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The analgesic action of morphine-N-oxide

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1. The analgesic activity of morphine-N-oxide in mice and rats has been investigated and compared with that of morphine.

2. Both morphine and morphine-N-oxide were more active when given subcutaneously than when given intraperitoneally.

3. Given subcutaneously, morphine was 11–22 times more potent than morphine-N-oxide and when given intraperitoneally it was 39–89 times more potent. The potencies depended on the test situation and the species of animal used.

4. In animals pretreated with amiphenazole or tacrine, the analgesic activities of morphine and morphine-N-oxide were increased. The potencies of these analgesic drugs given intraperitoneally were increased to a greater extent than were the potencies obtained by subcutaneous administration.

5. A possible explanation for the increase in analgesic potency of morphine-N-oxide produced by pretreatment with amiphenazole or tacrine may be that morphine-N-oxide is rapidly inactivated in the liver and this inactivation is impaired by amiphenazole and tacrine.

Morphine-N-oxide has been reported to possess little or no analgesic activity (Freund & Speyer, 1910; Rosenthaler, 1933; Keil, Schmidt & Günther, 1933; Anton, Theiss & Weissig, 1935). It is difficult, however, to evaluate these reports for they contain insufficient information concerning the species and numbers of animals used, the routes of administration and whether morphine-N-oxide was given as a solid, suspension or solution. Braenden, Eddy & Halbach (1955) predicted only a weak analgesic activity for morphine-N-oxide. They suggested that the tertiary nitrogen of morphine cannot be made quaternary, as in the formation of morphine-N-oxide, without a great diminution in analgesic activity. On the other hand Polonowsky, Nayrac & Tiprez (1930) reported that when administered subcutaneously, morphine-N-oxide possessed considerable analgesic activity.

In dogs, amiphenazole (2,4-diamino-5-phenylthiazole) and tacrine (1,2,3,4-tetrahydro-9-aminoacridine, THA) reversed morphine-induced sleep (Shaw & Bentley, 1952; Shaw, Gershon & Bentley, 1957). In man they have been found to reverse or prevent narcosis produced by morphine while analgesia has been maintained or even increased (Shaw & Shulman, 1955; Holmes, 1956; McKeogh & Shaw, 1956; Christie, Gershon, Gray, Shaw, McCance & Bruce, 1958; Stone, Moon & Shaw, 1961). McKenzie (1960) has reported that amiphenazole potentiated

morphine-induced analgesia in mice. It was suggested that amiphenazole combined with morphine to produce an intermediate complex which was analgesic but non-addicting (Gershon, Bruce, Orchard & Shaw, 1958). Bruce (1964) has shown that amiphenazole may be of use in the treatment of morphine and opium addiction. Since then, chromatographic studies of urine from patients treated with morphine and amiphenazole, and morphine and tacrine have revealed a substance distinct from these three drugs. This substance was identified as morphine-N-oxide, and it was suggested that this compound may be an intermediate product in the conversion of morphine to normorphine in the liver. Morphine-N-oxide has not been detected as a metabolite of morphine in normal circumstances ; this may be due to rapid breakdown by microsomal enzymes. Amiphenazole and tacrine may act by inhibiting an enzyme responsible for the conversion of morphine-N-oxide to normorphine with a consequent accumulation of morphine-N-oxide (Woo, Gaff & Fennessy, 1968).

This paper describes the analgesic actions of morphine and morphine-N-oxide and the effects of amiphenazole or tacrine on their actions.

Methods

The analgesic potencies and the duration of action of morphine and morphine-N-oxide given by the subcutaneous and intraperitoneal routes were compared in mice with the hot plate method and in rats with the tail pinch method. The potency and duration of action of these analgesics were also compared in animals treated with amiphenazole and tacrine.

Hot plate method

The method of Eddy & Leimbach (1953) was used. The criterion of analgesia was the absence of hind paw licking within 30 sec after placing the mouse on the hot plate. Reaction times were recorded 30 min before drug administration and at 30, 60, 90, 120, 180 and 240 min after injection. The ED50 for analgesia was determined from the reaction times recorded 30 min after drug administration. The experimental animals were white Swiss mice (Commonwealth Serum Laboratories) of both sexes and weighing between 20 and 25 g. A group of ten was used for each dose level.

Tail clip method

The method was that of Bianchi & Franceschini (1954). A large artery clip, with its jaws covered by thin silicone rubber tubing, was applied to the base of the tail of a rat. Only animals which made repeated attempts to remove the clip within 15 sec were used. The criterion of analgesia was the absence of any attempt to remove the clip within 30 sec. The determination of the ED50 for analgesia and the duration of action was the same as that used in mice with the hot plate method. Albino Wistar rats of both sexes weighing between 200 and 250 g were used. A group of eight was used for each dose level.

The doses producing analgesia (at the criterion levels adopted) in 50% of animals (ED50) together with the 95% confidence limits were determined according to the method of Lichfield & Wilcoxon (1949).

Drugs

The drugs used were morphine sulphate (D.H.A.), morphine-N-oxide, amiphenazole hydrochloride (Nicholas Pty. Ltd.) and tacrine (H. W. Woods Pty. Ltd.). The concentration of amiphenazole has been expressed in terms of the salt, and those of morphine, morphine-N-oxide and tacrine in terms of base. Morphine-N-oxide was prepared according to the method of Freund & Speyer (1910). Solutions for injections were made by dissolving it in 0.1 N-HCl with adjustment to pH5 with NaOH; they were freshly prepared on the day of each experiment.

Results

Morphine analgesia

In both rats and mice, morphine was approximately 1.5 times more potent in producing analgesia when given subcutaneously than when given intraperitoneally (Fig. 1). This small difference was not statistically significant ($P \geqslant 0.05$). At the ED50, maximum reaction times were observed 30 min after the injection of morphine and, at this dose level, the duration of analgesia was approximately 2 hr by either route.

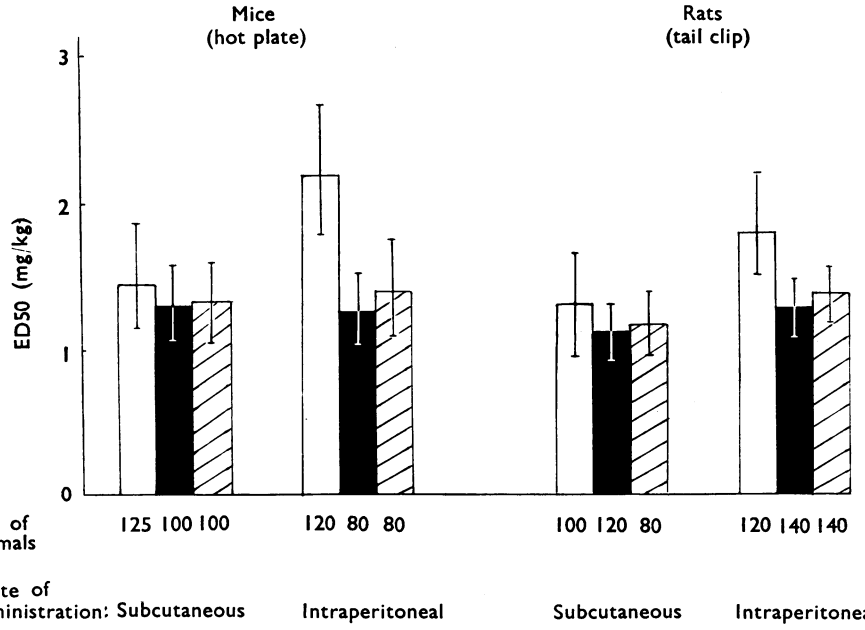

FIG. 1. Analgesic activity of morphine administered intraperitoneally or subcutaneously in rats and mice, and the effects of pretreatment with amiphenazole (20 mg/kg) or tacrine (1.5 mg/kg), injected intraperitoneally 30 min before the morphine. Analgesia was tested 30 min after administration of morphine, and the ED50 was calculated from the proportions of animals exhibiting analgesia at the criterion level in each dose group. The vertical lines represent the 95% confidence limits. Open columns, morphine; closed columns, morphine + amiphenazole; hatched columns, morphine + tacrine.

Intraperitoneal injections of amiphenazole (20 mg/kg) or tacrine (1.5 mg/kg) were without analgesic effect when given alone, but significantly increased the analgesic potency of intraperitoneally administered morphine ($P \leq 0.05$). There was little change in the analgesic activity of subcutaneously injected morphine. In

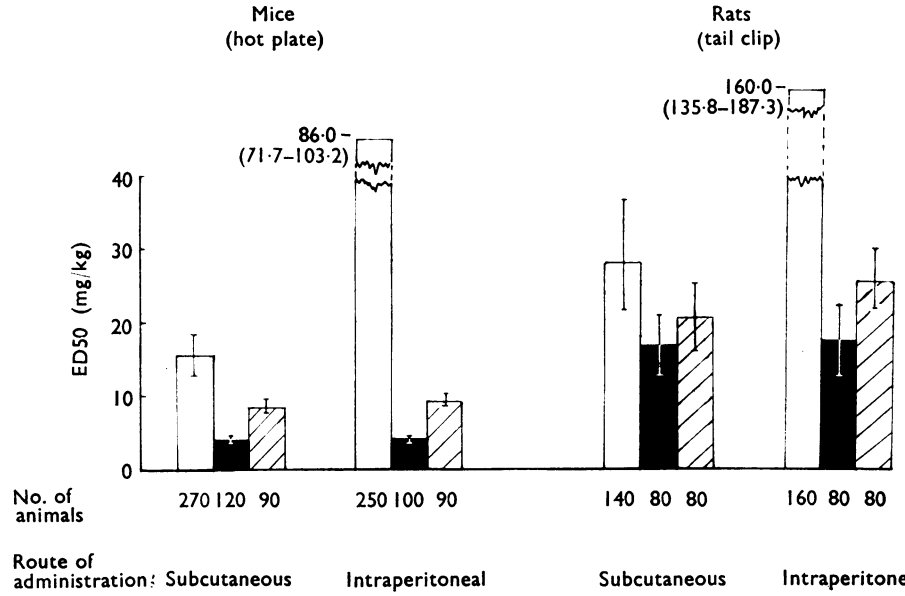

FIG. 2. Analgesic activity of morphine-N-oxide as affected by various factors: for details see legend to Fig. 1. Open columns, morphine-N-oxide; closed columns, morphine-N-oxide + amiphenazole; hatched columns, morphine-N-oxide + tacrine.

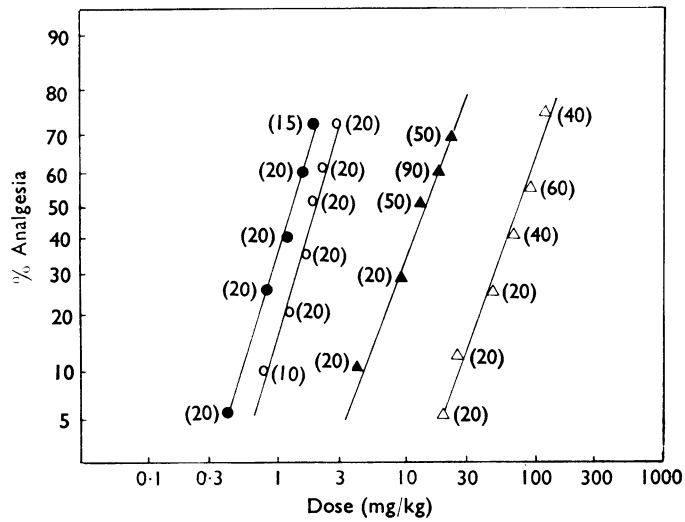

FIG. 3. Analgesic activities of morphine (circles) and morphine-N-oxide (triangles) (MNO) in mice 30 min after subcutaneous (closed symbols) or intraperitoneal (open symbols) injection. Abscissa: dose in mg/kg on log scale. Ordinate: percentage of mice in each group exhibiting analgesia on a provisional probit scale. The figures in parentheses refer to the number of mice used for determining each point.

animals pretreated with amiphenazole or tacrine there was virtually no difference in the potency of morphine when given intraperitoneally or given subcutaneously—that is, the ED50 values for subcutaneously and intraperitoneally administered morphine were the same (Fig. 1). The duration of analgesia in the pretreated animals was extended by about 1 hr. Amiphenazole and tacrine were just as effective in increasing analgesia and prolonging duration of action when they were given 0.5, 18 or 24 hr before morphine.

Morphine-N-oxide analgesia

In both rats and mice, morphine-N-oxide given subcutaneously was about 5.5 times more powerful in producing analgesia than when given intraperitoneally (Fig. 2). The differences were statistically significant ($P=0.05$). The duration of analgesia induced by the ED50 of morphine-N-oxide was similar to that of the ED50 of morphine, being approximately 2 hr. Pretreatment of animals with amiphenazole or tacrine significantly decreased the analgesic ED50 values for morphine-N-oxide and increased the duration of action by about 1 hr after subcutaneous or

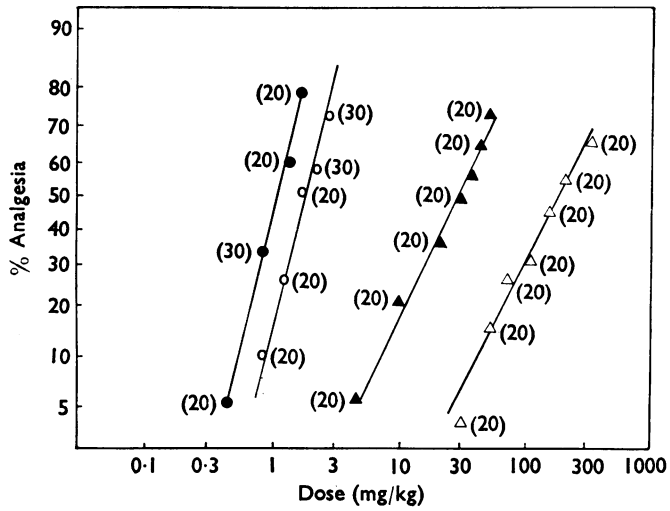

FIG. 4. Analgesic activities of morphine (circles) and morphine-N-oxide (triangles) in rats 30 min after subcutaneous (filled symbols) or intraperitoneal (open symbols) injection. Abscissa: dose in mg/kg on log scale. Ordinate: percentage of rats in each group exhibiting analgesia on a provisional probit scale. The figures in parentheses refer to the number of rats used at each dose level.

TABLE 1. *Ratio of the analgesic activities in mice and rats of morphine and morphine-N-oxide, given alone and after pretreatment with amiphenazole or tacrine injected intraperitoneally 30 min previously*

	Mouse (Hot plate)		Rat (Tail clip)	
Pretreatment	Subcutaneous	Intraperitoneal	Subcutaneous	Intraperitoneal
None	10·7	39·0	22·0	88·9
Amiphenazole (20 mg/kg)	3·3	3·3	14·4	14·1
Tacrine (1·5 mg/kg)	6·5	6·8	18·3	18·9
Ratio of analgesic potency: $\frac{\text{ED50 morphine}}{\text{ED50 morphine-N-oxide}}$				

intraperitoneal injection (Fig. 2). As with morphine, the ED50 values for subcutaneously and intraperitoneally administered morphine-N-oxide were almost identical in the presence of amiphenazole and tacrine when these drugs were given 0.5, 18 or 24 hr before morphine-N-oxide. Amiphenazole was more effective than tacrine in lowering this ED50.

Linear relationships were observed between the logarithm of the dose and the probits corresponding to the proportion of animals exhibiting analgesia at each dose level for both morphine and morphine-N-oxide, as shown for mice in Fig. 3 and for rats in Fig. 4. The slopes of the lines do not differ from parallelism ($P=0.05$). These graphs also show the relative potencies of morphine and morphine-N-oxide given subcutaneously and intraperitoneally. Table 1 shows that morphine was 11 to 89 times more potent than morphine-N-oxide depending on the species used and the route of administration. Both amiphenazole and tacrine reduced the difference in potency between morphine and morphine-N-oxide, amiphenazole being the more effective.

Discussion

Morphine-N-oxide has been found to possess analgesic activity. Depending on the test situation and the species used, morphine was 39–89 times more potent than morphine-N-oxide when given intraperitoneally, but only 11–22 times more potent when given subcutaneously. The analgesic activity of morphine was greater when it was injected subcutaneously than when injected intraperitoneally, confirming a previous report by Bianchi & Franceschini (1954). It has been shown that the liver rapidly destroys analgesic drugs (Sung & Way, 1950; Bonnycastle & Delia, 1950). It has been suggested that morphine may be partly N-demethylated to normorphine in liver and brain of several species (March & Elliot, 1954; Axelrod, 1956; Mellet & Woods, 1961; Misra, Mule & Woods, 1961; Milthers, 1962a, b). Glucuronide formation in the liver is, however, probably the most significant factor in the termination of the actions of morphine (Way & Adler, 1961). The fact that morphine is metabolized in the liver explains the difference in activity with different routes of administration. When morphine is injected intraperitoneally, it is absorbed into blood vessels that join the portal system and the morphine passes directly to the liver where it is presumably partly destroyed. On the other hand morphine absorbed from a subcutaneous injection reaches the brain without first passing through the liver.

Morphine-N-oxide was 5.5 times more effective when given subcutaneously than when given intraperitoneally, indicating that it is rapidly inactivated by the liver. It has been suggested that morphine-N-oxide is an unstable intermediate product of morphine metabolism (Woo, Gaff & Fennessy, 1968) and the reductive demethylation of morphine-N-oxide to normorphine may be due to an enzymatic step.

Others have considered the possibility that morphine metabolites may play a prominent part in mediating the analgesia produced by morphine administration. Thus, Beckett, Casy & Harper (1956) related N-demethylation to analgesia and suggested that normorphine mediated the analgesic responses. Although Axelrod & Cochin (1957) indicated that N-demethylation is not of great significance in the production of morphine analgesia, we suggest the individual steps in the conversion of morphine to normorphine are of some consequence.

A possible explanation for the increase in analgesic activities of morphine and morphine-N-oxide produced by pretreatment with amiphenazole or tacrine may be inhibition of an enzyme that converts morphine-N-oxide to normorphine: the accumulation of morphine-N-oxide would then result in increased analgesic activity. Another possibility is that the conversion of morphine to its N-oxide may constitute a minor pathway of its metabolism while conjugation with glucuronic acid may be a major pathway. If this major pathway is inhibited by amiphenazole or tacrine then the minor pathway would become more important and have more morphine to deal with, so that there could then be an accumulation of the N-oxide. A still further possibility is that morphine-N-oxide is reduced to morphine. The low level of activity of the N-oxide could then be due to an equilibrium between morphine-N-oxide and morphine. This, however, does not explain why the N-oxide is much more active subcutaneously than intraperitoneally.

The impairment by amiphenazole or tacrine of an enzymatic pathway that is involved in the elimination of morphine-N-oxide (or of morphine) would explain not only the increased analgesic activity of morphine and morphine-N-oxide in animals but also the increase in analgesia reported by cancer patients treated with a combination of morphine and amiphenazole (Gershon *et al.*, 1958) or morphine and tacrine (Shaw, 1960). The observations that morphine and morphine-N-oxide (*a*) produced a greater degree of analgesia when given subcutaneously than when given intraperitoneally, (*b*) possessed a similar duration of analgesic action and (*c*) had parallel slopes when the probits corresponding to the proportion of animals showing analgesia were plotted against the logarithm of the dose indicates that these drugs may be acting on the same receptors and that their metabolic pathways may be the same. An alternative explanation, however, is that morphine-N-oxide is converted to morphine and that the same drug (morphine or its cation) is produced in each case, under which conditions the same receptors are involved.

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