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Inhibition of Oxytocin Release by Morphine and Its Analogs (40080)

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Nutt and Jasinski (1) observed that administration of the morphine analog, oxilorphan, produced hypotonic polyuria in a group of heroin addicts. More recently Miller (2) reported that oxilorphan has a similar effect in Brattleboro rats heterozygous for the diabetes insipidus trait. He showed further that butorphanol, another morphine analog, was even more potent in causing polyuria in these rats. Both these investigators concluded that the analogs caused polyuria by inhibiting the release of the antidiuretic hormone (ADH) from the pituitary gland. Although it is well established that ADH and oxytocin (OT) can be released independently (3, 4) of each other, the cells producing these hormones are anatomically similar and closely associated. We thus thought it would be of interest to investigate how these two morphine analogs might affect release of OT in response to the physiological stimulus of suckling.

Materials and methods. All experiments were performed on Swiss Webster mice between the 11th and 22nd day of lactation. After the babies were born they were kept undisturbed for 10 days. On day 11 of lactation mothers were separated at about 10:00 AM and were reunited with their babies 4 hr later. They were then allowed to nurse their young for 1 hr. Babies were weighed just before and at the end of this hour of suckling. The difference of weight between the first and second weighings was assumed to be due to milk yield and was designated as "initial milk yield". All mothers then received an ip injection of 0.25 U of OT and were put back with their babies for another ½ hr of nursing. Babies were weighed again and the difference of weight between the second and third weighings was designated as the "residual milk yield". Babies were left with their mother overnight and separated the following morning. The same schedule was followed every day. On day 1 of experimentation mothers showed restlessness for about half an hour and they did not always provide a good initial milk yield. From day 2 on they seemed to be better adjusted to the experimental manipulations. Thus we always discarded the first day's results and the reported experiments were continued from day 2 for 10 days. In these experiments we did not cut down litter size to equal numbers but, instead, expressed the milk yield as g/100 g body weight of the litter.

Animals were divided in groups according to the design of the experiment. To avoid repetition of drug administration for two consecutive days to the same mice we altered the sequence of injection, i.e. the group which served as control on day 1 received butorphanol the next day and the group which received butorphanol on day 1 received oxilorphan the next day and so on.

Butorphanol, oxilorphan and morphine were always freshly diluted in isotonic saline and usually injected subcutaneously (s.c.) to the mother immediately before the 1 hr suckling period. In one set of experiments with butorphanol the drug was injected at different times to determine the best time for producing maximum inhibition. Control animals were injected with saline. Naloxone solutions were also made freshly every day and injected s.c. immediately before the 1 hr suckling period. An exception was the group which received both naloxone and morphine, where naloxone was given 10 min before morphine. Oxytocin, 0.25 U was injected intraperitoneally at the end of the 1 hr suckling period.

Butorphanol and oxilorphan are two newly synthesized morphine analogs. Their structures are given in Fig. 1. For comparison, structures of morphine and naloxone are also given. Naloxone, butorphanol and oxilorphan differ from morphine mainly by possessing a hydroxyl group at the 14 position. Butorphanol and oxilorphan can be fully synthesized whereas naloxone must be derived from thebaine, a natural alkaloid, as starting material.

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Butorphanol and oxilorphan were gifts from Bristol Laboratories, Syracuse, N.Y. Naloxone was a gift from Endo Lab, Inc., Garden City, N.Y. Oxytocin (Syntocinon) was a gift from Sandoz Pharmaceuticals. Morphine sulphate was purchased from Merck and Co., Inc.

Results. Figure 2 illustrates the effects of butorphanol and oxilorphan on initial milk yield. Both butorphanol and oxilorphan inhibited initial milk yield although the former appeared to be a much more potent inhibitor.

To determine whether the reduced milk yield in the presence of butorphanol and oxilorphan might reflect a reduction of milk in the mammary gland, 0.25 U OT was injected intraperitoneally in all mice, treated and control, after the initial 1 hr milk yield period. Table I shows the total milk yield, i.e., initial plus residual. Total milk yield in butorphanol and oxilorphan-treated mice was no less and actually a little more than in control mice. Thus the reduced milk yield in the presence of the drugs is not due to a reduction of milk in the gland but is more likely due to inhibition of milk ejection mediated by OT.

To find out the time of injection needed to obtain maximum inhibition of initial milk yield we injected 10 mg/kg doses of butorphanol at 0, 30, 60 and 120 min before the suckling period. The result is illustrated in Fig. 3. Maximum inhibition of 85% was obtained when butorphanol was injected immediately before suckling. Little more than 50% inhibition was observed when the drug was injected 30 min before and only 20% inhibition when given 120 min before the onset of suckling.

Pharmacological studies on oxilorphan and butorphanol clearly demonstrate that both drugs possess a mixture of analgesic and antagonistic properties (5, 6). To determine whether the analgesic or the antagonistic property of these drugs was responsible for the inhibition of OT release, we tested the effects of morphine, a purer agonist, and of naloxone, a purer antagonist, on OT release. The results of such experiments are given in Fig. 4. Naloxone at a dose of 5 mg and 10 mg/kg did not inhibit initial milk yield. However almost 95% inhibition was observed when mice were given 10 mg/kg of morphine. Thus morphine inhibited initial milk yield as much as or even more than butorphanol. If this inhibitory effect of morphine was a spe-

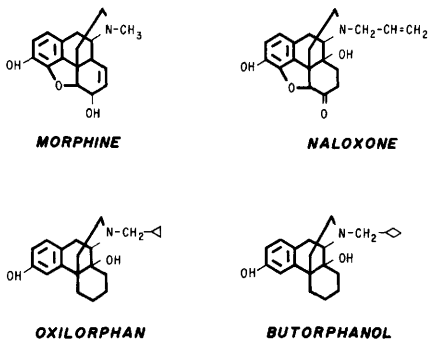

FIG. 1. Structures of oxilorphan and butorphanol compared with those of morphine and naloxone.

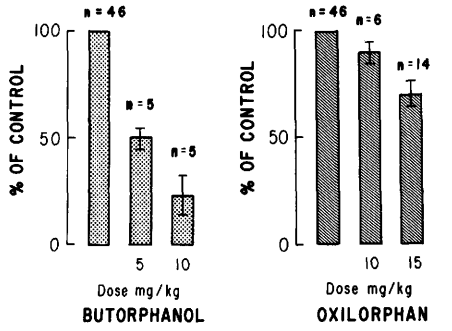

FIG. 2. Effects of butorphanol and oxilorphan on initial milk yield. Initial milk yield was calculated as described in Methods. Butrophanol and oxilorphan were injected subcutaneously immediately before the 1 hr suckling period. One hundred percent milk yield of control ranged from 1.14–5.03 g with a mean of 2.92 ± 0.15.

TABLE I. EFFECTS OF BUTORPHANOL AND OXILORPHAN ON TOTAL MILK YIELD.

	Total milk yield (g/100 g body weight)		
Control (10)[a]	2.40 ± 0.29[b]	control (12)	3.45 ± 0.50
Butorphanol (6) 5 mg/kg	2.88 ± 0.46	oxilorphan (5) 10 mg/kg	3.88 ± 0.68
Butorphanol (6) 10 mg/kg	2.94 ± 0.44	oxilorphan (14) 15 mg	4.35 ± 0.69

[a] Number of animals is given in parenthesis.
[b] Mean ± SE.

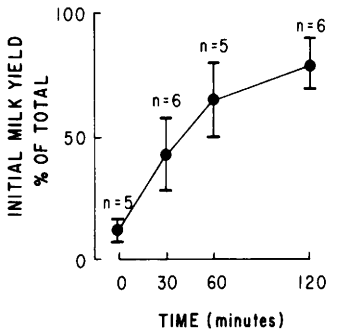

FIG. 3. Effect of time of injection of butorphanol on inhibition of initial milk yield. Butorphanol was injected at 0, 30, 60 and 120 min before the 1 hr suckling period. Initial milk yield is expressed as percentage of total. Dose of butorphanol, 10 mg/kg body weight.

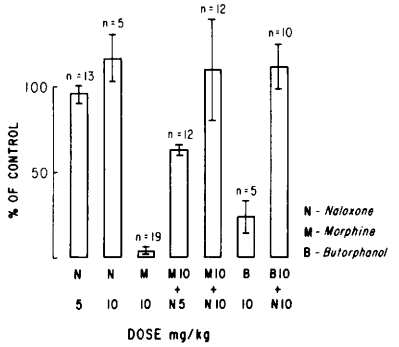

FIG. 4. Inhibitory effects of morphine and butorphanol on initial milk yield and their reversal by naloxone. In groups which received either naloxone or morphine, injection was made immediately before suckling. In groups which received both naloxone and morphine or butorphanol, naloxone was injected 10-min prior to morphine or butorphanol. The capital letters on the abscissa represent drugs and the numbers represent dose of the drug in mg/kg body weight.

cific effect of the narcotic it should be prevented by naloxone. As can be seen in Fig. 4, naloxone in a dose of 5 mg/kg partially prevented the inhibitory effect of morphine and a complete reversal of morphine inhibition was achieved by a dose of 10 mg/kg. Figure 4 illustrates further that the higher dose of naloxone effectively reversed butorphanol induced inhibition also.

Experiments were designed to determine whether the inhibition of milk yield in response to morphine was due to central inhibition of OT release or due to unresponsiveness of the mammary gland to endogenous OT. The response to intravenous injections of OT was tested in anesthetized lactating rats in a set-up similar to that described by Bisset *et al.* (7). Oxytocin was injected before and after subcutaneous administration of morphine at a dose of 10 mg/kg. Figure 5 illustrates the result of one such experiment. Oxytocin was administered in a dose of 0.1 mU, 1 min after and at 5-min intervals up to 75 min following morphine administration. The magnitude of milk ejection responses due to OT were not altered by preinjection of morphine.

Discussion. The results presented in this paper clearly demonstrate that both butorphanol and oxilorphan inhibit initial milk yield in lactating mice. They also show that butorphanol is a stronger inhibitor than oxilorphan.

The finding that babies were able to get a considerable amount of milk following OT injection indicates that a lack of circulating OT was probably responsible for the reduced milk yield in the drug treated animals. Thus the inhibition of milk yield was apparently due to inhibition of OT release into the blood and not due to unresponsiveness of the mammary gland to endogenous OT. Results obtained with lactating rats in which morphine failed to alter oxytocin-induced milk ejection fit nicely with this view.

The pharmacological studies of Pircio and Glyss (5) on oxilorphan and of Pircio et al. (6) on butorphanol show that both of these compounds possess a mixture of agonistic and antagonistic activities. Butorphanol is a stronger analgesic while oxilorphan is a stronger antagonist. Our finding that mor-

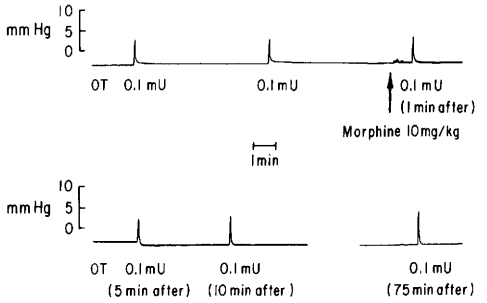

FIG. 5. Effect of subcutaneous injection of morphine, 10 mg/kg, on milk ejection response obtained by 0.1 mU OT injected intravenously. Following morphine injection OT was administered 1 min after and at every 5 min until 75 min.

phine, but not naloxone, was a potent inhibitor of OT release explains why butorphanol was a stronger inhibitor than oxilorphan.

The results presented in this paper, however, do not shed any light on how oxytocin release is inhibited by the agonist drugs. We know from the classical work of Douglas (8) and of Douglas and Poisner (9) with isolated pituitary glands that Ca^{2+} is extremely important for the release of posterior pituitary hormones. Thus a certain amount of Ca^{2+} must be present in order to have successful hormone release. Although morphine has been reported to lower Ca^{2+} in different regions of brain, particularly in the hypothalamus (10), it is unlikely that the local Ca^{2+} levels would ever be low enough to interfere with OT release.

It has been reported by several investigators (11–15) that morphine releases ADH. However, the mechanism of such release is not clear. According to Schmidt and Livingston (16), the antidiuretic action of morphine is at least partly due to its cardiovascular effects. On the other hand, morphine has been reported to release catecholamines from the hypothalamus both in rats (17) and mice (18). Since there are several lines of evidence that catecholamines cause release of ADH (19, 20) it is possible that the release of ADH by morphine is an indirect one via the release of catecholamines.

Cross (21), in his elegant experiments on rabbits, showed how catecholamines can block OT release centrally. Activation of the sympatheticoadrenal system causing release of epinephrine or norepinephrine was sufficient to block OT release. More recently he showed that norepinephrine causes inhibition of firing rates when applied iontophoretically to the cell bodies of the paraventricular nuclei (22). Thus it is possible, in the experiments reported here, that the observed inhibition of OT release by morphine is due to the release of catecholamines.

Whatever may be the exact mechanism of inhibition it is certain that the inhibitory effects of morphine and butorphanol are specific ones since they are completely blocked by preinjection of a proper amount of naloxone. It may be noted that the dose of naloxone, i.e., 10 mg/kg, needed to achieve complete blockade of morphine inhibition is significantly higher than the blocking dose reported by Markowitz *et al.* (23). In their experiment 0.1 mg/kg naloxone could block the analgesic effect of 10 mg/kg morphine. However, it is hard to compare analgesic effects of morphine to its other effects since varying amounts of naloxone are needed to block analgesia when this is assessed by different methods.

This paper presents clear evidence of a new effect of morphine which was hitherto unknown. Inhibition of oxytocin release by morphine raises the questions whether endorphins, which have morphine-like properties, will also inhibit OT release and whether endorphins may participate in the physiological regulation of OT release.

Summary. Two synthetic morphine analogs, butorphanol and oxilorphan have been found to inhibit suckling induced oxytocin (OT) release in lactating mice. Morphine when tested in a similar experimental set-up produced even greater inhibition. Naloxone could effectively block inhibitions induced both by butorphanol and by morphine. When injected alone, naloxone did not produce any inhibition. These results indicate that (a) butorphanol, oxilorphan and morphine inhibit suckling induced OT release in mice, (b) the analgesic property of these compounds is responsible for the inhibition, and (c) inhibition produced by morphine and butorphanol is specific in nature since it could be reversed by naloxone.

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