayed a pattern of reflex milk-ejection responses similar to that found in conscious rats. This reflex was significantly inhibited in a dose-related, naloxone-reversible manner by buprenorphine (ED_{50} 0.18 mg kg^{-1}), meptazinol (ED_{50}: 14.0 mg kg^{-1}), morphine (ED_{50}: 0.67 mg kg^{-1}), pentazocine (ED_{50}: 15.0 mg kg^{-1}) and pethidine (ED_{50}: 7.9 mg kg^{-1}).

6 Although intraperitoneal injection of morphine (5 mg kg^{-1}) abolished the increase in intramammary pressure occurring at reflex milk-ejection, that evoked by intravenous oxytocin (0.5–1 mu) was unaffected.

7 Each opiate also caused significant, dose-related, naloxone-reversible increases in tail-flick latency. The ED_{50} doses were buprenorphine (ED_{50}: 0.14 mg kg^{-1}), meptazinol (ED_{50}: 12.5 mg kg^{-1}), morphine (ED_{50}: 5.0 mg kg^{-1}), pentazocine (ED_{50}: 12.5 mg kg^{-1}) and pethidine (ED_{50}: 6.1 mg kg^{-1}).

8 The order of potency for analgesia and for suppression of oxytocin release were identical, namely: buprenorphine > morphine > pethidine > meptazinol > pentazocine.

9 The results obtained with lactating rats suggest that secretion of the hormone oxytocin is substantially reduced during opiate-induced analgesia.

Introduction

Oxytocin is a hormone produced by magnocellular neurones in the paraventricular and supraoptic nuclei of the hypothalamus and released from the nerve terminals in the posterior pituitary. Its established functions relate to the female reproductive system. Oxytocin causes contractions of the uterus during labour and of myoepithelial cells in the mammary gland to cause milk ejection or 'let down'.

There is considerable evidence to suggest that opioid peptides are involved in the control of oxytocin release. Endogenous opioid peptides are found in close proximity to the magnocellular neurones containing oxytocin (Rossier *et al.*, 1980) and abundant opioid binding sites are present both in the hypothalamus (Atweh & Kuhar, 1983) and pituitary (Simantov & Snyder, 1977). Furthermore morphine (Clarke *et al.*, 1979; Clarke & Patrick, 1983) and opioid peptides (Wright *et al.*, 1983) inhibit oxytocin release. This inhibition may occur concomitantly with opiate-induced analgesia and thus could be an important consideration, for example, in the management of labour. In order to examine this possibility several clinically used opiates were tested to determine their potency in suppressing oxytocin release relative to their potency as analgesics in lactating rats.

Methods

Lactating Wistar rats were used 7–10 days *post-*

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partum. They were kept under constant environmental conditions with a 14 h light period and were supplied with food and water *ad libitum*. All rats except those in which oxytocin release was assessed by measurement of pup weight gain, were separated from all but one of their pups the evening before the experiment. They were then divided into two groups for investigation of the effect of opiates either in an antinociceptive test or on oxytocin release.

Measurement of oxytoxin release in unanaesthetized animals

Animals in this group were separated from their pups for 3 h only. Intraperitoneal injections of morphine or 0.9% saline were made at the beginning of a 30 min suckling period and the effect on the reflex release of oxytocin was assessed by measurement of pup weight gain during this period. Four groups of 8 rats (2 control and 2 treated groups) were used; 4 individual rats failed to suckle their young and the data were excluded from the results.

Measurement of oxytocin release in anaesthetized animals

These animals were lightly anaesthetized with urethane (1.1 g kg^{-1}, i.p.) and 10 hungry pups were placed on the nipples 3 h later. The reflex ejection of milk usually commenced within 30 min and continued for a period of several hours. Each milk ejection, occuring at regular intervals of approximately 5 min (as a consequence of the pulsatile release of 1–2 mu of oxytocin, Wakerley & Lincoln, 1971), was detected by the characteristic behaviour of the young (Lincoln *et al.*, 1973). Opiates were administered after 5 or 6 milk-ejection responses. Significant suppression of the release of oxytocin was considered to have occurred if 3 or more consecutive milk ejections were abolished. Intervals of this magnitude represent an increase over the mean milk-ejection interval before opiate administration of greater than the 99% probability estimate (see Clarke *et al.*, 1975).

An investigation was made to determine whether opiates exert a direct action on the mammary gland. The experimental protocol was the same as before but for these experiments intramammary pressure was also recorded. Halothane (0.5–1.0% in oxygen/nitrous oxide) was used to provide additional (surgical) anaesthesia for cannulation of a saphenous vein and in inguinal mammary gland. Intravenous injections of oxytocin (0.5–1.0 mu) were made 5–10 min prior to (between reflex milk-ejection responses), and 15–20 min after, the administration of morphine (5 mg kg^{-1}, i.p.) and the effect on intramammary pressure was determined.

Measurement of antinociceptive effect of opiates

Nociceptive thresholds were measured by the tail-flick method. The rat was placed in a restrainer and the distal third of its tail positioned unrestricted at the point of focus of a noxious radiant heat source (a focussed projector bulb). Switching on the light source started an electronic timer and movement of the tail exposed a photosensor to the light which stopped the clock; the period from heat application to tail-flick was recorded automatically. Following a 30 min acclimatization period within the restrainer, the test was conducted at 5 min intervals with a lamp intensity set to give baseline response latencies of 5–6 s. All latencies were expressed as a % change from the mean baseline latency. To avoid thermal damage to the tail a cut-off limit was set at twice this value. The analgesic ED_{50} was taken (as the dose that in 50% of the animals caused a 100% increase in response latency from baseline, for 3 or more consecutive (5 min) intervals. This represented an in-

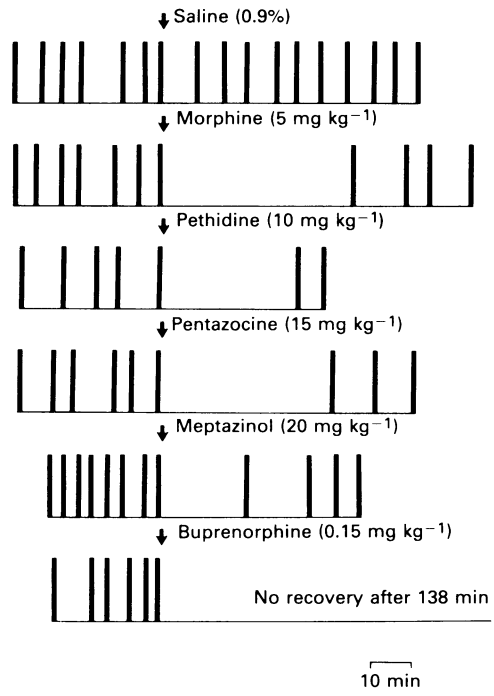

Figure 1 Effect of opiates on the release of oxytocin evoked during suckling in rats. The pattern of the milk-ejection response is shown for 6 different anaesthetized rats; each vertical column corresponds to an individual response. Each opiate, administered by intraperitoneal injection at the dose indicated, caused a significant ($P<0.01$) inhibition of the reflex.

crease above the 99% probability level (statistical analysis as for oxytocin release in anaesthetized animals).

In an additional series of experiments, animals were lightly anaesthetized with urethane for 'milk ejection' experiments but after two milk ejections the animal was transferred to the restrainer and tail-flick latencies were measured before and after pethidine administration.

Administration of an opiate antagonist

In an additional series of experiments each opiate, at the dose found to cause significant effects in all animals tested, was administered simultaneously with the opiate antagonist naloxone (5 mg kg^{-1} i.p.). Responses in tests to measure reflex milk ejection (anaesthetized rats) or tail-flick latencies (conscious rats) were measured.

Drugs

The following drugs were used: morphine sulphate (MacFarlan Smith), pethidine (Arnolds), pentazocine lactate (Fortral, Winthrop), meptazinol hydrochloride (Wyeth), buprenorphine (Temgesic, Rickett and Colman) and naloxone hydrochloride (Endo). They were dissolved or diluted (if proprietary preparations) in 0.9% saline and administered in a volume of 1 ml by intraperitoneal injection. Drug doses are expressed in terms of the base.

Results

Effect of morphine on milk yield in unanaesthetized rats

The intake of milk by the pups was determined by weighing them before and after a 30 min suckling period. Pups gained 3.5 ± 1.17 g per pup (6 litters of 10 pups) in saline control groups but only 1.0 ± 0.58 g per pup (7 litters of 10 pups) in groups in which the doe had received 1 mg kg^{-1} (i.p.) morphine, a difference of 71.4%, significant at $P<0.02$ (Mann-Whitney U test). The suppressive effect of morphine was still significant ($P<0.01$ Mann-Whitney U test) but less marked at a dose of 5 mg kg^{-1}; pups (8 litters of 10) gained 2.12 ± 0.99 g per pup which was 48.8% less than those in their saline control group (7 litters of 10).

Table 1 Suppressive effect of opiates on oxytocin release (milk-ejection reflex)

Drug	*Dose* (mg kg^{-1}, i.p.)	*Mean suppression of milk-ejection reflex* *Duration* (min)	*% change from basal interval*	*% of animals showing significant suppression**	n
Saline 0.9%		8.0 ± 1.1	13 ± 14	0	6
Buprenorphine	0.09	5.5–0.7	30 ± 40	0	5
	0.15	>80	>1320	88	8
	0.45	>79	>1270	88	8
Meptazinol	10	7.6 ± 1.4	50 ± 20	0	5
	20	30.7 ± 10.2	530 ± 250	80	5
	30	42.8 ± 10.3	560 ± 250	100	5
Morphine	0.33	7.9 ± 2.2	33 ± 25	0	8
	0.67	25.3 ± 6..4	300 ± 70	43	7
	1	26.3 ± 3.9	300 ± 60	80	10
	5	>75	>880	100	5
Pentazocine	5	11.6 ± 3.8	150 ± 110	20	5
	15	39.0 ± 12.5	550 ± 200	57	7
	30	70.8 ± 10.8	930 ± 210	100	5
Pethidine	5	33.1 ± 6.7	340 ± 70	43	7
	10	36.0 ± 8.9	320 ± 30	67	6
	15	53.7 ± 7.8	960 ± 190	100	5

± s.e.mean.

* $P<0.01$ (see methods).

Effect of opiates on the milk ejection reflex in anaesthetized rats

Rats, lightly anaesthetized with urethane, displayed a pattern of milk ejection similar to that found in conscious rats. For example the mean interval between reflex milk ejections was 6.3 ± 0.3 min ($n = 116$), which is very similar to that reported for conscious rats from the same colony: 6.6 ± 0.6 min (Lincoln *et al*, 1973). Each of the opiates used in this study significantly inhibited the milk-ejection reflex (Figure 1). Thus morphine (0.6–5 mg kg^{-1}, i.p.), pethidine (5–15 mg kg^{-1}), pentazocine (5–30 mg kg^{-1}), meptazinol (10–30 mg kg^{-1}) and buprenorphine (0.045–0.9 mg kg^{-1}) all caused a dose-dependent suppression of the milk-ejection reflex; these results are summarised in Table 1. This action of the opiates was completely antagonized by naloxone (5 mg kg^{-1}, i.p.).

The sensitivity of the mammary gland to oxytocin was unaffected by intraperitoneal injection of morphine. The mean increase in intramammary pressure induced by oxytocin before and after intraperitoneal injection of morphine (5 mg kg^{-1}), which completely abolished the milk-ejection reflex in 5/5 rats, was identical (see Figure 2). After the milk-ejection response had been abolished for a significant ($P > 0.01$) period (30–35 min), naloxone (1 mg kg^{-1}, i.p.) was injected and in 5/5 rats reflex milk-ejection resumed 8.7 ± 2.6 min later. However, in animals not treated with naloxone reflex milk-ejection was abolished for >75 min (see Table 1).

Dose-response curves were constructed (Figure 3) and the doses required to cause a significant inhibition ($P \leq 0.01$) in half the animals (ED_{50}) were obtained. This gave the following potency order: buprenorphine (ED_{50} 0.18 mg kg^{-1}, i.p.) > morphine (0.67 mg kg^{-1}) > pethidine (7.9 mg kg^{-1}) > meptazinol (14.0 mg kg^{-1}) > pentazocine (15.0 mg kg^{-1}). At the ED_{50} dose the mean duration of the inhibition was calculated to be 19 min for meptazinol, 25.5 min for morphine, 35 min for pethidine and 39 min for pentazocine. Buprenorphine caused the longest suppression of reflex milk injection (>80 min) at the ED_{50} dose and only rarely was recovery observed during the 3 h observation period.

Effect of opiates in the tail-flick test

All the opiates used caused significant ($P \leq 0.01$) increases in the latency to the tail-flick response to noxious heat application (Figure 4) at doses which had depressed milk-ejection. These antinociceptive effects of morphine (1–10 mg kg^{-1}, i.p.), pethidine (5–15 mg kg^{-1}), pentazocine (5–30 mg kg^{-1}), meptazinol (10–30 mg kg^{-1}) and buprenorphine (0.045–0.45 mg kg^{-1}) were dose-dependent (Table 2) and could be antagonized by naloxone (5 mg kg^{-1} i.p.). The ED_{50} i.e. the dose which caused significant

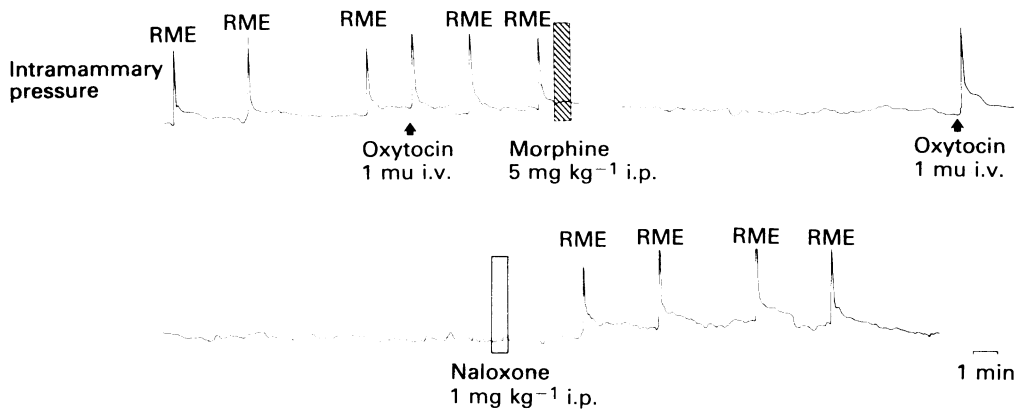

Figure 2 Effect of morphine on the milk-ejection reflex and the sensitivity of the mammary gland to oxytocin. The figure shows a continuous intramammary pressure recording from a urethane anaesthetized lactating rat. The reflex milk-ejection response (RME) shown at the start of the trace was the fourth and occurred 17 min after applying 10 hungry pups to the nipples. After intraperitoneal injection of morphine (5 mg kg^{-1} the milk-ejection reflex was abolished whereas the response to intravenous oxytocin (1 mu) was unaltered and hence the sensitivity of the mammary gland was unaffected. Naloxone (1 mg kg^{-1}, i.p.) antagonized the opiate-induced inhibition of oxytocin release. The pups responded at reflex milk-ejection and to intravenous oxytocin (1 mu) in a similar manner; the magnitude of the intramammary pressure was equivalent to 10mmHg.

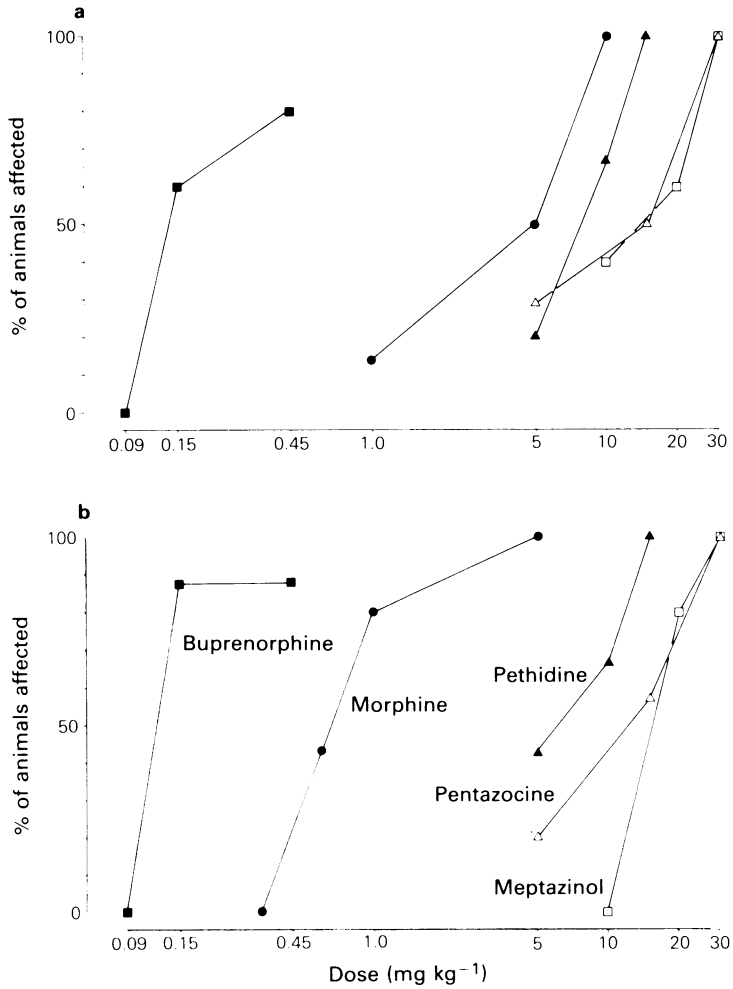

Figure 3 Dose-response plots for opiate-induced analgesia (a) and opiate-induced inhibition of oxytocin release (b) in the lactating rat. Ordinate scale gives percentage of animals significantly ($P<0.01$) affected by opiates. Each point represents the mean of 5–10 rats.

($P \leq 0.01$) increases in tail-flick latency in 50% of the animals was obtained from the dose-response curve (Figure 3). This gave the following potency order: buprenorphine (ED_{50} 0.14 mg kg^{-1}, i.p.) morphine (5.0 mg kg^{-1}) > pethidine (6.1 mg kg^{-1}) > meptazinol (12.5 mg kg^{-1}) > pentazocine (15.2 mg kg^{-1}). The mean duration of maximum effect (i.e. $\geq$100% increase in response latency) was calculated at the ED_{50} dose and found to be shortest for pethidine (8 min) and longest for buprenorphine (>112 min). In comparison the duration for metazinol was 23.5 min, pentazocine was 27 min and morphine was 33.1 min.

Effect of pethidine in 'tail-flick' tests on anaesthetized rats

The mean baseline tail-flick latency of rats which had been lightly anaesthetized with urethane was 5.78 ± 0.37 s which was very similar to that of conscious rats tested in parallel at the same lamp intensity (mean = 5.79 ± 0.31 s). Pethidine (0.5–5 mg kg^{-1}, i.p.) caused dose-dependent increases in the tail-flick latency of anaesthetized rats. The ED_{50} was 0.86 mg kg^{-1}.

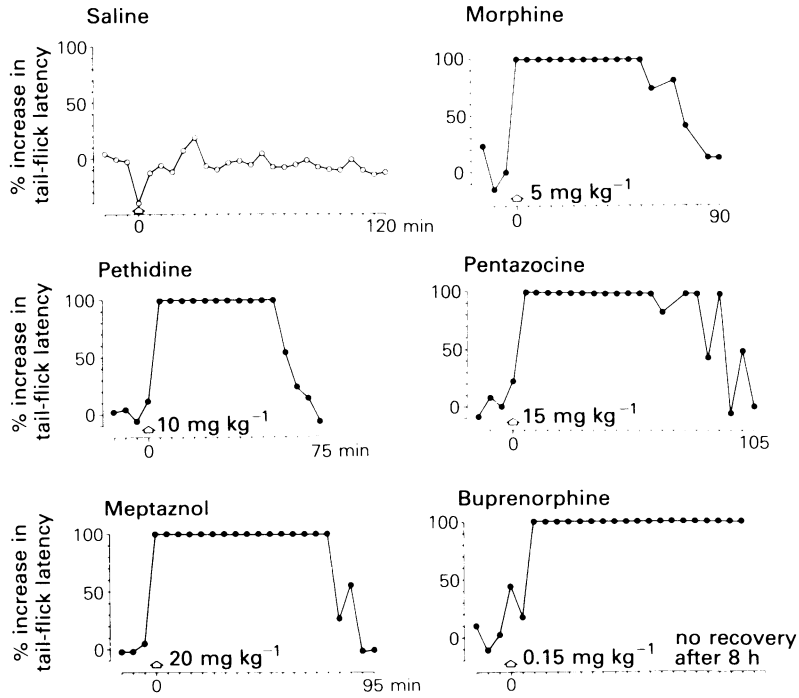

Figure 4 Effect of opiates on tail-flick latency in unanaesthetized lactating rats. Results are from 6 different rats. Each point represents the response latency to a noxious stimulus applied every 5 min and is expressed as a percentage change from the mean basal response. If this value were greater than 100% the noxious stimulus was automatically terminated. The results show that each opiate, at the dose (i.p.) indicated, had a significant ($P<0.01$) antinociceptive effect.

Discussion

In the rat in common with other mammals (including man) oxytocin is released in a pulsatile manner during suckling and causes contraction of the myoepithelial cells of the mammary gland, which ejects milk from the alveoli. Our results obtained using the lactating rat suggest that secretion of this hormone is substantially reduced during opiate-induced analgesia.

Morphine, pethidine, pentazocine, meptazinol and buprenorphine all caused a significant inhibition of oxytocin release; this action was dose-dependent and was antagonized by naloxone. It is unlikely that the sensitivity of the mammary gland to oxytocin is altered by any of the opiates for morphine did not alter the pressure response to intravenous injection of oxytocin at a dose that blocked reflex hormone release. One probable site at which the opiates exerted an inhibitory effect is in the posterior pituitary, where the nerve terminals of oxytocinergic neurones are located. Endogenous opioids are present in the locality (Goldstein & Ghazarossian, 1980; Rossier *et al.*, 1980) as are opiate binding sites (Simantov & Snyder, 1977). Furthermore, it has been shown that the release of oxytocin evoked by electrical stimulation in this region is blocked by morphine, both *in vivo* following intracerebroventricular injection (Clarke *et al.*, 1979) and *in vitro* using the isolated neural lobe preparation (Bicknell & Leng, 1982; Clarke & Patrick, 1983).

Opiate suppression of suckling-induced release of oxytocin was achieved within the dose-ranges that caused significant analgesia as indicated by dose-related increases in the latency of the tail-flick response to noxious heat application. The sensitivity of lactating rats to the analgesic effect of opiates appeared to be similar to that reported for male rats (Cowan *et al.*, 1977; Tyers, 1980; Bill *et al.*, 1983). However, the animals used in the experiments in which the milk-ejection response was monitored were lightly anaesthetized; observations of the behavioural response of the pups is easier under these conditions, the effect of opiates on maternal behaviour (Russell & Spears, 1984) is avoided and the doe is less readily disturbed by extraneous stimuli.

Table 2 Analgesic effect of opiates (tail-flick test)

		Mean analgesia effect			
Drug	*Dose* ($mg\,kg^{-1}$ i.p.)	*No. of readings 100% above baseline response latency*	*Duration* (min)	*% of animals showing significant* analgesia*	n
Saline 0.9%		0	0	0	7
Buprenorphine	0.045	0	0	0	5
	0.09	0	0	0	5
	0.15	>23	>113	60	55
	0.45	>28	>124	80	5
Meptazinol	10	3.8±2.1	18.0±10.3	40	5
	20	8.7±2.4	36.7±12.7	60	5
	30	12.2±2.0	56.0±10.2	100	5
Morphine	1	3.4±3.3	19.3±19.3	14	7
	5	6.9±2.6	33.1±13.1	50	8
	10	14.0±1.8	68.8± 9.0	100	5
Pentazocine	5	2.4±1.5	10.0± 6.8	29	7
	15	6.7±2.4	26.7±10.8	50	6
	30	12.3±3.1	57.5±15.5	100	5
Pethidine	5	2.0±0.9	4.0±4.0	20	5
	10	4.8±1.5	14.8±9.1	67	6
	15	7.0±1.2	30.8±6.5	100	6

± s.e. mean.
* $P < 0.01$ (see methods)

The latter point is important since in the rat, oxytocin release occurs only during periods of synchronous EEG activity akin to slow wave sleep (Lincoln *et al.*, 1980) not during periods of desynchrony when the rat is alert or aroused. When these lightly anaesthetized rats were used in 'tail-flick' experiments the dose of pethidine required to cause significant antinociceptive effects was less than that for unanaesthetized rats. Thus although the ED_{50} for suppression of oxytocin release was very similar to that for 'analgesia' for each opiate, it is possible that under anaesthesia the dose-response curve (for suppression of oxytocin release) was shifted to the left. If such a shift had occurred it would appear to be small, for it was found that the same low dose of morphine significantly suppressed oxytocin release in both anaesthetized and conscious rats; milk yield and pattern of milk ejection are similar in urethane-treated and conscious rats (see Lincoln *et al.*, 1973).

In addition to its involvement in lactation, oxytocin may have an important role in parturition. It is known to promote uterine contractions, this may occur both as a consequence of direct action and by prostaglandin release (Husslein *et al.*, 1981), and may be crucial for the initiation of labour (Fuchs *et al.*, 1982). An opiate, usually pethidine, is often given for pain relief during labour although it is thought that opiates diminish uterine activity and prolong labour (Friedman, 1978). Our results, which suggest that oxytocin release is substantially reduced during opiate-induced analgesia, provide a possible explanation for these clinical observations.

Our experiments have shown that the dose of each opiate required for analgesia was similar to that for suppression of oxytocin release and the potency order for each action was identical. Thus there appears to be a parallelism between these two diverse actions of the opiates, whereas in obstetrics a dissimilarity might prove clinically advantageous.

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