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A sex difference in the interaction between promethazine and morphine in the mouse

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The effects of promethazine on the antinociceptive and respiratory actions of morphine have been examined in the mouse. Moderate doses of promethazine (5 and 10 mg kg^{-1}) potentiated morphine's action in male mice but inhibited it in female mice. Gonadectomy abolished the interaction between promethazine and morphine in both sexes, although the intensity and duration of morphine's activity was greatly enhanced in these mice. Replacement of oestradiol in ovariectomized mice restored morphine's activity to intact female control values. However, interactions between promethazine and morphine required progesterone, as well as oestradiol, replacement to obtain results approaching those obtained in intact female mice.

Soon after the introduction of chlorpromazine, Laborit, Jaulmes & Benitte (1952) reported that opiate-induced analgesia was potentiated by phenothiazine derivatives. The combination was soon widely used for pre-anaesthetic medication, pain relief in labour (Zardu, 1967) and in the relief of pain in gynaecological tumours (Ullery, 1964).

One of the most frequently used phenothiazines for this purpose is promethazine. Whilst clinical reports frequently re-iterate that promethazine potentiates narcotic analgesic activity (Pena, 1965), several controlled trials of experimental pain in man have suggested that, not only does promethazine reduce the analgesic activity of narcotic analgesics, but also that it is capable of lowering the pain threshold by itself (Moore & Dundee, 1961; Dundee, Nicholl & others, 1965). Indeed Dundee, Love & Moore (1963) found that promethazine was one of the most 'antanalgesic' compounds of the phenothiazines tested. However, they pointed out the importance of both dose and time course of activity in these effects. Some phenothiazines were shown to exhibit both analgesic and antanalgesic properties dependent upon dose and time after administration.

The present study was designed to follow the time course of the effects of promethazine on morphine's antinociceptive activity in the mouse and to determine whether promethazine has similar modifying effects upon the respiratory effects of morphine.

MATERIALS AMD METHODS

Groups of 12 albino mice (Manchester strain), 25–35 g, were used.

Antinociceptive activity was measured using the hot plate (55°) reaction time test as described by Bousfield & Rees (1969). To avoid tissue damage, any mouse not responding within 45 s was removed. Reaction times were measured at 15 min intervals after the intraperitoneal administration of drugs. Experiments were continued until drug-treated mice had reaction times not significantly different from concurrently tested saline-treated controls.

Respiratory frequency was measured by placing the mouse's snout in the barrel of a 5 ml syringe connected to a pressure transducer and pen recorder. Respiratory movements were recorded for at least 10 s. Respiratory frequency was measured before injection of drug and subsequently just before each measurement of hot plate reaction time.

Results are expressed as means ± standard error and significance was calculated using the Students *t*-test. For non-parametric data, the Mann-Whitney 'U' test was used.

Bilateral ovariectomy or orchidectomy was carried out under ether anaesthesia. The mice were allowed to recover for a minimum of three weeks before any further experiments were carried out.

Drugs used were: morphine hydrochloride (20 mg kg^{-1}) (MacFarlane Smith); promethazine hydrochloride (5–20 mg kg^{-1}) (May & Baker); oestradiol monobenzoate (10 μg kg^{-1} daily for three days) (Organon) and progesterone (5–10 mg kg^{-1} daily for three days) (BDH). Progesterone (1 mg ml^{-1}) and oestradiol monobenzoate (2 μg ml^{-1}) were dissolved in arachis oil. All other drugs were dissolved in saline. Doses in the text refer to salts.

RESULTS

Intact male and female mice

A clear sex difference in interactions between promethazine and morphine is shown in Fig. 1. Prome-

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thazine (10 mg kg^{-1}) inhibited both the antinociceptive activity and the respiratory effects of morphine (20 mg kg^{-1}) in females, but enhanced these actions of morphine in males. Morphine alone had a similar intensity and duration of action in both sexes and promethazine alone had no significant effects on reaction time or respiratory frequency when compared with saline controls in both males and females.

A qualitatively similar sex difference was seen in interactions between 5 mg kg^{-1} promethazine and morphine. However 20 mg kg^{-1} promethazine appeared to enhance the actions of morphine in both sexes, but at this dose promethazine caused significant depression of respiratory frequency when administered alone (a fall of 56 ± 15 breaths min^{-1} compared with a rise of 2 ± 11 breaths min^{-1} for saline-treated female mice) and mice given both 20 mg kg^{-1} promethazine and morphine exhibited gross ataxia and sat on the hot plate for more than 45 s.

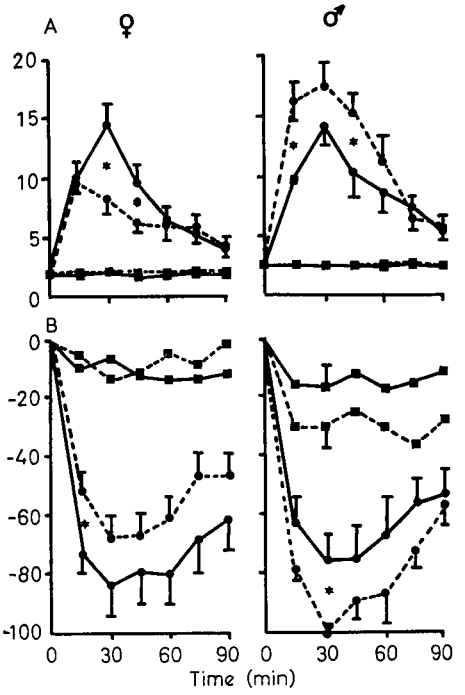

FIG. 1. The effects of promethazine, 10 mg kg^{-1} (■ - - ■) on the actions of morphine, 20 mg kg^{-1} (●—●) in male and female mice. Morphine and promethazine in combination (●- -●). A. Antinociceptive activity measured as hot plate reaction times. B. Effects on respiratory frequency measured as change (breaths min^{-1}) from pre-injection control values. Results are means ± standard errors of 12 experiments. Standard errors have been omitted from most of the saline (■—■) and promethazine alone values for clarity. * Significant difference between morphine and morphine promethazine-treated mice ($P < 0.05$). Ordinates, A—reaction time(s). B—change in respiratory frequency.

Gonadectomized mice

In both ovariectomized and orchidectomized mice, promethazine (10 mg kg^{-1}) had no significant effects upon the actions of morphine. 30 min after injection, orchidectomized mice given morphine showed a fall in respiratory frequency of 75 ± 9 breaths min^{-1}, whilst similar mice given morphine and promethazine (10 mg kg^{-1}) showed a fall of 71 ± 10 breaths min^{-1}. This is the time after injection when the maximum enhancement of morphine's action was seen in intact male mice.

However the actions of morphine alone were increased both in intensity and duration in gonadectomized mice. This is shown in Fig. 2 for intact males and orchidectomized mice.

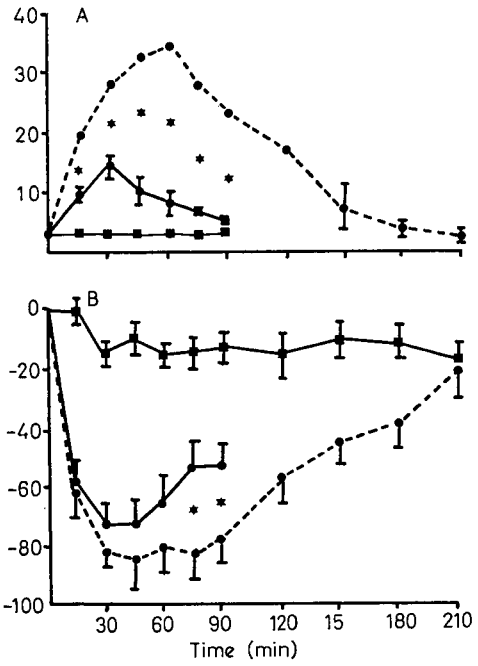

FIG. 2. The effects of orchidectomy (● - - ●) on the response of mice to morphine 20 mg kg^{-1}. A. Antinociceptive activity. B. Effects on respiratory frequency. Results are means ± standard errors of 12 experiments. Means without standard errors include mice which had reaction times above the arbitrary cut off time of 45 s. * Significant difference between the actions of morphine in orchidectomized mice and in intact males ($P < 0.05$ or < 0.01). ■—■—saline treated orchidectomized mice. ●—●—intact mice. Ordinates, A—reaction time(s). B—change in respiratory frequency.

Similarly Fig. 3 shows that morphine's activity is enhanced in ovariectomized mice. Fig. 3 also shows the effects of oestradiol pretreatment on the activity of morphine in ovariectomized mice. The duration and intensity of morphine's actions in these mice reverted to a pattern almost identical to that obtained in intact female mice.

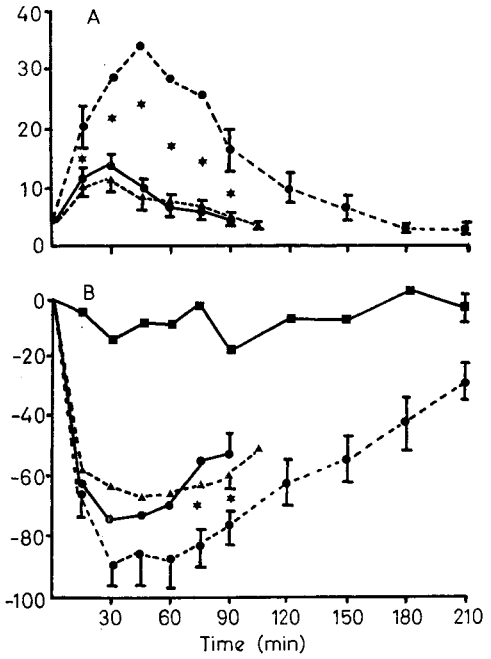

FIG. 3. The effects of ovariectomy (●– –●) and oestradiol pretreatment 10 μg kg^{-1} × 3 days of ovariectomized mice (▲- -▲) on the response of the mouse to morphine 20 mg kg^{-1}. A. Antinociceptive activity. B. Effects on respiratory frequency. Results are means ± standard error of 12 experiments. Values without standard errors in A include mice with reaction times above 45 s. Some standard errors have been omitted from B for clarity. * Significant difference between the effects of morphine in ovariectomized mice and intact females ($P < 0.05$ or < 0.01). ■—■—ovariectomized mice treated with saline. ●—●—Intact mice. Ordinates, A—reaction time(s). B—change in respiratory frequency.

The dose regimen used for the oestradiol pretreatment increased the uterine weights of the ovariectomized mice to values midway between the values for mice in oestrus and dioestrus (Table 1).

Pretreatment of ovariectomized mice with oestradiol, however, did not revert the promethazine morphine interaction to a pattern similar to intact females (cf. Figs 1 and 4). As in intact males, promethazine enhanced the actions of morphine in overiectomized mice treated with oestradiol

A pattern of interaction between promethazine and morphine closer to that in intact females, was obtained by pretreating ovariectomized mice with both oestradiol and progesterone. Fig. 4 also shows the results obtained in ovariectomized mice pretreated with 10 μg kg^{-1} oestradiol and 10 mg kg^{-1} progesterone. The addition of progesterone, however, reduced the antinociceptive activity of mor-

Table 1. *The effects of ovariectomy and oestradiol treatment on uterus weights in mice.*

Mice	No.	Uterus weight mg ± s.e.
Intact		
mixed cycle	22	234 ± 35
oestrus*	6	266 ± 19
dioestrus*	6	93 ± 20
Ovariectomized	24	28 ± 4
+ oestradiol 10 μg kg^{-1} × 3 days	48	135 ± 8

* Cycle stage determined by cerivcal smear.

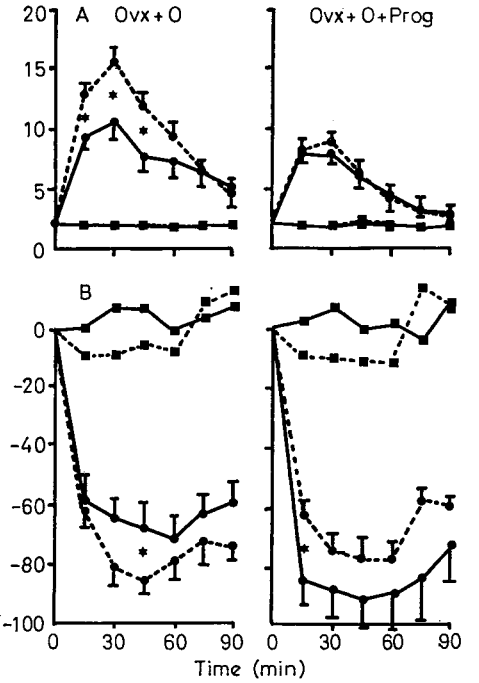

FIG. 4. The interaction between promethazine, 10 mg kg^{-1} (■- -■) and morphine 20 mg kg^{-1} (●—●) in ovariectomized mice pretreated with either oestradiol, 10 μg kg^{-1} × 3 days (Ovx + O) or both oestradiol 10 μg kg^{-1} × 3 days and progesterone 5 mg kg^{-1} × 3 days (Ovx + O + Prog.) Morphine and promethazine in combination (●- -●). A. Antinociceptive activity. B. Effects on respiratory frequency. Results are means ± standard error of 6 or 12 experiments. Standard errors have been omitted from the values for saline (■—■) or promethazine treated mice for clarity. * Significant difference between mice given morphine alone and mice given morphine and promethazine ($P < 0.05$). Ordinates, A—reaction time(s). B—change in respiratory frequency.

phine. The duration of morphine's antinociceptive activity, measured as the time that each individual mouse had a reaction time of 5 s or above, was significantly less ($P < 0.05$) in ovariectomized mice treated with oestradiol and 10 mg kg^{-1} progesterone, than it was in ovariectomized mice treated with oestradiol alone or in intact female mice. 5 mg kg^{-1} progesterone had less inhibitory effects on morphine's antinociceptive activity, but it was also less effective in reverting the promethazine morphine interaction to the values obtained for intact females.

DISCUSSION

The present study has shown that moderate doses of promethazine antagonize the antinociceptive and respiratory depressant activity of morphine in female mice and potentiates morphine's actions in male mice. Using only female mice, Leslie & Nunn (1968) also demonstrated antagonism of morphine's antinociceptive activity by 10 mg kg^{-1} promethazine and it is interesting that most of the clinical reports of antagonism by promethazine of narcotic analgesic activity involved female patients (Dundee & others, 1963, 1965).

The high dose of promethazine (20 mg kg^{-1}) used increased morphine's activity in both sexes. However promethazine at this dose level significantly depressed respiratory frequency alone and thus the effects of the combination of promethazine and morphine may have been a summation of the separate depressant effects of the two drugs. Furthermore the gross ataxia exhibited by mice given both morphine 20 mg kg^{-1} and promethazine 20 mg kg^{-1} may have prevented the mice from exhibiting the required end point response for the hot plate reaction time test.

In gonadectomized mice promethazine neither potentiated nor antagonized the actions of morphine in the dose used. This suggests that both the potentiation of morphine by promethazine and the antagonism depend on the presence of sex hormones. The experiments reported suggest that progesterone is important in the production of morphine antagonism by promethazine. Oestradiol replacement alone in ovariectomized mice produced a pattern of promethazine morphine interaction in these mice similar to that obtained in male mice, i.e. potentiation. Progesterone was required to obtain a promethazine morphine interaction similar to that obtained in intact female mice.

However, interpretation of the effects of gonadectomy, oestradiol and progesterone on the promethazine-morphine interaction are complicated by the effects of these procedures on the actions of morphine alone. Both the antinociceptive and respiratory activity of morphine was enhanced in ovariectomized and orchidectomized mice.

The oestradiol pretreatment used in this study restored the uterus weight in ovariectomized mice to values comparable to intact female mice. Oestradiol pretreatment also restored the activity of morphine in ovariectomized mice to that obtained in intact female mice. Thus it appears that, in the mouse, circulating sex hormones must play an important role in the determination of the duration and intensity of morphine's action. Furthermore progesterone reduced the antinociceptive activity of morphine still further in oestradiol-replaced, ovariectomized mice.

Oestrogens, androgens and progestogens are all capable of stimulating drug metabolism (Mannering, 1968). Thus the rate of morphine's metabolism in the 'normal' adult mouse may be enhanced by circulating sex hormones. Removal of these hormones might then revert the rate of morphine's metabolism to a slower 'basal' rate. Such a mechanism could explain some of the findings in this study.

If circulating sex hormone concentrations were equally important in determining the duration and intensity of narcotic analgesic activity in man, this might explain, in part, the enhanced susceptibility of the very young and aged to narcotic analgesics and other drugs.

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