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A comparison of the antinociceptive actions of cholinomimetic and morphine-like drugs

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Summary

1. The antinociceptive activity of morphine, nalorphine, oxotremorine and eserine has been examined in mice in electroshock and phenylbenzoquinone writhing tests.

2. The effectiveness of these drugs alone, in combination with each other, and in combination with the muscarinic antagonist atropine sulphate, and with the narcotic antagonist naloxone has also been investigated.

3. In both tests morphine was effective and antagonized by naloxone.

4. Nalorphine was active in the phenylbenzoquinone test but only slightly active in the electroshock test: it was antagonized by naloxone in both tests.

5. Morphine was potentiated by nalorphine in the phenylbenzoquinone test, but antagonized by it in the electroshock test.

6. Oxotremorine was effective in both tests, and was antagonized by atropine sulphate.

7. Eserine was active only in the phenylbenzoquinone test, and was antagonized by atropine sulphate.

8. Oxotremorine was potentiated by eserine in the phenylbenzoquinone test, but antagonized by it in the electroshock test.

9. Crossed agonist and partial agonist experiments produced enhancement.

10. No antagonism was seen in the crossed antagonist experiments.

11. The similarities between the effects of the two classes of drugs are discussed, and the conclusion drawn that they act by separate mechanisms.

Introduction

The first observations that cholinomimetic drugs produce central behavioural effects, especially analgesia, were made in man (Pellanda, 1933; Flodmark & Wramner, 1945). Since then many workers, including Hendershot & Forsaith (1959), Herz (1962), Jacob & Barthelemy (1965), Leslie (1969), and Metys, Wagner, Metysova & Herz (1969), have shown various cholinomimetic drugs to have antinociceptive actions in a variety of tests involving several species.

The present experiments were performed to investigate the nature of the antinociceptive effect, and its relation to that observed with narcotic analgesics such as morphine, using specific partial agonists and antagonists.

Methods

Two tests for antinociception were used, the electroshock test of Burn, Finney & Goodwin (1950), and the phenylbenzoquinone writhing test of Parkes & Pickens (1965). In both tests albino mice, weight range 18–22 g, were used, and at least eight groups of animals were employed at each dose level.

Electroshock test

Electric shocks of 100 μA were applied at 1 Hz to the tails of mice, and vocalization was taken as the nociceptive response. If a mouse failed to vocalize after five consecutive shocks, it was considered to be insensitive, and not used in the investigation. The number of shocks (n) required to produce vocalization in each mouse was recorded and the mouse marked for identification. Groups of ten mice were then injected subcutaneously with the drug under examination, and 30 min later were again tested for their reaction to electric shocks of the same magnitude and rate. Failure to vocalize after 5 plus the original number of shocks needed to produce vocalization ($5+n$) was taken as indicative of the antinociceptive activity of the drug. The number of mice in each group which failed to produce a nociceptive

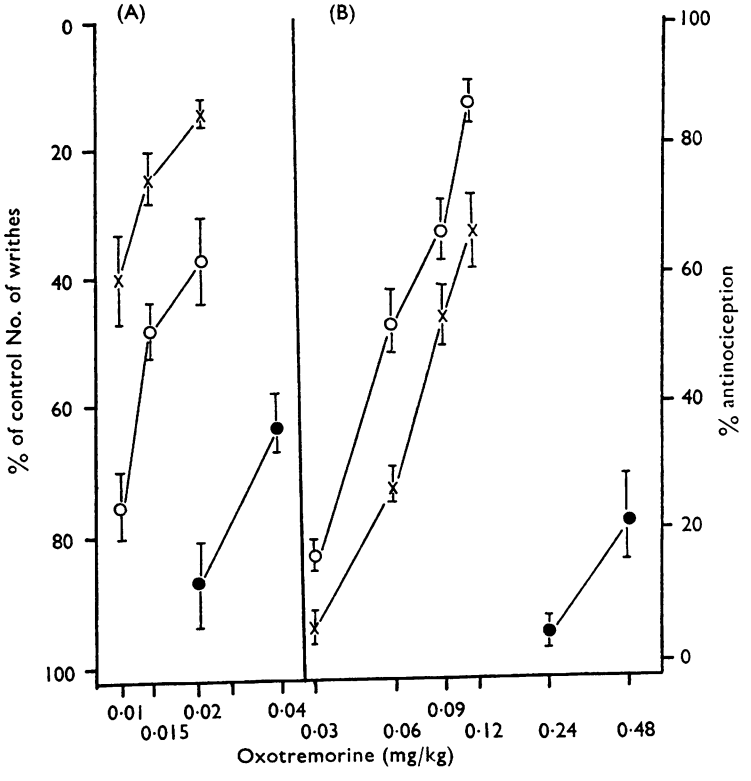

FIG. 1. Antinociceptive effect of oxotremorine (—○—) is enhanced by 0·03 mg/kg eserine (—×—) in the phenylbenzoquinone test (A) and antagonized by 0·1 mg/kg eserine in the electroshock test (B), and antagonized in both tests by atropine sulphate, 0·5 mg/kg (—●—). The vertical lines on either side of each point represent one standard error.

response was expressed as a percentage. With a high degree of antinociception most of the animals in a group would not vocalize, and a high percentage response was recorded.

Phenylbenzoquinone writhing test

When mice are injected intraperitoneally with 2 mg/kg of a solution of 2-phenyl-1-4-benzoquinone they writhe. A writhe is a contortion of the mouse resulting from a contraction of the abdominal musculature, often associated with an extension of the hind limbs, and giving the appearance of an elongation of the body. This is a nociceptive response. A group of five mice was injected intraperitoneally

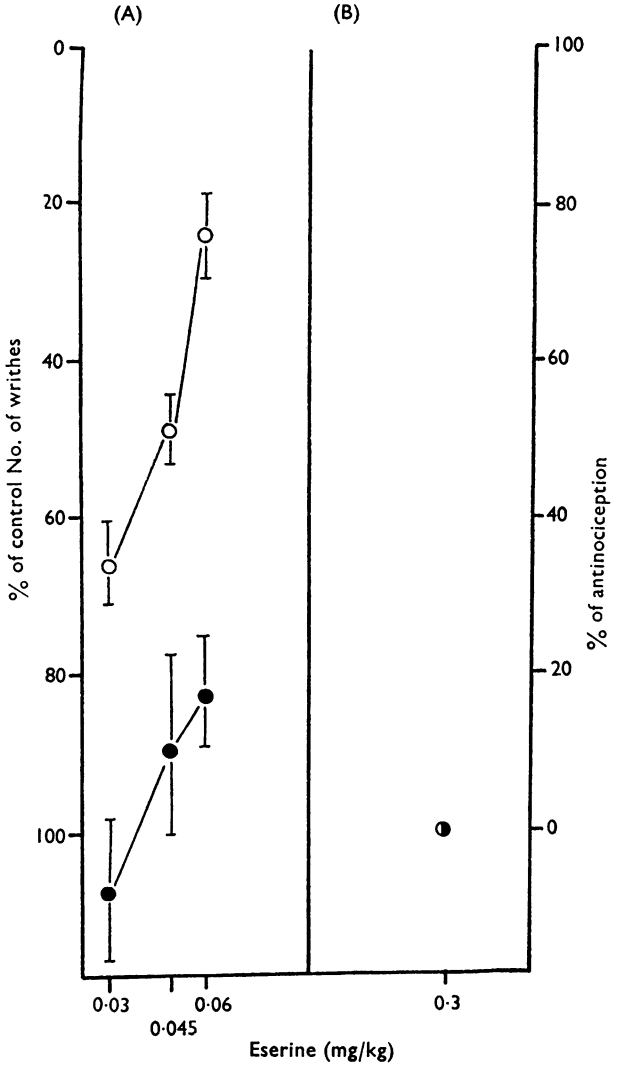

FIG. 2. Eserine shows no antinociceptive activity in the electroshock test (B) but is effective in the phenylbenzoquinone test (A) (—○—), and is antagonized by atropine sulphate, 0·5 mg/kg (—●—). The vertical lines on either side of each point represent one standard error.

with phenylbenzoquinone. Five minutes later the mice were placed in a Perspex box and the number of writhes occurring in the following 10 min counted. Similarly, groups of five mice were premedicated with the drug under investigation instead of saline. The number of writhes obtained from the treated groups is expressed as a percentage of the number of writhes of the control group. The greater the antinociceptive activity of the drug, the fewer the number of writhes and the lower the percentage.

Two classes of drugs were investigated, the cholinomimetics oxotremorine hydrochloride and eserine sulphate, with the muscarinic antagonist atropine sulphate ; and the narcotic analgesic morphine sulphate, the partial agonist nalorphine hydrobromide, and the pure antagonist naloxone hydrochloride. In all experiments involving the cholinomimetic drugs, the mice were premedicated with the quaternary muscarinic receptor blocking drug atropine methylbromide (0·5 mg/kg) in order to prevent the agonists from producing muscarinic effects peripherally. Atropine methylbromide does not antagonize the antinociceptive action of the drugs (Koster, Anderson & De Beer, 1959 ; Herz, 1962). Atropine methylbromide does not affect the antinociceptive action of cholinomimetic drugs, since it has only poor penetration

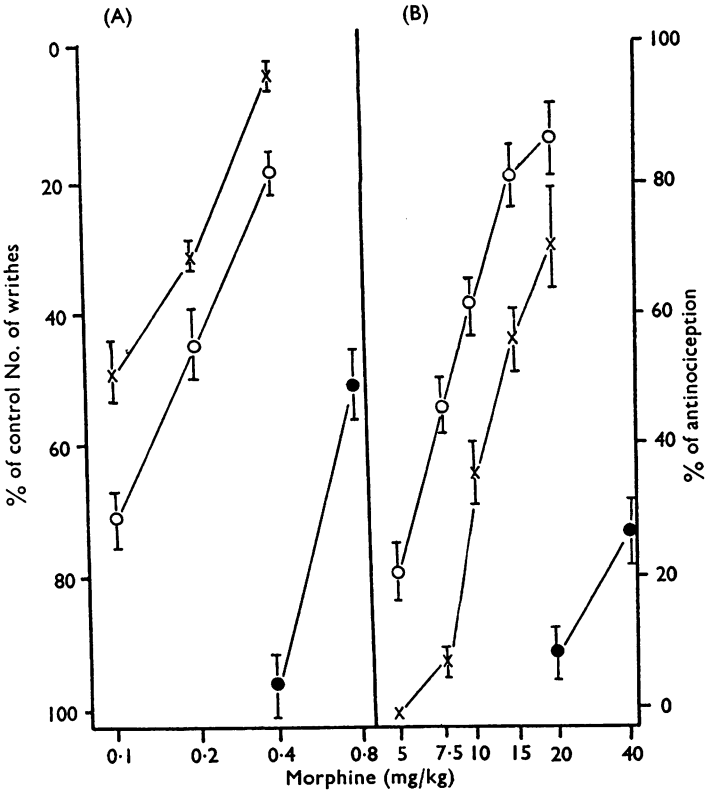

FIG. 3. Antinociceptive effect of morphine (—○—) is enhanced by 0·05 mg/kg nalorphine (—×—) in the phenylbenzoquinone test (A) and antagonized by 50 mg/kg nalorphine in the electroshock test (B), and antagonized in both tests by naloxone (—●—), 2·5 mg/kg in the electroshock test and 0·025 mg/kg in the phenylbenzoquinone test. The vertical lines on either side of each point represent one standard error.

to the brain (Koster *et al.*, 1959 ; Herz, 1962). All test drugs were administered subcutaneously. In all animals receiving a combination of drugs, the drugs were administered simultaneously. Antinociception was rated 15 min after treatment.

Results

In order to compare the results from the two tests, the ordinate axis in those graphs pertaining to the phenylbenzoquinone test has been inverted ; thus in all graphs a shift to the right denotes antagonism, and a shift to the left summation or potentiation.

The first series of experiments was concerned with the response to the cholinomimetic drugs. It can be seen in Fig. 1 that oxotremorine was effective in both tests, at 0·01–0·02 mg/kg in the phenylbenzoquinone test, and at 0·03–0·12 mg/kg in the electroshock test, and was antagonized in both tests by 0·5 mg/kg atropine sulphate. Oxotremorine was antagonized by eserine (0·1 mg/kg) in the electroshock test, but potentiated by it (0·03 mg/kg) in the phenylbenzoquinone test. Eserine (Fig. 2) was ineffective in the electroshock test (0·3 mg/kg) but active in the phenylbenzoquinone test (0·03–0·06 mg/kg), where antagonism was observed with atropine sulphate (0·5 mg/kg).

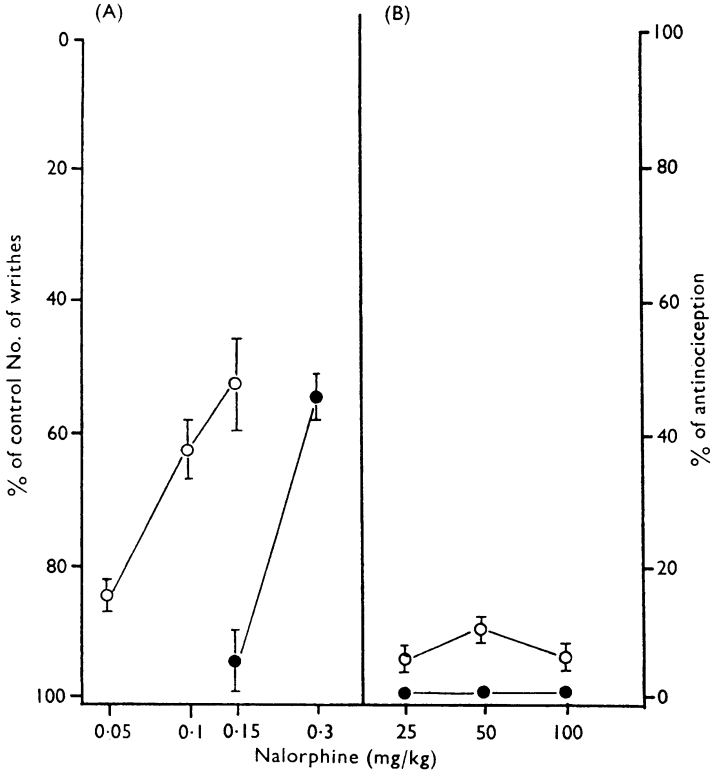

FIG. 4. Nalorphine (—○—) shows antinociceptive activity which is antagonized by naloxone (—●—), 2·5 mg/kg in the electroshock test (B) and by 0·025 mg/kg in the phenylbenzoquinone test (A). The vertical lines on either side of each point represent one standard error.

In the second series of experiments the same pattern of effects was observed with the narcotic agonists and antagonist. Morphine (Fig. 3) was active in both tests, at 5–20 mg/kg in the electroshock test, and at 0·1–0·4 mg/kg in the phenylbenzoquinone test. Antagonism was obtained with naloxone at 2·5 mg/kg in the electroshock test, and at 0·025 mg/kg in the phenylbenzoquinone test. As was observed with oxotremorine and eserine, whilst antagonism of morphine occurred with 50 mg/kg of nalorphine in the electroshock test, there was summation or potentiation between morphine and 0·05 mg/kg nalorphine in the phenylbenzoquinone test. In Fig. 4 it can be seen that nalorphine was active in the phenylbenzoquinone test at 0·05–0·15 mg/kg, but showed only very slight activity in the electroshock test (100 mg/kg). In both tests antagonism by naloxone, at the previously mentioned dose levels, was observed.

In the third series of experiments the interaction of the two classes of drugs on the response was observed. Eserine at 0·045 mg/kg potentiated morphine in both tests (Fig. 5). Oxotremorine was potentiated by nalorphine, 0·05 mg/kg in the phenylbenzoquinone test, and 50 mg/kg in the electroshock test (Fig. 6). Nalorphine was potentiated by eserine (0·045 mg/kg) in both tests (Fig. 7). Although there was

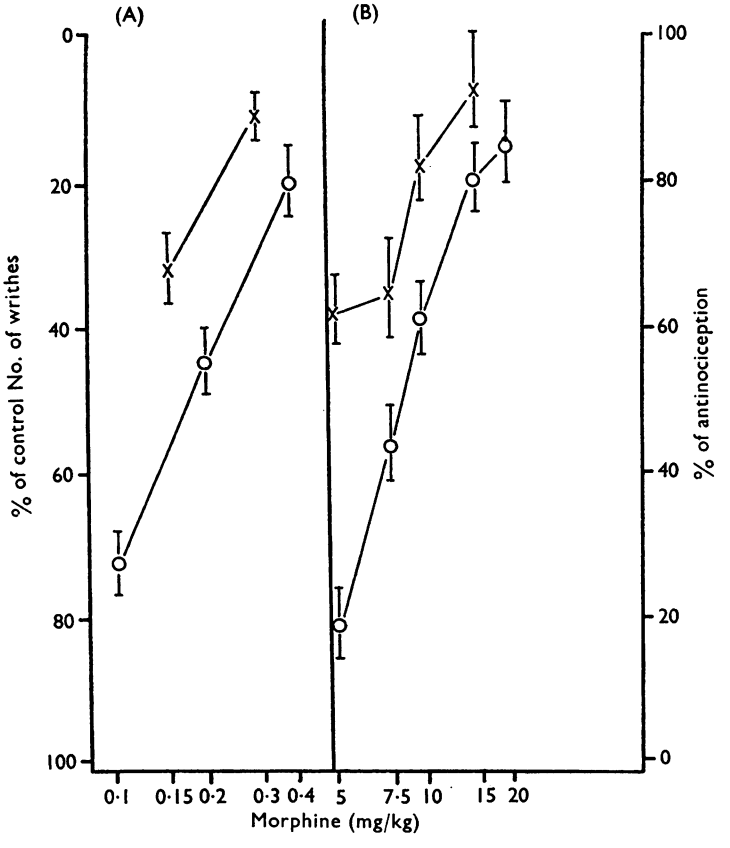

FIG. 5. Antinociceptive effect of morphine (—○—) is enhanced by combination with 0·045 mg/kg eserine (—×—). A, Phenylbenzoquinone test; B, electroshock test. The vertical lines on either side of each point represent one standard error.

interaction between the agonists and partial agonists from the two classes of drugs there was no interaction between crossed antagonists. Neither morphine nor nalorphine was affected by 0·5 mg/kg atropine sulphate in either test, nor was the response to oxotremorine or eserine reduced by 2·5 mg/kg naloxone in either test (Fig. 8).

It is therefore evident from the results that within each class of drugs studied there is a similar pattern of effect with the agonist, partial agonist, antagonist, and combinations of two of these, in both tests. Agonists of one class are able to enhance the effects of the agonists of the second class. Antagonists of one class do not reduce the effectiveness of the agonists of the second class.

Discussion

In this investigation of the nature of the antinociceptive action of cholinomimetics, two important characteristics have been demonstrated; the similarity in effect compared with morphine-like analgesics, and the interrelation between the two classes of drugs.

It can be seen from the results, by comparing Figs. 1 and 3, and Figs. 2 and 4, that the nature of the antinociceptive effects of the cholinomimetic and morphine-

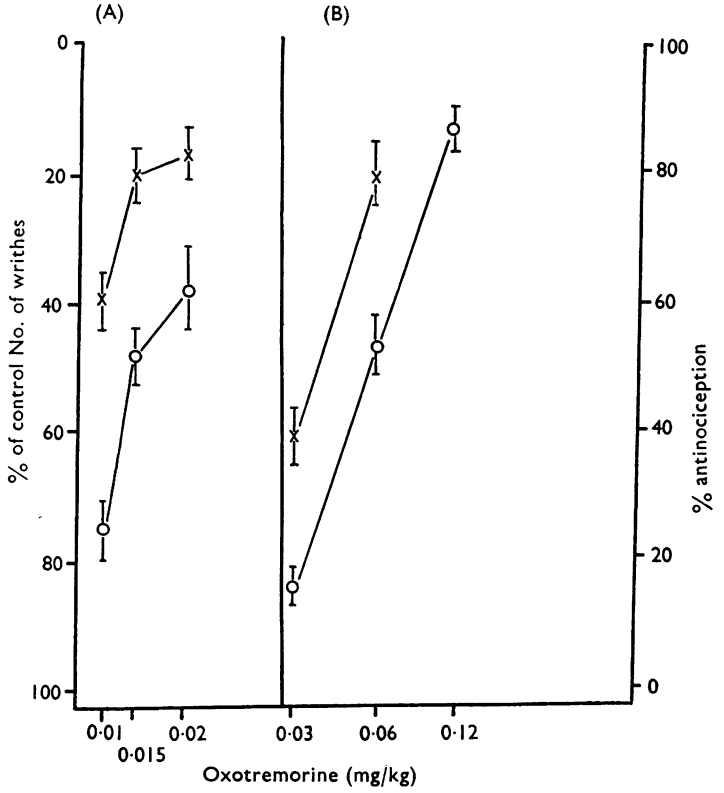

FIG. 6. Antinociceptive effect of oxotremorine (—○—) is enhanced by combination with nalorphine (—×—), 0·05 mg/kg in the phenylbenzoquinone test (A) and 50 mg/kg in the electroshock test (B). The vertical lines on either side of each point represent one standard error.

like drugs are very similar. Jaffe (1965) used the hypothesis of nalorphine being a partial agonist to explain its effectiveness as an analgesic but morphine antagonist in man. Such a hypothesis of agonist and partial agonist could explain the antagonism observed between oxotremorine and eserine and morphine and nalorphine in the electroshock test, and the potentiation of oxotremorine by eserine and of morphine by nalorphine in the phenylbenzoquinone test. If in producing its antinociceptive effect, oxotremorine stimulated the majority of the receptors, then eserine, having a high affinity for the receptors, would compete with oxotremorine, and since it has a relatively low intrinsic activity, the result would be a diminution of the effect. This might explain what is seen in the electroshock test, where the noxious stimulus used is powerful, and a large dose of oxotremorine is required to produce an antinociceptive effect. In the phenylbenzoquinone test, where much lower doses of the agonists are required to produce antinociceptive effects, one might assume that in order to produce its effect oxotremorine need only affect a proportion of the receptors. In this situation, eserine might still compete with the oxotremorine, but may also be stimulating some of the free receptors, and the net effect may be an increased antinociception, as seen. The hypothesis that eserine and nalorphine are both partial agonists would also explain the anomalous finding that both drugs alone are ineffective in the electroshock test but active when used in combination.

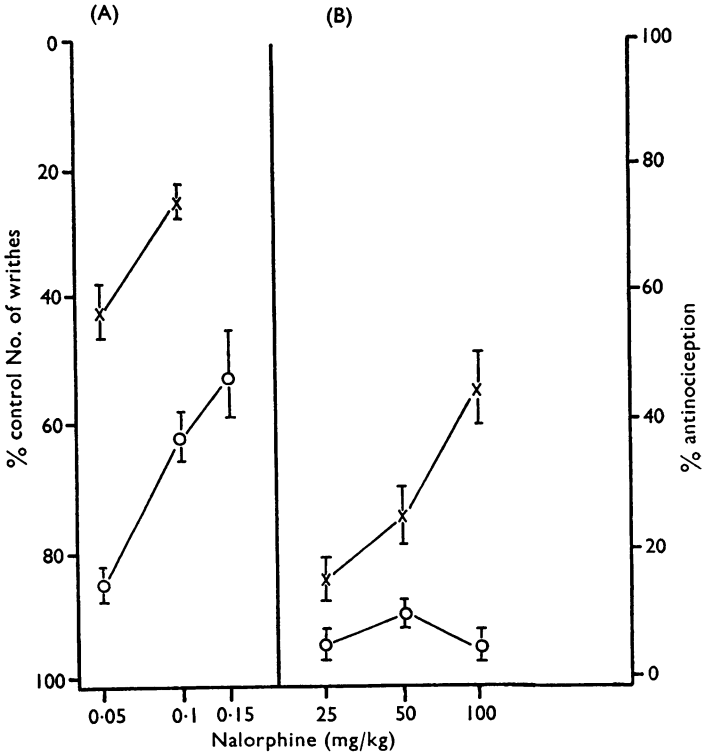

FIG. 7. Antinociceptive action of nalorphine (—○—) is enhanced by combination with 0·045 mg/kg eserine (—×—). A, Phenylbenzoquinone test; B, electroshock test. The vertical lines on either side of each point represent one standard error.

On this simple basis of agonist, partial agonist and antagonist, it is not surprising that the two classes of drugs examined should produce such strikingly similar pictures.

From data dealing with the antinociceptive effectiveness of cholinomimetic and morphine-like narcotic drugs, and their relative activities in influencing the different nociceptive responses in a variety of species, Metys *et al.* (1969) have postulated that " the substrate which is affected by cholinomimetic agents and by morphine is not completely different ". Such an hypothesis could gain support from our observations involving crossed agonists and partial agonists, where the effectiveness of the combined drugs from the two classes is always greater than the effectiveness of each alone, summation or potentiation occurring. Despite the similarities, however, the results obtained from the crossed antagonists experiments would indicate that these two classes of drugs have different mechanisms of action. The results indicate that oxotremorine and eserine produce their antinociceptive effect by an action on a centrally-sited muscarinic receptor, whereas morphine and nalorphine do not.

It would appear that at least two separate systems are involved, but each can complement the other. Such a theory would explain the observations that cholinomimetic drugs potentiate morphine-like antinociceptive drugs.

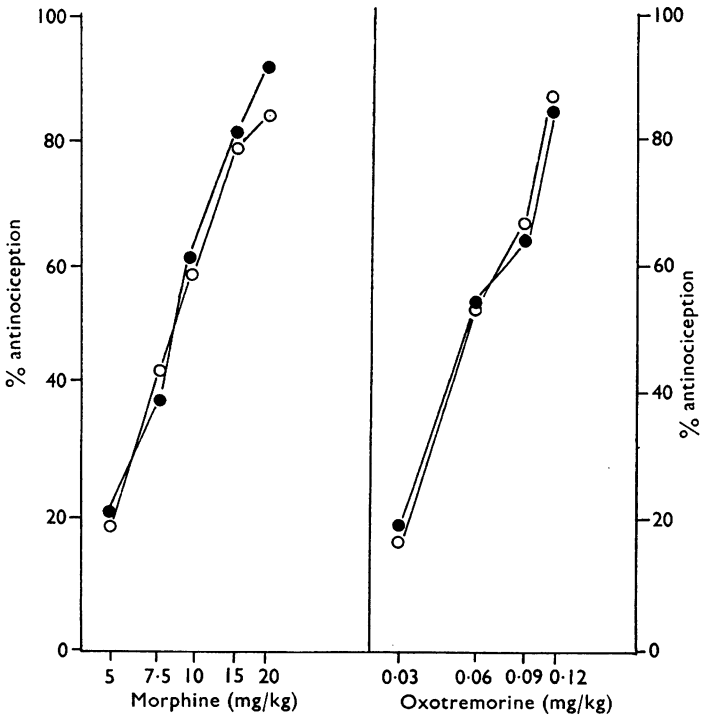

FIG. 8. In the electroshock test, the antinociceptive activity of morphine alone (—○—) is not affected by 0·5 mg/kg atropine sulphate (—●—) nor is the antinociceptive activity of oxotremorine (—○—) affected by 2·5 mg/kg naloxone (—●—).

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