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Modification of morphine analgesia by drugs affecting adrenergic and tryptaminergic mechanisms

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The effects of drugs that modify adrenergic or tryptaminergic mechanisms were tested on the analgesic action of morphine in mice. Analgesia was assessed by the hot plate method and phenylquinone-writhing method. Reserpine antagonized the analgesic action of morphine in both tests, the maximal effects occurring 6–8 h after the administration of reserpine. *p*-Chlorophenylalanine antagonized the analgesic action of morphine as assessed by the writhing method but not by the hot plate method. The analgesic action of morphine was not modified in either test by pretreatment with α-methyl-*p*-tyrosine, propranolol, phentolamine or methysergide. These results suggest that the analgesic action of morphine, as measured in the writhing test, may be mediated by 5-hydroxytryptamine but that other mechanisms may be involved in the hot plate test.

Conflicting reports have appeared concerning the effect of reserpine on morphine analgesia. Most authors have reported antagonism (Schneider, 1954; Takagi, Tashima & Kimura, 1964; Verri, Graeff & Corrado, 1968), whereas potentiation of the analgesic action of morphine by reserpine was observed when heat was employed as the nociceptive stimulus (Tripod & Gross, 1957; Garcia Lema & Rocha e Silva, 1961). This effect of reserpine on morphine analgesia, be it antagonism or potentiation, has led most workers to conclude that the analgesic action of morphine may be mediated by the release of catecholamines within the central nervous system. This view has been reinforced by a number of other findings: (a) morphine releases noradrenaline in the central nervous system (Vogt, 1954; Maynert & Klingman, 1962); (b) morphine analgesia is antagonized by pretreatment with α-methyl-*p*-tyrosine, a specific depletor of catecholamines (Verri & others, 1968); (c) intracerebral injection of catecholamines produces analgesia in mice (Handley & Spencer, 1969); (d) morphine analgesia is antagonized by phenoxybenzamine (Heller, Saavedra & Fischer, 1968).

On the other hand, some authors have suggested that the antagonism of morphine analgesia by reserpine may involve changes in tryptaminergic mechanisms. There is evidence in support of this contention: (a) morphine reduces the levels of 5-hydroxytryptamine (5-HT) in the central nervous system (Türker & Akçasu, 1962); (b) *p*-chlorophenylalanine, a specific depletor of 5-HT, antagonizes morphine analgesia (Tennen, 1968); (c) intraventricular administration of 5-HT not only prolongs morphine analgesia but abolishes the effect of reserpine in antagonizing morphine analgesia (Sparkes & Spencer, 1969).

The levels of 5-HT in mouse brain were depressed by morphine in a dose of 0·85 mg/kg, that corresponded to the ED50 for analgesia in the phenylquinone-writhing test, whereas a higher dose (8·5 mg/kg), which was the ED50 for analgesia in the hot plate test, depressed levels of both 5-HT and noradrenaline (Lee & Fennessy, 1970).

It was suggested that a tryptaminergic mechanism may be involved in the analgesic action of morphine when phenylquinone is the stimulus, whereas a combination of adrenergic and tryptaminergic mechanisms may be involved when heat is the stimulus. In view of these findings we decided to study the effect of reserpine on morphine analgesia in both the hot plate and phenylquinone-writhing tests. In addition we have investigated the effects of a number of drugs that interfere with adrenergic and tryptaminergic functions on the analgesic activity of morphine.

EXPERIMENTAL

Swiss albino mice (Commonwealth Serum Laboratories strain) of either sex and weighing 18–25 g were randomly assigned to groups of 10. Two analgesic tests were used: the hot plate method (Eddy & Leimbach, 1953) and the phenylquinone-writhing method (Hendershot & Forsaith, 1959). The criteria used for assessing analgesia in these tests were as described by Lee & Fennessy (1970). The ED50 values for morphine analgesia in all tests were determined 30 min after subcutaneous administration of morphine and were calculated by the method of Litchfield & Wilcoxon (1949).

p-Chlorophenylalanine (BDH) was dissolved in half the required volume of 0·9% NaCl solution adjusted to pH 10 with 5M NaOH titrated to pH 4·5 with 5M HCl and two drops of polysorbate 80 were added; α-methyl-*p*-tyrosine (Aldrich) was suspended in 0·5M phosphate buffer at pH 7·4 in a concentration of 50 mg/ml and was dissolved by the addition of 3M NaOH, then the pH was adjusted to 7·4 with M HCl; phenylquinone (phenyl-*p*-benzoquinone, Sigma) was dissolved in 5% ethanol in water; these solutions were injected intraperitoneally. All other drugs were dissolved in 0·9% NaCl solution and were injected subcutaneously in a volume of 0·1 ml/10 g of mouse. Doses of morphine are expressed in terms of morphine sulphate (DHA); doses of propranolol hydrochloride (ICI), phentolamine mesylate (Ciba), reserpine (Ciba), 5-hydroxytryptamine creatine phosphate complex (Sigma), noradrenaline bitartrate monohydrate (Winthrop) and methysergide maleate (Sandoz) are expressed in terms of the base.

RESULTS

Phenylquinone-writhing test

The time course of the antagonistic effect of reserpine (1 mg/kg) on morphine analgesia is shown in Fig. 1. The increase in the analgesic ED50 of morphine was significantly ($P \leqslant 0{\cdot}05$) above the control ED50 of morphine between 2 and 48 h after reserpine administration. Maximal antagonism of morphine analgesia was observed about 8 h after reserpine when the analgesic ED50 value for morphine was 2·55 mg/kg, compared with the control value of 0·85 mg/kg. Reserpine produced marked sedation but did not affect the writhing response to phenylquinone.

Pretreatment of mice with three doses each of 316 mg/kg of *p*-chlorophenylalanine, injected 72, 48 and 24 h before morphine, produced a significant ($P < 0{\cdot}05$) increase in the analgesic ED50 of morphine (Fig. 2). This dose regime of *p*-chlorophenylalanine has been shown by Koe & Weissman (1966) to inhibit tryptophane hydroxylase selectively. These mice did not differ noticeably in behaviour from control animals, nor was there any effect on the writhing response to phenylquinone.

Treatment with α-methyl-*p*-tyrosine (100 mg/kg) has been reported to inhibit noradrenaline synthesis specifically by inhibition of dopa decarboxylase (Spector, Sjoerdsma & Udenfriend, 1965). This dose, given 2 h before morphine did not

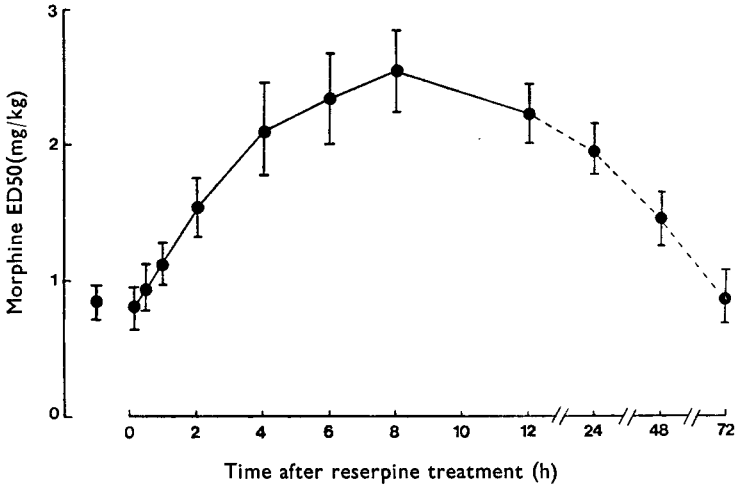

FIG. 1. The analgesic ED50 of morphine in the phenylquinone-writhing test at various times after subcutaneous injections of reserpine (1 mg/kg). Vertical lines are the 95% confidence limits.

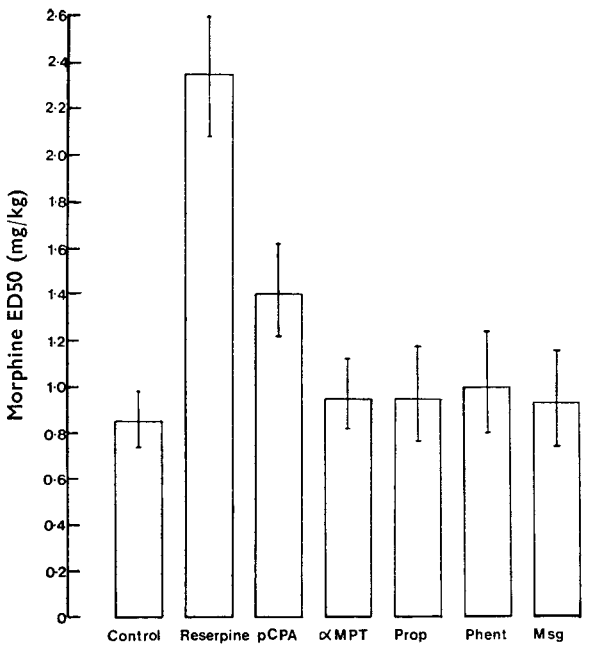

FIG. 2. The effects of pretreatment with various drugs on the ED50 value of morphine in the phenylquinone-writhing test. The doses of drugs and the intervals between pretreating for morphine and analgesia are as follows: reserpine, 1 mg/kg, 6 h pretreatment; '*p*-chlorophenylalanine (pCPA), 316 mg/kg, 72, 48 and 24 h pretreatment; α-methyl-*p*-tyrosine (αMPT), 400 mg/kg, 2 h pretreatment; propranolol (Prop), 5 mg/kg, 30 min pretreatment; phentolamine (Phent), 10 mg/kg, 30 min pretreatment; methysergide (Msg), 5 mg/kg, 30 min pretreatment. Vertical lines are the 95% confidence limits.

significantly affect the analgesic action of morphine (Fig. 2). α-Methyl-*p*-tyrosine did not possess any analgesic activity by itself; however, the mice were sedated and they had an increased frequency of defaecation.

The blocking drugs, phentolamine, propranolol and methysergide, when used in doses reported to produce receptor blockade, did not significantly ($P \geqslant 0{\cdot}7$) affect morphine analgesia (Fig. 2).

Hot plate test

Pretreatment with reserpine (1 mg/kg) caused a slight and statistically insignificant enhancement of the analgesic action of morphine after 0·5 and 1 h and then antagonized significantly ($P < 0{\cdot}05$) the analgesic action of morphine from 4 to 18 h after administration of reserpine (Fig. 3). Maximal antagonism was observed between 6 to 8 h after reserpine. The analgesic ED50 for morphine 6 h after reserpine pretreatment was 19·3 mg/kg compared to the control ED50 of 8·5 mg/kg.

Neither *p*-chlorophenylalanine, α-methyl-*p*-tyrosine, propranolol, phentolamine nor methysergide affected morphine analgesia (Fig. 4).

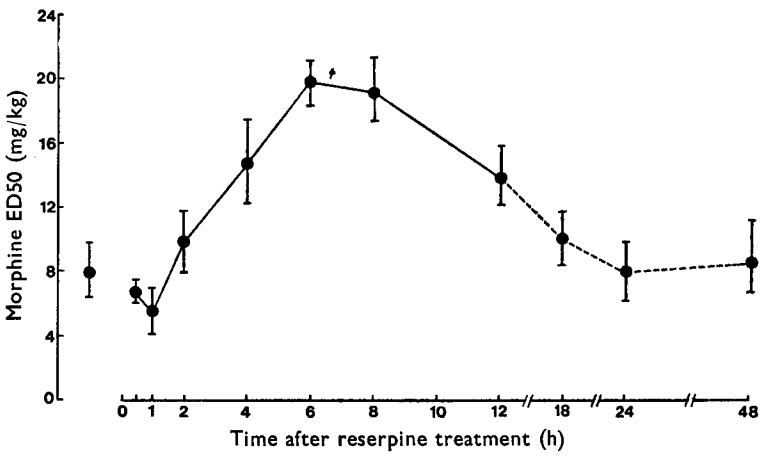

FIG. 3. The analgesic ED50 of morphine in the hot-plate test at various times after reserpine (1 mg/kg), subcutaneously. Vertical lines are the 95% confidence limits.

DISCUSSION

Reserpine antagonized the analgesic action of morphine in mice when either a heat method or a writhing method was used in the assessment of analgesia. The antagonism of morphine analgesia in the hot plate test with mice after pretreatment with reserpine confirms the results of Verri & others (1968) and Medaković & Banic (1964) but disagrees with the reports of Garcia Lema & Rocha e Silva (1961), Tardos & Jobbágyi (1958) and Ross & Ashford (1967) who demonstrated potentiation of the analgesic action. The conflicting reports of the effect of reserpine on morphine analgesia using the hot plate method do not appear to depend on the laboratory of origin since Verri & others (1968) obtained opposite results to Garcia Lema & Rocha e Silva (1961) even though both groups worked in the same laboratory and used the same strain of mice. Antagonism of morphine analgesia after reserpinization of mice using the writhing method has previously been reported by Rudzik & Mennear (1965).

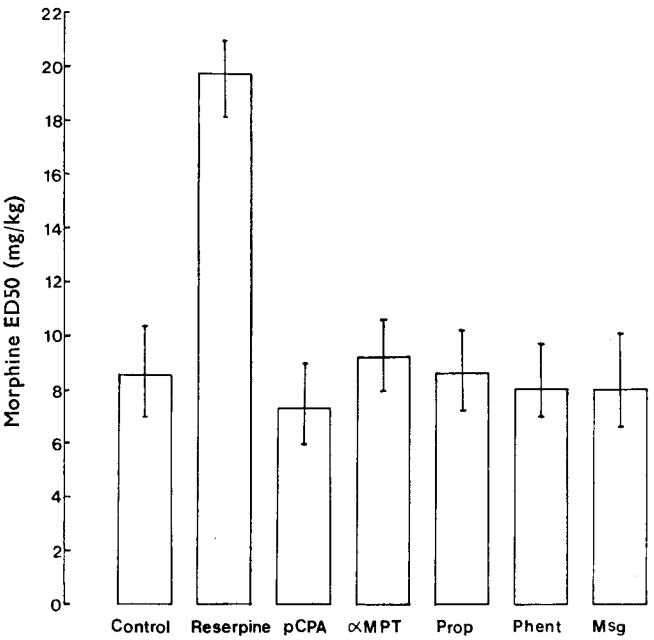

FIG. 4. The effects of pretreatment with various drugs on the ED50 value of morphine in the hot plate test. The doses of drugs and intervals between pretreatment and testing of morphine analgesia are as follows: reserpine, 1 mg/kg, 6 h pretreatment; *p*-chlorophenylalanine (pCPA), 316 mg/kg, 72, 48 and 24 h pretreatment; α-methyl-*p*-tyrosine (αMPT), 400 mg/kg, 2 h pretreatment; propranolol (Prop), 5 mg/kg, 30 min pretreatment; phentolamine (Phent), 10 mg/kg, 30 min pretreatment; methysergide, (Msg) 5 mg/kg, 30 min pretreatment. Vertical lines are the 95% confidence limits.

It is possible that the antagonism of morphine analgesia produced by reserpine in the writhing test may be due to decreased levels of 5-HT in the central nervous system since *p*-chlorophenylalanine, but not α-methyl-*p*-tyrosine, antagonized morphine analgesia. *p*-Chlorophenylalanine has been shown to inhibit tryptophane decarboxylase specifically, resulting in depletion of 5-HT (Koe & Weissman, 1966), whereas α-methyl-*p*-tyrosine specifically inhibits dopa decarboxylase and leads to a decrease in levels of noradrenaline (Spector & others, 1965). We have suggested previously (Lee & Fennessy, 1970) that morphine analgesia as determined by the writhing method may involve 5-HT since the ED50 of morphine caused a reduction in brain levels of this amine but not of noradrenaline. The present findings support this suggestion since the analgesic action of morphine was depressed by drugs which depleted the brain levels of 5-HT.

The observation that reserpine, but not *p*-chlorophenylalanine or α-methyl-*p*-tyrosine, reduced the analgesic action of morphine when the hot plate method was used is more difficult to explain. We were unable to confirm the findings of Medaković & Banic (1964) and Verri & others (1968) who reported an antagonism to morphine analgesia after pretreatment of mice with α-methyl-*p*-tyrosine using the hot plate method. Doses of morphine required to produce analgesia by this method significantly reduced brain levels of both 5-HT and noradrenaline (Lee & Fennessy, 1970). If both of these amines were concerned conjointly in morphine analgesia as determined in the hot plate method, then neither *p*-chlorophenylalanine nor α-methyl-*p*-tyrosine would be expected to cause antagonism. There is other evidence for difference

between the two tests. The ED50 of morphine was ten times greater and the persistance of effect for the reserpine-induced antagonism was shorter when the nociceptive stimulus was thermal than when it was chemical. In the hot plate test, there was slight early enhancement of analgesia that was not observed in the writhing test. The time course of antagonism of analgesia in the hot plate test by reserpine was closely parallel to the effect of reserpine in depleting catecholamines (Iversen, Glowinski & Axelrod, 1965).

Phentolamine, an α-adrenoreceptor antagonist, propranolol, a β-adrenoreceptor antagonist, and methysergide, a 5-HT antagonist, did not affect the analgesic action of morphine in either of the two tests. A possible explanation for the lack of effect is that the adrenergic and tryptaminergic receptors in the central nervous system, if they are involved in the production of analgesia, may be different to those in peripheral systems. However, other workers have observed effects of blocking drugs on morphine analgesia, although the reports are conflicting. Tolazoline was reported to antagonize morphine analgesia whereas phenoxybenzamine was without effect (Contreras & Tamayo, 1966). On the other hand, Gupta & Deshpande (1965) reported potentiation of morphine analgesia by phenoxybenzamine and tolazoline and antagonism by pronethalol.

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