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Quantitative studies on the antagonism by naloxone of some narcotic and narcotic-antagonist analgesics

S. E. SMITS* AND A. E. TAKEMORI

Department of Pharmacology, College of Medical Sciences, University of Minnesota, Minneapolis, Minnesota 55455, U.S.A.

Summary

1. Naloxone was used to study the antagonism of the analgesic effects of some narcotics (morphine sulphate, levorphanol tartrate, and methadone hydrochloride) and narcotic antagonists (pentazocine, cyclazocine, and nalorphine hydrochloride). The analgesic assay used was the mouse phenylbenzoquinone stretching test.

2. The *in vivo* equivalent of a pA_2 value (apparent pA_2) for naloxone was determined with each agonist. These values were found to be significantly larger with the narcotics than with the narcotic antagonists.

3. The slopes in the apparent pA_2 plots were also found to be significantly different. It was concluded that this difference in slopes was probably not due to a lack of equilibrium in one of the two groups of analgesics.

4. The results suggest that the narcotic and the narcotic-antagonist analgesics may inhibit stretching in this assay by interacting either with two different receptors or with the same receptor in a different manner.

Introduction

It is well known that most narcotic antagonists also possess significant narcotic (agonistic) activity, including analgesia in man (Lasagna & Beecher, 1954 ; Keats & Telford, 1956 ; DeKornfeld & Lasagna, 1963 ; Keats & Telford, 1964). Unlike the narcotic analgesics, however, the narcotic-antagonist analgesics induce very little tolerance or physical dependence, as shown by a mild withdrawal syndrome which is qualitatively different from that seen with the narcotics (Martin, Fraser, Gorodetzky & Rosenberg, 1965 ; Martin & Gorodetzky, 1965). Also, the narcotic antagonists show little or no analgesic activity in animals in assays using heat as the noxious stimulus (Green, Ruffel & Walton, 1954 ; Harris & Pierson, 1964 ; Winter, Orahovats & Lehman, 1957). These marked contrasts suggest a fundamental difference in the mechanism of action of these two groups of analgesics.

Schild (1947a) has suggested the determination of pA_x values as a convenient and quantitative measure of drug antagonism. In subsequent papers (Schild, 1947b ; Arunlakshana & Schild, 1959) the usefulness of these determinations for

* Present address: The Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, Indiana 46206, U.S.A.

the identification of agonists which act on the same receptor population was described. Although this method has usually been applied *in vitro*, several studies were reported recently in which the equivalent of pA_2 values were determined *in vivo* (Cox & Weinstock, 1964 ; Green & Fleming, 1967 ; Blane, Boura, Fitzgerald & Lister, 1967 ; Takemori, Kupferberg & Miller, 1969).

Since the analgesic test involving the induction of writhes or stretches in rodents is quite sensitive to the effects of both the narcotics and the narcotic antagonists (Taber, Greenhouse & Irwin, 1964 ; Blumberg, Wolf & Dayton, 1965), it provides a convenient assay for comparative studies of these two groups of compounds. Naloxone has been reported to be a potent antagonist of both narcotic and narcotic-antagonist analgesics (Blumberg, Dayton & Wolf, 1966) and to possess no analgesic activity in this assay (Blumberg, Dayton, George & Rapaport, 1961). Therefore, this agent could be used to study the quantitative antagonism of both groups of analgesics without interference from agonistic activity.

In the present study, experiments were performed to determine whether or not these two groups of analgesics interact with similar receptors. This was done by utilizing the phenylbenzoquinone method of assaying analgesia and the antagonist, naloxone, to obtain the *in vivo* equivalent of pA_2 values.

A preliminary report was presented at the meeting of the Federation of American Societies for Experimental Biology (Smits & Takemori, 1969).

Methods

Animals

Male Swiss-Webster albino mice weighing 15 to 22 g (Simonson Laboratories, White Bear, Minnesota) were used in all experiments. They were housed for at least 1 day after arrival but were used within 3 days thereafter. Each mouse was used only once.

Chemicals and drugs

The narcotic agents used in these experiments were levorphanol tartrate (Hoffman-La Roche, Inc.), methadone hydrochloride (Mallinckrodt), and morphine sulphate (Mallinckrodt). The narcotic antagonists were cyclazocine (Win 20,740, Sterling Winthrop Research Institute), nalorphine hydrochloride (Merck and Co., Inc.), pentazocine (Win 20,228, Sterling Winthrop Research Institute) and naloxone hydrochloride (Endo Laboratories, Inc.). The irritant used to induce writhing or stretching was phenylbenzoquinone (2-phenyl-1,4-benzoquinone, Eastman Organic Chemicals) (PBQ). This agent was initially dissolved in a small amount of 95% ethanol and then diluted with warm water to give a final PBQ solution of 0·2 mg/ml in 5% ethanol. This solution was prepared fresh daily and kept in a dark brown bottle. All other drugs were dissolved in distilled water except cyclazocine and pentazocine which were initially dissolved in a minimum amount of dilute HCl, then partially neutralized with NaOH, and finally diluted to volume with distilled water (final pH 5·0–6·0). All solutions were prepared so that the injection volume was 10 ml/kg. Dosages were given in the form in which the drug was obtained—as either the salt or the free base.

PBQ analgesic assay

The inhibition of PBQ-induced stretching responses was determined by a modification of the method of Hendershot & Forsaith (1959). The response is characterized by a wave of contraction of the abdominal musculature followed by extension of the hind limbs. At least ten mice were used for each treatment and were assayed in groups containing five mice each. The test drugs were injected subcutaneously. When two treatments were given to the same animal, they were injected at contralateral sites. Unless otherwise noted, 23 min later the mice received an intraperitoneal injection of the PBQ solution (2·0 mg/kg). The mice were then placed in individual transparent containers and the total number of stretching responses was determined for the group during a 4 min observation period. This period began 5 min after the injection of the PBQ solution. Thus, the interval from 28 to 32 min after drug treatment served as the assay time. The midpoint of the observation period was at 30 min.

This assay is known to be influenced by numerous factors, including room temperature, dieting, fasting (Parkes & Pickens, 1965) and aggregation (Okun, Liddon & Lasagna, 1963). To control these and other variables, the conditions of the assay were standardized as much as possible. Room temperature was kept between 24° and 26° C. The assay was always performed in the morning with ordinary laboratory noise and activity kept to a minimum. Free access to food and water was maintained before the assay and aggregation of the mice was avoided.

Calculations and statistical analyses

From a control group which had received either water or naloxone, the % inhibition of PBQ-induced stretching responses was calculated as follows:

$$\%\ \text{inhibition of PBQ-induced stretching responses} = 100 - \left[\frac{S_x}{S_c}(100)\right]$$

where S_x=total number of stretching responses per ten mice in the test group and S_c=total number of stretching responses per ten mice in the control group.

Whenever apparent pA_2 values were determined, the validity of the assay, the test of parallelism, the slopes, and the ED50s were estimated for each drug by a CDC 3300 (Control Data Corporation) computer using the parallel line assay of Finney (1964). For each shift in the dose-response curve, the ratio of the ED50 of the drug in the presence of naloxone to the ED50 of the drug alone was calculated. The equivalent of a pA_2 value was estimated by plotting log (ED50 ratio−1) against the negative log (dose of naloxone in mol/kg). For this plot the Programma 101 (Olivetti Underwood) computer was used to determine a straight line regression and its slope and S.E. The intercept on the abscissa gives the apparent pA_2 values. Student's *t* test was used to compare the slopes.

Results

Apparent pA_2 values for naloxone with several narcotic and narcotic-antagonist analgesics

The average number of stretching responses per group of ten control mice was 95 ± 12 (mean $\pm$ S.D.). There was no difference between the number of stretches

TABLE 1. *Summary of the dose-response curves for narcotic and narcotic-antagonist analgesics*

Analgesic	ED50 (95% confidence limits) mg/kg	Slope ±S.E.
Morphine sulphate	0·27 (0·23–0·32)	70·4± 3·9
Levorphanol tartrate	0·059 (0·047–0·073)	80·8± 4·8
Methadone hydrochloride	0·40 (0·34–0·48)	111·2±10·7*
Pentazocine (free base)	2·7 (2·1–3·4)	72·0± 4·7
Cyclazocine (free base)	0·026 (0·021–0·031)	79·6± 4·3
Nalorphine hydrochloride	0·28 (0·20–0·39)	68·1± 6·3

* The slope for methadone was the only one significantly different from that for morphine ($P<0·01$).

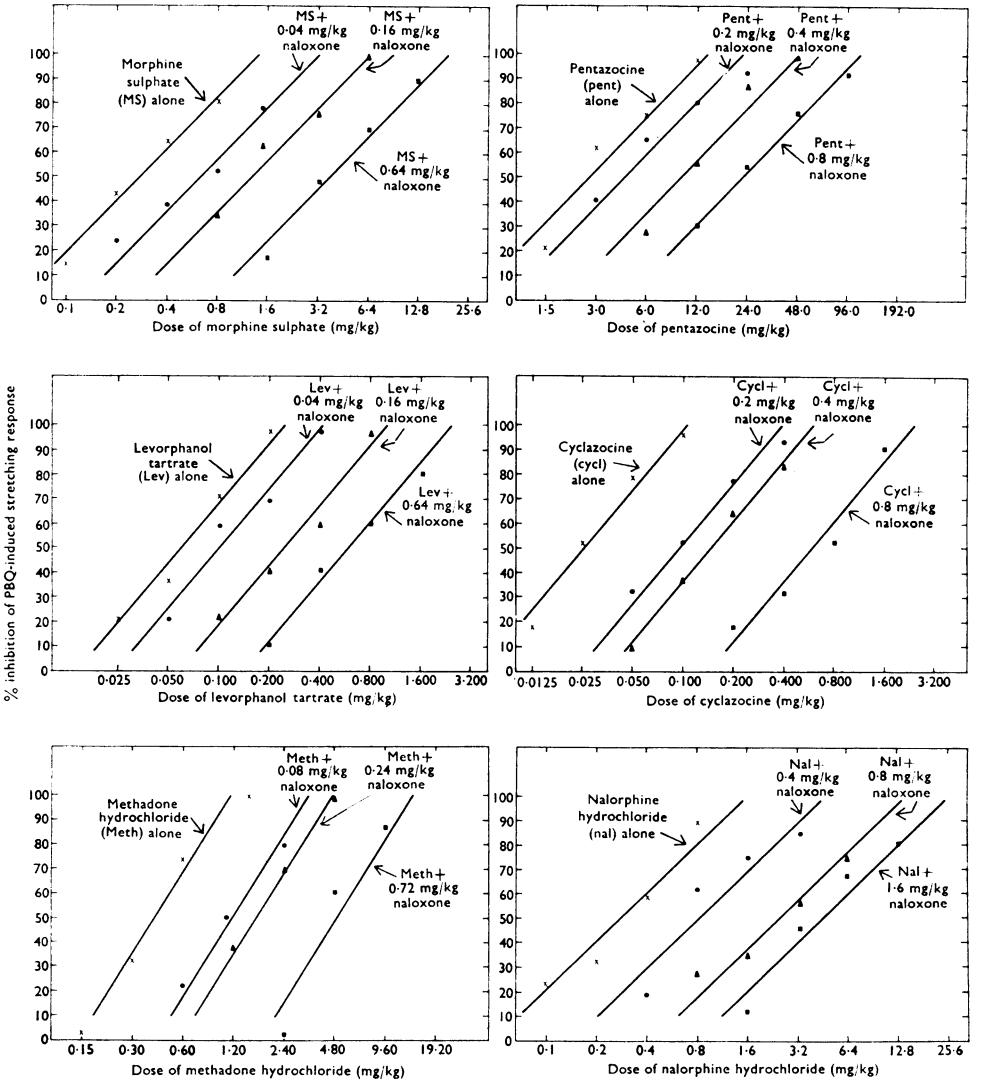

FIG. 1. Dose-response curves for the inhibition of PBQ-induced stretching by some narcotic (left-hand graphs) and narcotic-antagonist (right-hand graphs) analgesics in the absence and presence of various doses of naloxone.

for control mice treated with water and that for mice treated with naloxone, showing that naloxone had no significant agonistic activity in this assay at any of the doses used in this study. This is in agreement with previous reports (Blumberg *et al.*, 1961, 1966).

The three narcotics studied were morphine sulphate, levorphanol tartrate and methadone hydrochloride while the three narcotic antagonists were pentazocine, cyclazocine, and nalorphine hydrochloride. The ED50 and the slope of the dose-response curve for the inhibition of stretching for each drug are shown in Table 1. The range in ED50s is nearly 7-fold for the narcotics and more than 100-fold for the narcotic antagonists. The slopes of the dose-response curves for morphine, levorphanol and the three narcotic antagonists were similar. Only the slope of the curve for methadone differed significantly from that of morphine.

Figure 1 illustrates the shift in the dose-response curves when naloxone is used to antagonize the action of the six analgesics used in the present study. For each drug the first curve represents the inhibition of stretching due to the agonist alone while the remaining three curves were obtained with the agonist plus three different doses of naloxone. Since statistical analysis showed that the dose-response curves for each drug did not deviate from parallelism, all four curves for each drug are drawn parallel using the average slope of the four individual curves. Note that for each narcotic the lowest dose of naloxone was 0·04 mg/kg and that it produced approximately a 2-fold shift in the ED50. For each narcotic antagonist, however, the lowest dose of naloxone which produced approximately a 2-fold shift in the ED50 was either 0·20 mg/kg (for pentazocine and cyclazocine) or 0·40 mg/kg (for nalorphine).

The apparent pA_2 plots for the narcotics and the narcotic antagonists are shown in Fig. 2 and the summary of the results and the statistical analyses are shown in Table 2. The apparent pA_2 values for the narcotic agents were not significantly

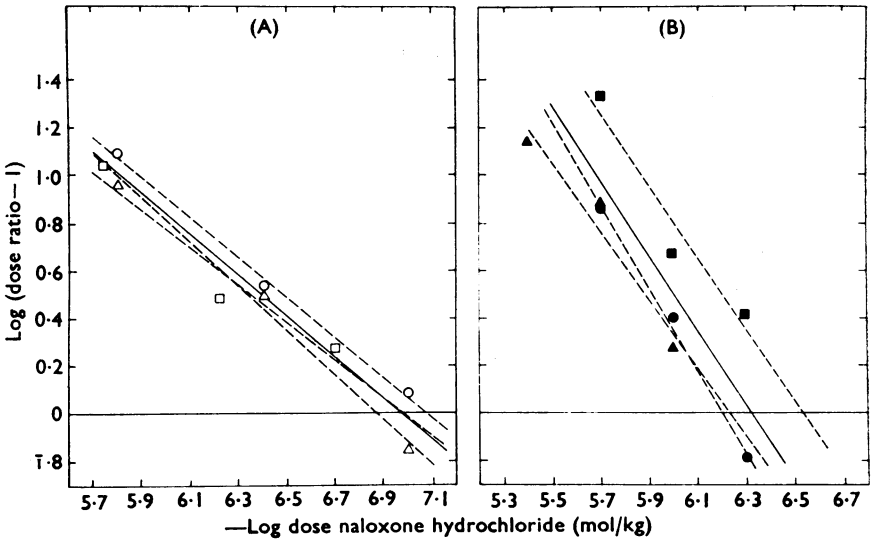

FIG. 2. Apparent pA_2 plots using naloxone and (A) some narcotic analgesics (○, morphine sulphate; □, methadone sulphate; △, levorphanol tartrate; −, average regression line) and (B) narcotic-antagonist analgesics (●, pentazocine; ■, cyclazocine; ▲, nalorphine hydrochloride; −, average regression line).

different from each other and those for the narcotic antagonists were also similar. The average apparent pA_2 for the narcotics was $6{\cdot}98 \pm 0{\cdot}06$ (mean $\pm$ S.E.) while the average for the narcotic antagonists was $6{\cdot}32 \pm 0{\cdot}11$ (mean $\pm$ S.E.). The two groups of analgesics were also distinguished in the apparent pA_2 plots by having different slopes. The slopes for the narcotics and the narcotic antagonists were $-0{\cdot}85 \pm 0{\cdot}04$ and $-1{\cdot}58 \pm 0{\cdot}08$ (mean $\pm$ S.E.), respectively. The differences between the two groups for both the apparent pA_2 values and the slopes were statistically significant.

Peak effect of morphine, pentazocine and naloxone

The slope of the line in a pA_2 plot can be affected by the extent to which equilibrium has been attained between the agonist, the antagonist and the receptor (Arunlakshana & Schild, 1959). To determine whether the difference in slopes for the apparent pA_2 plots of the two groups of analgesics could be explained by a lack of equilibrium in one of the groups, the time for the peak effect was determined for one narcotic and one narcotic-antagonist analgesic. Fig. 3 shows the time for the peak effect of morphine (0·5 mg/kg) and of the antagonism of this effect by naloxone (0·04 mg/kg). It can be seen from the upper curve that the inhibition of PBQ-induced stretching by morphine is greatest at 30 min after its injection. The bottom curve represents the antagonism by naloxone of the inhibition of stretching due to morphine. It was obtained by keeping the time after the injection of morphine constant at 30 min and performing the assay at various times after the injection of naloxone. In all cases the time refers to the interval from the injection of the test drug to the midpoint of the 4 min observation period. The observation period was always begun 5 min after the injection of PBQ as described in **Methods**. The effect of naloxone appears to be maximal between 20 and 30 min. It appears, therefore, that the original apparent pA_2 determination for morphine-naloxone was performed quite close to the time for the peak effect of both drugs.

Fig. 4 shows the results of a similar peak effect estimation using pentazocine (5 mg/kg) and naloxone (0·2 mg/kg). It can be seen from the upper curve that although pentazocine is still quite effective at 30 min, its peak effect occurs between 10 and 20 min. The bottom curve was obtained by keeping the time after the injection of pentazocine constant at 15 min and varying the time after the injection

TABLE 2. *Summary and statistical analysis of the results presented in the apparent pA_2 plots*

Classification	Drug	Slope $\pm$ S.E.*	Mean $\pm$ S.E.*	Apparent pA_2* (95% confidence limits)	Mean $\pm$ S.E.†
Narcotic analgesic	Morphine sulphate	$-0{\cdot}83 \pm 0{\cdot}03$		7·08 (6·86–7·29)	
	Levorphanol tartrate	$-0{\cdot}93 \pm 0{\cdot}05$	$-0{\cdot}85 \pm 0{\cdot}04$	6·87 (6·65–7·10)	$6{\cdot}98 \pm 0{\cdot}06$
	Methadone hydrochloride	$-0{\cdot}79 \pm 0{\cdot}09$		6·98 (6·71–7·24)	
Narcotic-antagonist analgesic	Pentazocine (free base)	$-1{\cdot}74 \pm 0{\cdot}05$		6·20 (6·01–6·40)	
	Cyclazocine (free base)	$-1{\cdot}53 \pm 0{\cdot}12$	$-1{\cdot}58 \pm 0{\cdot}08$	6·50 (6·39–6·60)	$6{\cdot}32 \pm 0{\cdot}11$
	Nalorphine hydrochloride	$-1{\cdot}46 \pm 0{\cdot}10$		6·21 (5·99–6·42)	

* Significant differences in the slopes and apparent pA_2 values were not found within either group of analgesics ($P < 0{\cdot}05$).
† The differences in the means of the slopes and the apparent pA_2 values between the two groups were statistically significant ($P < 0{\cdot}005$).

of naloxone at which the PBQ assay was performed. Although there was good antagonism at 30 min, the peak effect of naloxone occurred at 20 min.

Determination of the apparent pA_2 for pentazocine-naloxone at the time of the peak effect

Because the peak effect of pentazocine occurred before 30 min, the original apparent pA_2 may not have been determined at the time of equilibrium for

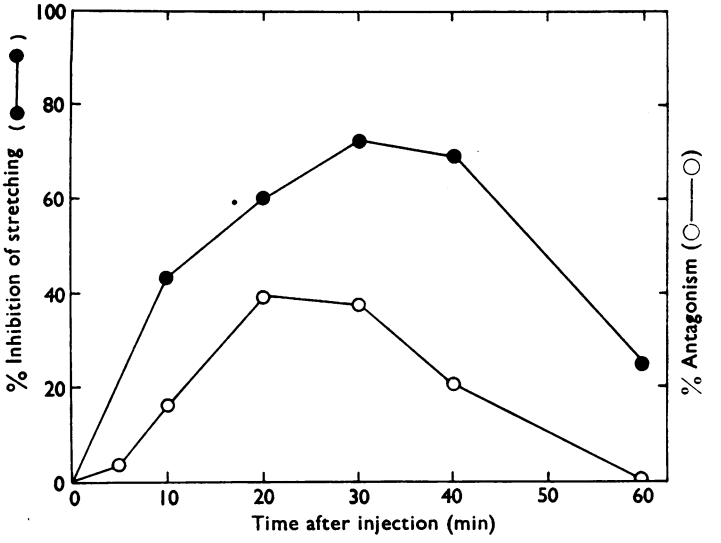

FIG. 3. Time course of the inhibition of stretching by morphine and of the antagonism of this inhibition by naloxone. ●—●, Morphine sulphate (0·5 mg/kg); ○—○, morphine sulphate (0·5 mg/kg)+naloxone (0·04 mg/kg). See text for experimental procedure.

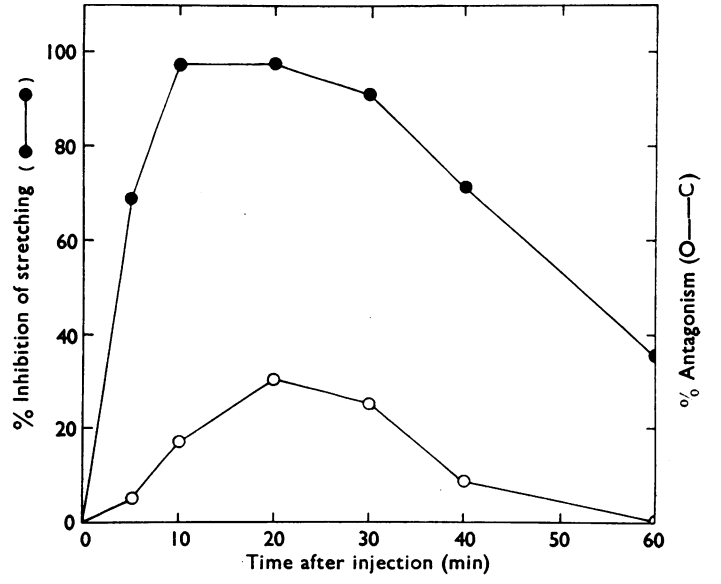

FIG. 4. Time course of the inhibition of stretching by pentazocine and of the antagonism of this inhibition by naloxone. ●—●, Pentazocine (5 mg/kg); ○—○, pentazocine (5 mg/kg)+naloxone (0·2 mg/kg). See text for experimental procedure.

pentazocine, naloxone, and the receptor. The apparent pA_2 was therefore determined again. This time the midpoint of the observation period was at 20 min after the injection of both pentazocine and naloxone. The new ED50 for pentazocine was 1·07 mg/kg with 95% confidence limits of 0·87–1·30 mg/kg. In the apparent pA_2 plot the new slope and S.E. were $-1·60 \pm 0·09$ while the new apparent pA_2 value and 95% confidence limits were 6·39 (6·24–6·54). These values are essentially the same as those obtained in the original experiment.

Further shifting of the dose-response curves for morphine and cyclazocine with high doses of naloxone

It was possible that with increasing doses of naloxone the receptors for morphine might become completely blocked and morphine might begin to combine with the narcotic-antagonist analgesic receptor. In this case the curve for morphine in the apparent pA_2 plot might show some indication of reverting to the curve for the narcotic antagonists. To determine whether the slopes in the apparent pA_2 plot remained constant for morphine and cyclazocine with higher doses of naloxone, additional dose-response curves were obtained for both of these agents.

The dose-response curves for morphine with doses of naloxone at 2·56 and 10·24 mg/kg are shown in Fig. 5. At these doses of naloxone the ED50s for morphine were 20·6 and 86·1 mg/kg with corresponding dose ratios of 75·1 and 313·4. Even with more than a 300-fold increase in the ED50 for morphine there was no significant deviation from parallelism for the dose-response curves.

The dose-response curves for cyclazocine with doses of naloxone at 1·6 and 6·4 mg/kg are shown in Fig. 6. The high doses of cyclazocine needed to overcome these doses of naloxone resulted in mild to moderate ataxia. The ED50s were 1·02 and 2·49 mg/kg. Since the curves did not deviate significantly from parallelism, dose ratios could be validly calculated and were found to be 38·4 and 93·7.

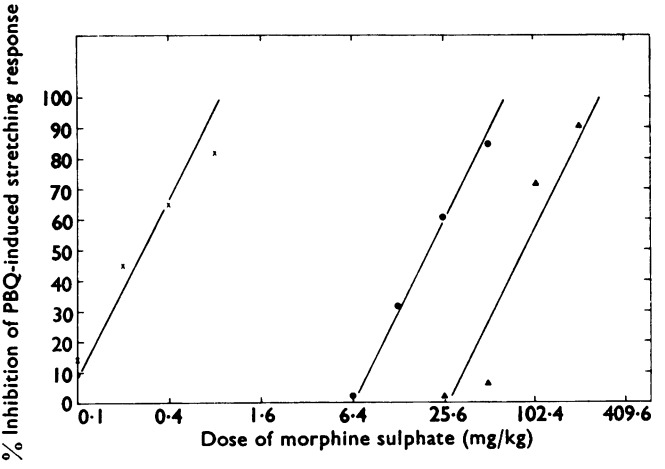

FIG. 5. Effect of high doses of naloxone on the dose-response curve of morphine. ×—×, Morphine sulphate alone; ●—●, morphine sulphate + 2·56 mg/kg naloxone; ▲—▲, morphine sulphate + 10·24 mg/kg naloxone.

Figure 7 shows the apparent pA_2 plots with the results from both the previous experiments with morphine and cyclazocine and the present results using higher doses of naloxone. Although the range of doses of naloxone was very wide (0·04 to 10·24 mg/kg), neither the slope nor the apparent pA_2 for morphine was different from that of the previous study using lower doses of naloxone. This is evidence that morphine was acting on the same receptors over this wide range of doses and that naloxone acted as a competitive antagonist at the doses used. For cyclazocine, however, the two higher doses of naloxone produced shifts in the dose-response curve

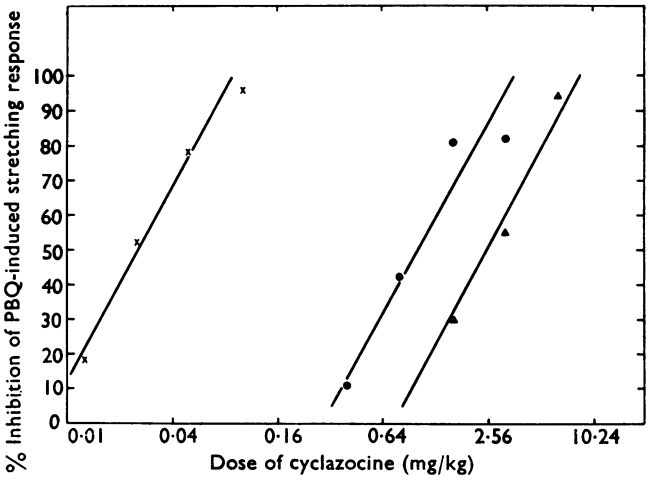

FIG. 6. Effect of high doses of naloxone on the dose-response curve of cyclazocine. ×—×, Cyclazocine alone; ●—●, cyclazocine+1·6 mg/kg naloxone; ▲—▲, cyclazocine+6·4 mg/kg naloxone.

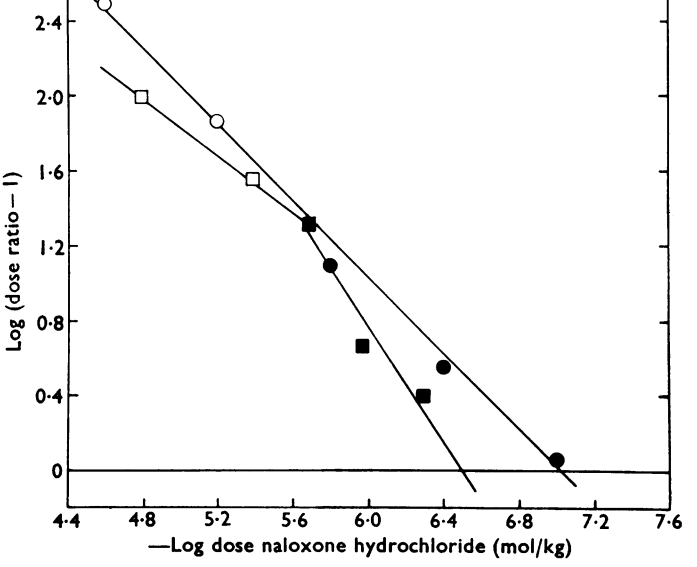

FIG. 7. Apparent pA_2 plots using additional doses of naloxone with morphine and cyclazocine. Morphine sulphate: ●, previous results; ○, high dose results. Cyclazocine: ■, previous results; □, high dose results.

which were relatively smaller than the shifts produced by increments of naloxone at the lower doses. This is manifest in the apparent pA_2 plot by a decrease in the slope. However, as mentioned above, the doses of cyclazocine needed to overcome the larger doses of naloxone resulted in mild to moderate ataxia in the mice. Since the toxic effect of cyclazocine on the number of stretching responses in the PBQ assay is not known, the significance of this change in slope is questionable.

Discussion

The results presented in this paper show that the action of naloxone in antagonizing the effect of different analgesics differs in two respects from the predictions for competitive antagonism (Schild, 1957). In the first place, the slope of the pA_x plot was not $-1{\cdot}0$. For the three narcotics—morphine, levorphanol and methadone—the average slope was $-0{\cdot}85$, which is quite close to the theoretical value, but for the narcotic antagonists—pentazocine, cyclazocine and nalorphine—the average slope was $-1{\cdot}58$. Also the apparent pA_2 value for naloxone tested against the narcotics was higher (6·98) than the value for naloxone tested against the narcotic antagonists (6·32). The pA_2 value for naloxone did not appear to be related to the potency of the agonist. This is in agreement with the results of Blumberg *et al.* (1966) who found that naloxone was equally effective against nalorphine, pentazocine, cyclazocine and cyclorphan in the mouse writhing test.

The differences in the pA_x plots for the two groups of drugs could be explained by : (1) lack of complete equilibrium with some of the drugs ; (2) the existence of two distinct types of receptor for the two classes of analgesic, both of them blocked by naloxone ; (3) a single type of receptor that interacts in different ways with the two classes of analgesics.

We have found that the naloxone-pentazocine interaction did not depend critically on the timing of the experiment, and conclude that lack of complete equilibrium could not satisfactorily account for the anomalous pA_x plots. Martin (1967) has postulated the existence of two receptors, suggesting that morphine-like and nalorphine-like drugs produce their analgesic effects by interacting with different receptors, and that the nalorphine-like drugs also act as antagonists at the morphine receptor. The present results would be consistent with this scheme if it were assumed that naloxone acted as an antagonist at both sites.

Taber, Greenhouse & Irwin (1965) also concluded that morphine and nalorphine exerted their effects by different mechanisms ; but more recently (Taber, Greenhouse, Rendell & Irwin, 1969) reinterpreted these results in the light of findings on the cross-tolerance of nalorphine and morphine, and proposed that both drugs act on the same receptor, as full and partial agonist respectively. The results presented in this paper suggest that the mechanism cannot be as simple as this, for a competitive antagonist should give the same pA_2 value when tested against full and partial agonists acting at the same receptor, whereas we have found a difference of 0·87 between the pA_2 values for naloxone measured against morphine and nalorphine, as well as a marked difference in the slope of the pA_x plots.

It should be noted that a pA_x plot with a slope markedly different from $-1{\cdot}0$ needs to be interpreted with caution. With a curve of the theoretical slope, the pA_2 value gives a measure of the affinity constant for the antagonist (Schild, 1947a), but this is no longer true if the slope is greater or less than $-1{\cdot}0$. Thus the different

pA_2 values for naloxone in the two groups of experiments do not necessarily mean that naloxone is acting at different receptors. In principle, differences in the binding function for the agonists could explain our results without the need for postulating more than one receptor type. In Schild's analysis it is assumed that both agonist and antagonist combine with the receptors according to the Langmuir equation. If, in the present experiments, the nalorphine-like drugs did not obey the Langmuir equation but followed instead some steeper binding function, then the anomalous slope of the pA_x plot could be accounted for.

Other authors (Blane & Dugdall, 1968; Ward, Foxwell & Funderburk, 1965) have interpreted in different ways the interactions between narcotics and their antagonists. It appears at present impossible to say for certain whether there is more than one type of receptor. It seems possible that further quantitative studies using the pA_x analysis will throw more light on these interactions.

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REFERENCES

ARUNLAKSHANA, O. & SCHILD, H. O. (1959). Some quantitative uses of drug antagonists. *Br. J. Pharmac. Chemother.*, **14**, 48–58.

BLANE, G. F., BOURA, A. L. A., FITZGERALD, A. E. & LISTER, R. E. (1967). Actions of etorphine hydrochloride (M99): A potent morphine-like agent. *Br. J. Pharmac. Chemother.*, **30**, 11–22.

BLANE, G. F. & DUGDALL, D. (1968). Interactions of narcotic antagonists and antagonist-analgesics. *J. Pharm. Pharmac.*, **20**, 547–552.

BLUMBERG, H., DAYTON, H. B., GEORGE, M. & RAPAPORT, D. N. (1961). N-allyl-noroxymorphone: A potent narcotic antagonist. *Fedn Proc.*, **20**, 311.

BLUMBERG, H., DAYTON, H. B. & WOLF, P. S. (1966). Counteraction of narcotic antagonist analgesics by the narcotic antagonist naloxone. *Proc. Soc. exp. Biol. Med.*, **123**, 755–758.

BLUMBERG, H., WOLF, P. S. & DAYTON, H. B. (1965). Use of writhing test for evaluating analgesic activity of narcotic antagonists. *Proc. Soc. exp. Biol. Med.*, **118**, 763–766.

COX, B. M. & WEINSTOCK, M. (1964). Quantitative studies of the antagonism by nalorphine of some of the actions of morphine-like analgesic drugs. *Br. J. Pharmac. Chemother.*, **22**, 289–300.

DEKORNFELD, T. J. & LASAGNA, L. (1963). Win 20,740, a potent new analgesic. *Fedn Proc.*, **22**, 248.

FINNEY, D. J. (1964). *Statistical Methods in Biological Assay*, 2nd ed. New York: Hafner Publishing Company.

GREEN, A. F., RUFFELL, G. K. & WALTON, E. (1954). Morphine derivatives with antianalgesic action. *J. Pharm. Pharmac.*, **6**, 390–397.

GREEN, R. D. & FLEMING, W. W. (1967). Agonist-antagonist interactions in the normal and supersensitive nictitating membrane of the spinal cat. *J. Pharmac. exp. Ther.*, **156**, 207–214.

HARRIS, L. S. & PIERSON, A. K. (1964). Some narcotic antagonists in the benzomorphan series. *J. Pharmac. exp. Ther.*, **143**, 141–148.

HENDERSHOT, L. C. & FORSAITH, J. (1959). Antagonism of the frequency of phenylquinone-induced writhing in the mouse by weak analgesics and non-analgesics. *J. Pharmac. exp. Ther.*, **125**, 237–240.

KEATS, A. S. & TELFORD, J. (1956). Nalorphine, a potent analgesic in man. *J. Pharmac. exp. Ther.*, **117**, 190–199.

KEATS, A. S. & TELFORD, J. (1964). Studies of analgesic drugs VIII. A narcotic antagonist analgesic without psychotomimetic effects. *J. Pharmac. exp. Ther.*, **143**, 157–164.

LASAGNA, L. & BEECHER, H. K. (1954). The analgesic effectiveness of nalorphine and nalorphine-morphine combinations in man. *J. Pharmac. exp. Ther.*, **112**, 356–363.

MARTIN, W. R. (1967). Opioid antagonists. *Pharmac. Rev.*, **19**, 463–521.

MARTIN, W. R., FRASER, H. F., GORODETZKY, C. E. & ROSENBERG, D. E. (1965). Studies of the dependence-producing potential of the narcotic antagonist 2-cyclopropylmethyl-6,7-benzomorphan (cyclazocine, Win-20,740, ARC II-C-3). *J. Pharmac. exp. Ther.*, **150**, 426–436.

MARTIN, W. R. & GORODETZKY, C. W. (1965). Demonstration of tolerance to and physical dependence on N-allylnormorphine (Nalorphine). *J. Pharmac. exp. Ther.*, **150**, 437–442.

OKUN, R., LIDDON, S. C. & LASAGNA, L. (1963). The effects of aggregation, electric shock, and adrenergic blocking drugs on the inhibition of the "writhing syndrome". *J. Pharmac. exp. Ther.*, **139**, 107–109.

PARKES, M. W. & PICKENS, J. T. (1965). Conditions influencing the inhibition, by analgesic drugs, of the response to intraperitoneal injections of phenyl-benzoquinone in mice. *Br. J. Pharmac. Chemother.*, **25**, 81–87.

SCHILD, H. O. (1947a). pA, a new scale for the measurement of drug antagonism. *Br. J. Pharmac. Chemother.*, **2**, 189–206.

SCHILD, H. O. (1947b). The use of drug antagonists for the identification and classification of drugs. *Br. J. Pharmac. Chemother.*, **2**, 251–258.

SCHILD, H. O. (1957). Drug antagonism and pA_x. *Pharmac. Rev.*, **9**, 242–246.

SMITS, S. E. & TAKEMORI, A. E. (1969). Quantitative studies on the antagonism by naloxone of the anti-writhing activity of some narcotic and narcotic-antagonist analgesics. *Fedn Proc.*, **28**, 736.

TABER, R. I., GREENHOUSE, D. D. & IRWIN, S. (1964). Inhibition of phenylquinone-induced writhing by narcotic antagonists. *Nature, Lond.*, **204**, 189–190.

TABER, R. I., GREENHOUSE, D. D. & IRWIN, S. (1965). Interaction between narcotic analgesics, narcotic antagonists and other drugs on chemically-induced writhing in mice. *Pharmacologist*, **7**, 164.

TABER, R. I., GREENHOUSE, D. D., RENDELL, J. K. & IRWIN, S. (1969). Agonist and antagonist interactions of opioids on acetic acid-induced stretching in mice. *Pharmac. J. exp. Ther.*, **169**, 29–38.

TAKEMORI, A. E., KUPFERBERG, H. J. & MILLER, J. W. (1969). Quantitative studies on the antagonism of morphine by nalorphine and naloxone. *J. Pharmac. exp. Ther.*, **169**, 39–45.

WARD, J. W., FOXWELL, M. & FUNDERBURK, W. H. (1965). The detection of analgesia produced by morphine antagonists in laboratory animals. *Pharmacologist*, **7**, 163.

WINTER, C. A., ORAHOVATS, P. D. & LEHMAN, E. G. (1957). Analgesic activity and morphine antagonism of compounds related to nalorphine. *Archs int. Pharmacodyn. Thér.*, **110**, 186–202.

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