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Comparison of the dose-response effects of morphine on brain amines, analgesia and activity in mice

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Summary

1. Noradrenaline, dopamine, 5-hydroxytryptamine and 5-hydroxyindoleacetic acid concentrations in the mouse brain were measured 30 min after subcutaneous injection of doses of morphine ranging from 0·1 to 100 mg/kg: motor activiy was also measured.

2. The noradrenaline concentration in the mouse brain was reduced by moderate (2 to 20 mg/kg) but not by high (above 20 mg/kg) and low (below 2 mg/kg) doses of morphine.

3. The dopamine concentration in the mouse brain was reduced by moderate (1 to 20 mg/kg) doses but was raised by high doses (above 20 mg/kg) of morphine.

4. The 5-hydroxytryptamine concentration in the mouse brain was reduced by moderate (1 to 20 mg/kg) doses of morphine but not by high (above 20 mg/kg) and low (below 1 mg/kg) doses of morphine.

5. The 5-hydroxyindoleacetic acid concentration was not affected by low doses (0·1 to 2 mg/kg), raised by a dose of 5 mg/kg, lowered by doses of 10–50 mg/kg and not affected by 100 mg/kg of morphine.

6. These results are discussed with reference to the possible implication of changes in monoamines for the analgesic and behavioural effects of morphine.

Introduction

After many years of investigation the mechanisms of action of morphine are still the subject of numerous studies and lively discussion. Many investigations have been concerned with the possibility that a noradrenergic or a tryptaminergic mechanism is involved in the central actions of morphine. Most of this work has been prompted by the observations that reserpine alters the antinoceptive action of morphine (Schneider, 1954; Takagi, Tashima & Kimura, 1964; Tripod & Gross, 1957; Garcia Lema & Rocha e Silva, 1961; Verri, Graeff & Corrado, 1967, 1968; Fennessy & Lee, 1970).

The observations that reserpine lowers the brain concentration of noradrenaline and 5-hydroxytryptamine also suggest that the analgesic action of morphine may be related to its effects on these amines. Morphine has been shown to affect the brain concentration of noradrenaline (Holzbauer & Vogt, 1956; Chodera, Godlewski & Szczawinski, 1968; Gunne, 1959; Lee & Fennessy, 1970; Maynert & Klingman, 1962; Rethy, Smith & Villarreal, 1971; Vogt, 1954) and 5-hydroxy-

tryptamine (Brodie, Olin, Kuntzman & Shore, 1957; Lee & Fennessy, 1970; Türker & Akçasu, 1962). However, these investigations have used a variety of species of animals and have been confined to studies with only limited dose ranges of morphine. Thus it appeared desirable to determine the effects of a wide range of doses of morphine on brain amines: in addition we have studied the dose-response relations for the effects of morphine on motor activity.

Methods

Mice (Commonwealth Serum Laboratories strain) of either sex weighing between 18 and 25 g were randomly assigned to groups of 5 for the amine assays. For activity studies, groups of 12 mice were used. All injections were given subcutaneously in a volume of 0·1 ml/10 g of mouse. A constant pretreatment time of 30 min was chosen as this is the time of the maximal analgesic action of morphine (Lee & Fennessy, 1970).

The morphine was dissolved in 0·9% w/v NaCl solution (saline) and control mice received saline. Doses of morphine from 0·1 to 100 mg/kg were used. This dose range covers the reported analgesic activities of morphine in the commonly used analgesic tests (Table 1).

Amine assays

The mice were killed in groups of five. The brains were rapidly removed, and excess blood was blotted off. They were then weighed, immersed in 15 ml of ice cold 0·1 M HCl and homogenized. After centrifugation, a 10 ml portion of the supernatant was taken for assay by the method of Welch & Welch (1969) for noradrenaline, 5-hydroxytryptamine, dopamine and 5-hydroxyindoleacetic acid. The readings were made on a Hitachi–Perkin–Elmer spectrophotofluorimeter (model 203).

Activity determinations

Mice were placed in darkened individual cages (30 cm × 30 cm) with nine pressure-sensitive areas in the floor. Intrusions by the mouse on to the pressure-sensitive areas were electronically counted for a period of 30 min after injection. Activity was measured twice: first after saline injection; then, 24 h later, after either morphine or saline. The mean activity was determined from 12 mice with each dose of morphine and was expressed as a percentage of the mean activity of the group injected with saline on the second occasion.

TABLE 1. *Reported analgesic ED50 values of morphine given by subcutaneous injection*

Method	Animal	ED50	95% Confidence limits	Reference
Arachidonic writhing	Mice	0·4	0·3–0·7	Helfer & Jaques (1968)
Benzoquinone writhing	Mice	0·72	0·65–0·79	Takemori, Kapferberg & Miller (1969)
Phenylquinone writhing	Mice	0·85	0·74–0·95	Lee & Fennessy (1970)
Heat on tail	Rats	1·3	0·9–1·9	Miller, George, Elliot, Sang & Way (1955)
Electrical stimulation of tail	Mice	2·1	1·8–1·6	Paalzow (1969)
Hot Plate	Mice	2·1	2·0–2·2	Eddy & Leimbacch (1953)
Radiant Heat on Tail	Mice	2·3	2·0–2·5	Kraushaar (1953)
Radiant Heat on Tail	Rats	4·7	4·2–5·2	Tye & Christensen (1952)
Tail Clip	Mice	5·3	3·7–7·7	Milošević (1955)
Tail Clip	Mice	5·7	4·9–6·6	Bianchi & Franceschini (1954)
Hot Plate	Mice	8·5	6·7–10·8	Lee & Fennessy (1970)

Drugs

Morphine sulphate (Drug Houses of Australia), (−)-noradrenaline bitartrate (Levophed, Winthrop Laboratories), 5-hydroxytryptamine creatine sulphate complex (Sigma), dopamine hydrochloride (3-hydroxytyramine, Sigma) and 5-hydroxyindoleacetic acid cyclohexylammonium salt (5-hydroxyindol-3-acetic acid, Sigma) were used. Doses of morphine are expressed as the salt. Concentrations of the other substances in brain are expressed in terms of the base or the acid. Student's *t* test was used for the statistical analysis of results.

Results

The control values of noradrenaline, dopamine, 5-hydroxytryptamine and 5-hydroxyindoleacetic acid concentrations in the mouse brain 30 min after subcutaneous injection of saline are shown in Table 2.

TABLE 2. *Concentrations of amines in the brains of control mice. The values are expressed as μg/g of wet brain tissue* ±S.E.M. n *refers to the number of assays. Five mice were used in each assay*

Amine	*n*	amount of amine present
Noradrenaline	50	0·47±0·01
Dopamine	50	1·21±0·04
5-Hydroxytryptamine	50	0·77±0·02
5-Hydroxyindoleacetic acid	48	0·40±0·02

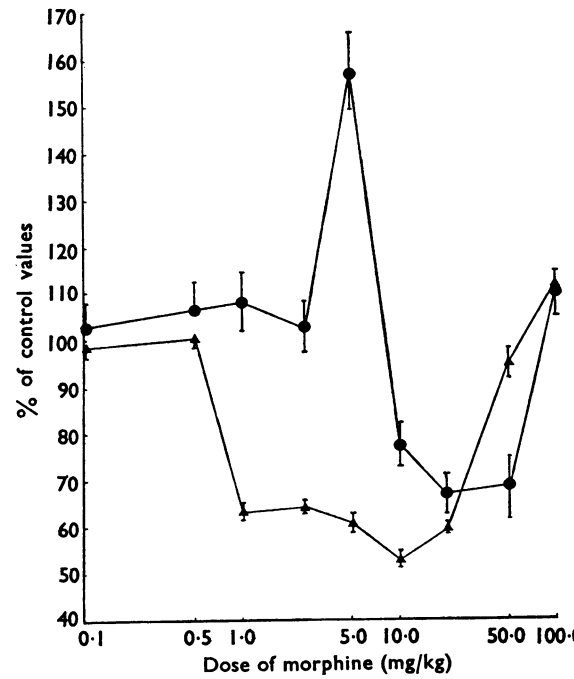

FIG. 1. Dose-response effects of morphine sulphate on the concentrations of 5-hydroxytryptamine (▲) and 5-hydroxyindoleacetic acid (●) in the mouse brain. The concentrations of these substances are expressed as a percentage of that present in the brains of control mice (Table 2). Brains were taken for assay 30 min after subcutaneous injection of morphine sulphate. The vertical bars represent standard errors of the means. The brains of 60 mice were used for each determination.

Effect of morphine on 5-hydroxytryptamine and 5-hydroxyindoleacetic acid concentrations in mouse brain

Figure 1 shows the effects of nine doses of morphine ranging from 0·1 to 100 mg/kg on the concentrations of 5-hydroxytryptamine and 5-hydroxyindoleacetic acid in the mouse brain. The 5-hydroxytryptamine concentration was greatly reduced after a dose of 1·0 mg/kg, and this effect was not substantially altered by increasing the dose to 20 mg/kg. However, after a dose of 50 mg/kg there was no significant change in the concentration of 5-hydroxytryptamine, and after a dose of 100 mg/kg the 5-hydroxytryptamine concentration was slightly greater than the control value.

There was no significant effect of morphine on the concentration of 5-hydroxyindoleacetic acid in the mouse brain until the dose was raised to 5 mg/kg, with which a significant increase was seen. However, a dose of 10 mg/kg of morphine produced a significant decrease in the concentration of 5-hydroxyindoleacetic acid. Further increases in the dose of morphine (20 and 50 mg/kg) did not greatly alter these reduced values. A dose of 100 mg/kg of morphine did not significantly affect the 5-hydroxyindoleacetic acid concentration in the mouse brain.

Effect of morphine on the concentration of dopamine in mouse brain

The smallest dose of morphine used (0·1 mg/kg) caused a significant increase in the concentration of brain dopamine (Fig. 2), whereas doses of 0·5 to 5·0 mg/kg of morphine produced a dose-dependent fall in dopamine concentration to approximately 80% of the control value. Increasing the dose of morphine to 10 mg/kg

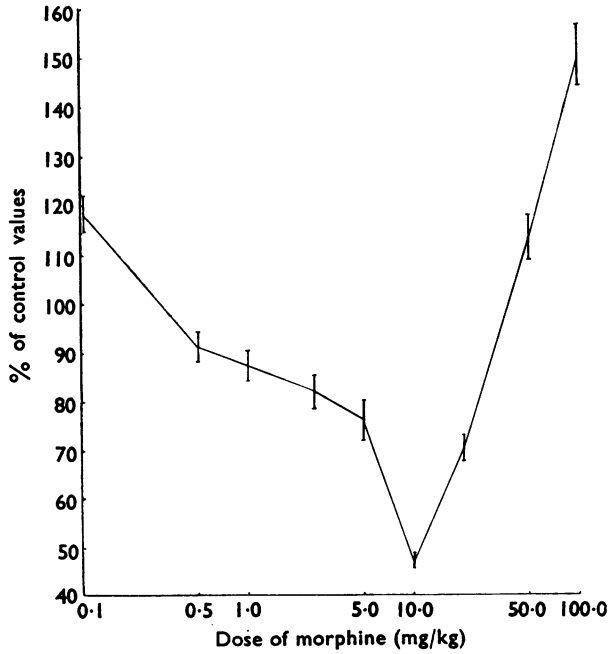

FIG. 2. Dose-response effects of morphine on dopamine concentrations in the mouse brain. The concentration of dopamine is expressed as a percentage of that present in the brains of control mice. Brains were removed for assay 30 min after subcutaneous injection of morphine sulphate. The vertical bars represent standard errors of the means. The brains of 60 mice were used for each determination.

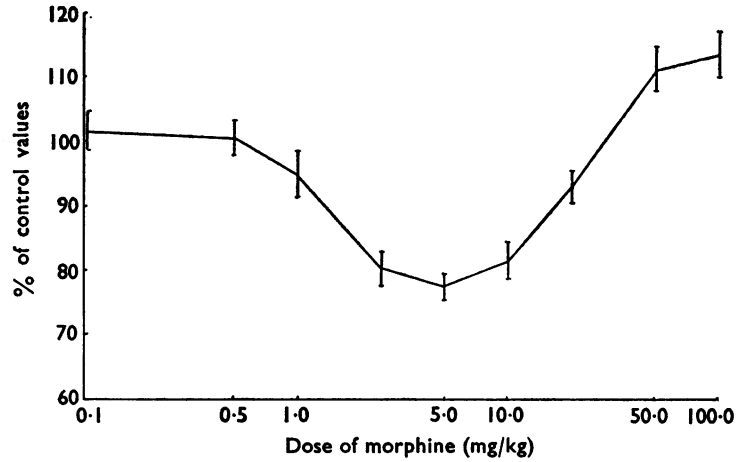

FIG. 3. Dose-response effects of morphine on noradrenaline concentrations in the mouse brain. The concentration of noradrenaline is expressed as a percentage of that present in brains of control mice. Brains were removed 30 min after a subcutaneous injection of morphine sulphate. The vertical bars represent standard errors of the means. The brains of 60 mice were used for each determination.

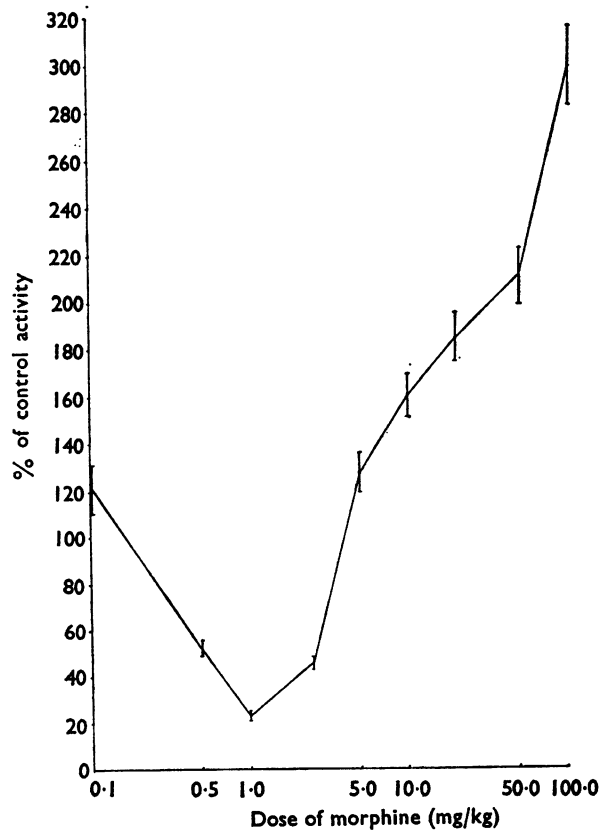

FIG. 4. Dose-response effects of morphine on the activity of mice. The activity is expressed as a percentage of that in control mice. Each point represents the mean activity of 12 mice which had been injected with morphine 30 min previously. The vertical bars represent standard errors of the means.

resulted in a sharper fall in the brain concentration of dopamine. With a further increase in dose to 20 mg/kg there was a smaller reduction in the concentration of dopamine. After 50 and 100 mg/kg there were significant increases in the cerebral concentration of dopamine.

Effect of morphine on the concentration of noradrenaline in the mouse brain

Morphine had no significant effect on the concentration of noradrenaline in the mouse brain in doses up to 1·0 mg/kg (Fig. 3). Doses of 2, 5 and 10 mg/kg produced falls to about 80% of control values. After 20 mg/kg, the noradrenaline concentration did not differ significantly from the control value, but after 50 and 100 mg/kg of morphine there were significant increases ($P<0·05$).

Effect of morphine on mouse activity

The lowest dose of morphine used (0·1 mg/kg) caused a slight increase in activity, but it was not statistically significant. Doses of 0·5, 1·0 and 2·5 mg/kg caused decreases in activity. There was no significant effect on activity with 5 mg/kg of morphine. Doses of morphine above 5 mg/kg caused increased activity (Fig. 4).

Discussion

It was suggested by Tenen (1968) and Fennessy & Lee (1970) that changes in brain 5-hydroxytryptamine are related to the analgesic effects of morphine. The deduction was based on the findings that the analgesic action of morphine was altered by drugs which affected 5-hydroxytryptamine concentrations in the brain. For example after *p*-chlorophenylalanine, a specific depletor of 5-hydroxytryptamine, the analgesic activity of morphine was reduced. Furthermore, Samanin, Gumulka & Valzelli (1970) reported a decreased analgesic activity of morphine in rats after the destruction of 5-hydroxytryptamine-containing neurones in the forebrain.

The effects of morphine (0·85 and 8·5 mg/kg) on the 5-hydroxytryptamine concentrations in the mouse brain are short lasting: the maximal decrease occurs 30 min after the subcutaneous administration of morphine but the effect is over within 60 min (Lee & Fennessy, 1970). However, Türker & Akçasu (1962), who gave large doses of morphine to cats, observed a decrease in the concentration of 5-hydroxytryptamine for up to 34 hours. Maynert, Klingman & Kaji (1962), on the other hand, failed to find any effect of morphine on the 5-hydroxytryptamine concentrations in the brains of rats, dogs and rabbits, but their assays were performed 5 hours after the administration of morphine.

The present study showed that morphine produced a reduction in the 5-hydroxytryptamine concentration in the mouse brain in doses of 1·0 to 20 mg/kg. Lower (0·1 and 0·5 mg/kg) and higher (50 and 100 mg/kg) doses of morphine had no significant effects. The analgesic ED50 values of morphine in the writhing tests (Table 1) are approximately at the threshold dose for reduction of brain 5-hydroxytryptamine concentration; however, it is unlikely that the two effects are related since considerably higher doses of morphine (50 and 100 mg/kg) do not produce a fall in 5-hydroxytryptamine concentration but still exert an analgesic action.

There is no apparent correlation between the effects of a range of doses of morphine on motor activity and on 5-hydroxytryptamine concentration in the brain.

The effect of morphine on the 5-hydroxyindoleacetic acid concentration in mouse brain has been studied previously by Haubrich & Blake (1969), who found a 32% increase at an unstated time after an injection of 20 mg/kg of morphine. Bowers & Kleber (1971) showed that methadone, in doses of 5, 10 and 15 mg/kg, produced increases of 45, 64 and 74% respectively in 5-hydroxyindoleacetic acid concentrations in rat brain 2 h after the injection. The present findings are that 5·0 mg/kg caused an increase and that 10, 20 and 50 mg/kg caused decreases in the concentration of 5-hydroxyindoleacetic acid in the mouse brain.

In regard to the effects of morphine on cerebral dopamine concentrations, Takagi & Nakama (1966) reported a reduction to 68% of control by a dose of 20 mg/kg of morphine in the mouse, which is confirmed by our findings. Watanabe, Matsui & Iwata (1969) reported a potentiation of the analgesic action of morphine by sodium diethyldithiocarbamate, an inhibitor of dopamine-β-hydroxylase (Carlsson, Lindqvist, Fuxe & Hökfelt, 1966) which produces a rise in the dopamine concentration in the mouse brain. This suggests a possible relation between dopamine concentrations and analgesic activity. However, the finding that high doses of morphine cause a rise rather than a fall in brain dopamine concentration, but still exert an analgesic action, is not in accord with the suggestion.

The increase in dopamine concentration produced by a low dose (0·1 mg/kg) and by high doses of morphine (50 and 100 mg/kg) and the decrease produced by intermediate doses results in a dose-effect curve which resembles in general form the dose-effect curve for morphine on motor activity. However, there is a discrepancy in the range of 5 to 10 mg/kg; with these doses dopamine concentrations are reduced, but activity is increased. Rethy *et al.* (1971) obtained evidence that the effects of morphine on locomotor activity involved brain catecholamines but Chan & Webster (1971) found a relation between activity and brain catecholamines to exist for some drugs but not others.

Morphine produces a reduction in brain noradrenaline concentration in doses corresponding to the ED50 values for analgesia in various tests involving heat and pressure (Table 1). However, there is a rise in brain noradrenaline concentrations with higher doses of morphine (20, 50 and 100 mg/kg) which still exert an analgesic action. This rise in noradrenaline may be due to an increase in availability of dopamine resulting in increased noradrenaline synthesis. Chodera *et al.* (1968) found that noradrenaline concentrations in the rat brain were reduced 1 and 3 h after 40 mg/kg of morphine. Maynert & Klingman (1962) failed to find an effect of morphine in doses of 5, 60 and 125 mg/kg on the noradrenaline concentration in dog brain, but in rats, the concentration of noradrenaline was decreased 5 h after 60 to 200 mg/kg but not after 20 mg/kg of morphine. They presented evidence that 5 h was the time of maximal effect for morphine to alter brain noradrenaline concentrations but the earliest time of testing was 3 h after giving the morphine. These times are considerably longer than the time for the maximal effect on cerebral noradrenaline (30 min) in the mouse (Lee & Fennessy, 1970). The observation by Vogt (1954) that morphine reduced the 'sympathin' levels in the cat and dog brain stimulated much research directed towards attempts to implicate changes in brain noradrenaline levels in tolerance and addiction (Gunne, 1959). In asso-

ciation with this work the acute effects of morphine have been incidentally reported, bu most of it was concerned with very high doses and long pretreatment times and has little relevance to the production of acute effects.

The sum of the evidence presented on the effects of a wide range of doses of morphine on brain amines does not support suggestions that there is a simple relation between these effects and the actions of morphine in analgesic tests or on locomotor activity.

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