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Stimulus Effects of Morphine in the Monkey: Quantitative Analysis of Antagonism[1]

JANICE J. TEAL AND STEPHEN G. HOLTZMAN

Department of Pharmacology, Emory University School of Medicine, Atlanta, GA 30322

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TEAL, J. J. AND S. G. HOLTZMAN. *Stimulus effects of morphine in the monkey: Quantitative analysis of antagonism.* PHARMAC. BIOCHEM. BEHAV. 12(4)587–593, 1980.—The ability of narcotic antagonists to block the discriminative stimulus effects of 3.0 mg/kg (IM) of morphine was evaluated quantitatively in the squirrel monkey using a two-choice discrete-trial avoidance paradigm. The time-course and relative potency of naloxone and naltrexone for antagonizing morphine's stimulus effects in the squirrel monkey were similar to those determined in rats and pigeons. Complete blockade of morphine's effects was attained with 0.03 mg/kg of either antagonist when given simultaneously with morphine, but only naltrexone completely blocked the stimulus effects of morphine when the pretreatment interval was extended to 12 and 18 hr. A Schild plot derived from the degree of antagonism of graded doses of morphine by graded doses of naltrexone (0.003–0.1 mg/kg) yielded a line with a slope of -0.63 ± 0.2 and an apparent pA_2 value of 8.25 ± 0.2. These results demonstrate the feasibility of quantitatively assessing the drug-receptor interactions that subserve the discriminative stimulus effects of morphine in the squirrel monkey.

Drug discrimination Discriminative stimulus Naloxone pA_2 Naltrexone Morphine Narcotic antagonist

THE discriminative stimulus effects of morphine have been studied in several different species of animals including the rat [24,25], the squirrel monkey [20], the gerbil [14] and the pigeon [13]. Pure narcotic antagonists such as naloxone and naltrexone will completely block the stimulus effects of morphine in all of these species [3, 13, 14, 36] indicating that opiate receptors mediate the stimulus control of behavior by morphine. Although the stimulus properties of morphine appear to be qualitatively similar in the various species, there are also some dramatic differences, particularly in the quantitative dimension. For example, the duration of the stimulus effects of 3.0 – 6.0 mg/kg of morphine is approximately 180 min in the rat [24] and in the pigeon [13], whereas in the squirrel monkey the stimulus control of behavior by 3.0 mg/kg of morphine persists for as long as 14 hours [20]. The time course of naloxone and naltrexone for antagonizing the discriminative effects of morphine has been studied only in the rat [23] and in the pigeon [13]. In view of the marked disparity in the duration of action of the stimulus effects of morphine in those species as compared to the squirrel monkey, differences in the duration of action of narcotic antagonists among species might also be anticipated. In fact, differences in the urinary excretion profile of naltrexone in primate and rodent species have been reported [5]. Therefore, a principal objective of this study was to determine and compare the durations of action of naltrexone and naloxone in blocking the discriminative stimulus effects of morphine in the squirrel monkey.

Recently, analgesic tests [30,31] and other behavioral techniques such as the shock titration task [38] have been used to assess the interactions of narcotic antagonists and narcotic agonists with opiate receptors. Graded doses of the antagonist are tested for its ability to quantitatively shift the dose response curve of the agonist to the right along the abscissa. A dose ratio analysis, which was originally developed for in vitro assays [2, 21, 22], is used to determine the apparent pA_2 value for the antagonist-agonist pair. The apparent pA_2 value is defined as the negative logarithm of the dose of the antagonist (moles/kg) which reduces the effect of a double dose of an agonist to that of a single dose [1] and is believed to reflect the affinity of the antagonist for the receptor mediating the effects of the agonist [29]. Apparent pA_2 values have been determined in several species of animals for combinations of naloxone and morphine [26, 28, 32, 38], and in mice for diprenorphine and morphine [30]. Another objective of this study was to determine the apparent pA_2 value for the interaction between naltrexone and morphine in the squirrel monkey using a two-choice discrete-trial drug discrimination technique.

METHOD

Animals

The subjects were ten adult squirrel monkeys (*Saimiri sciureus*) of either sex. All animals had been used in other drug discrimination studies [20,35], and had been tested with a variety of narcotic agonists and antagonists. Between experimental sessions, animals were housed in individual cages in a colony room illuminated between 7:00 a.m. and 7:00

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p.m. Purina Monkey Chow-25 and water were continuously available in the home cage.

Apparatus

The apparatus used has been described previously by Schaefer and Holtzman [20]. Briefly, during an experiment each monkey was restrained in a small primate cockpit (No. 142-11, BRS/LVE, Beltsville, MD) which was housed in a light-proof, sound attenuating and ventilated isolation chamber. The monkey's tail was restrained by a Plexiglas stock so that two brass electrodes could be positioned over a shaved portion of the tail. An electric current of constant intensity could be delivered to the tail through the two electrodes without the need for electrode paste. Two levers (No. 121-05, BRS/LVE) were mounted 10 cm apart on the front panel of the test chamber facing the monkey. A Plexiglas barrier with two small arm holes (2.5×4 cm) in the right and left edges separated the monkey from the two levers. In order to press a lever, the monkey has to fully extend its arm through the hole which corresponded to that lever. This arrangement prevented the monkey from pressing both levers simultaneously. All schedule contingencies were programmed and data were recorded by automatic relay equipment.

Procedure

Discrimination training. The monkeys were trained to discriminate 3.0 mg/kg of morphine from saline as described by Schaefer and Holtzman [20]. A monkey was placed in the cockpit and the training drug (3.0 mg/kg of morphine) or saline was injected into the thigh muscle. The cockpit containing the monkey was then placed in a darkened isolation chamber and the first trial of the session began 15 min later. The monkeys were trained in a discrete-trial avoidance paradigm in which the monkey must press one of the two levers in order to avoid or escape an electric shock to its tail. During a training session, only one of the two levers (i.e., the correct lever) was electrically activated to terminate the trial, whereas, a response on the other lever (i.e., the incorrect lever) had no programmed consequences but was recorded as an incorrect response. The illumination of the houselight signaled the onset of a trial. The monkey then had 5 sec to press the correct lever to avoid a 3-mA electric shock which was delivered intermittently to the tail as 1 sec pulses every 2 sec until the monkey emitted the correct response to terminate the trial. Only the first response, correct or incorrect, emitted after the onset of a trial was recorded. A trial was defined as being correct if the first response of the trial was on the correct lever. Trials were separated by a 50-sec intertrial interval (ITI) during which the chamber was dimly illuminated by a yellow stimulus lamp located on the front panel of the test chamber between the two levers. Each response on either lever during the ITI resulted in the delivery of a single 30 msec, 3-mA shock to the monkey's tail. This contingency discouraged continuous lever pressing during a session and helped ensure that responses would be emitted only during a trial. In fact, the most common posture of the monkeys during the ITI was to sit with both arms withdrawn from the openings of the Plexiglas barrier. Sessions ended after 25 trials or 40 min, whichever came first.

The correct lever for a training session was determined by the current drug state of the animal. For four of the ten monkeys, the right lever was the correct lever on days when the animal was administered 3.0 mg/kg of morphine before the session, whereas the left lever was correct on days when saline was administered. The opposite conditions held for the other six monkeys. The lever which was correct for a given monkey during a morphine or saline training session will be referred to as the morphine-appropriate or saline-appropriate lever, respectively.

Monkeys were trained five days a week receiving morphine or saline according to a double alternation schedule (i.e., morphine, morphine, saline, saline, morphine...). After an animal completed four consecutive sessions in which at least 88% of the trials were correct (i.e., 22 out of 25 trials), the next two sessions, one morphine and the other saline, were conducted as test sessions. Test sessions were identical to training sessions except that during test sessions both of the levers were electrically activated so that a trial would always be terminated by the first response of a trial. This was done to verify that the behavior of the animals was under the stimulus control of saline and morphine by minimizing the possibility of within session learning. If a monkey completed both of these test sessions with at least 88% of the trials correct, the animal was said to have acquired the discrimination and could be used in test procedures.

Time-related antagonism of morphine. Test sessions (i.e., both levers electrically activated) were conducted on Tuesdays and Fridays, whereas training sessions (i.e., only one lever electrically activated) with morphine and saline administered on a double alternation schedule were conducted on Mondays, Wednesdays and Thursdays to maintain stable behavioral performance. During training sessions, a monkey was injected first with saline and then was returned to its home cage for 0–6 hr. After the interval had elapsed, the monkey was placed in the test chamber and injected with either the training drug or saline; 15 min later the session began. If an animal did not complete at least 88% of the trials of a training session on the correct lever, a training session was conducted in place of the next scheduled test session. All doses of the test drugs were given in a random sequence that also included saline + saline and saline + 3.0 mg/kg of morphine. During test sessions, the pretreatment times for the antagonists and saline controls ranged from 0–18 hours. With the exception of the group pretreated at 0 hr, the animals were returned to their home cages during the pretreatment interval. After the pretreatment time had elapsed, the animals were placed in the test chamber and injected with 3.0 mg/kg of morphine or saline (saline + saline control group); this was defined as time 0. The test session began 15 min after the second injection. For the zero hour pretreatment group, both the antagonist (or saline) and morphine (or saline) were administered into different intramuscular sites 15 min before the session began. Because 3.0 mg/kg of morphine can produce prominent respiratory depression in the squirrel monkey, 0.4 mg of naltrexone was routinely administered to the animals following any session in which morphine had been administered.

Effect of naltrexone on the morphine dose-response curve. Dose-response curves for morphine were determined in six monkeys in the presence of saline and four different doses of naltrexone. Test sessions were conducted in a manner similar to the morphine antagonism tests except that morphine (or saline) and the antagonist naltrexone (or saline) were both always injected 30 min before the onset of the first trial of the session. After drug administration, the animals were returned to their home cage for 15 min. The animals were then restrained and placed in the darkened test chamber for the remainder of the pretreatment interval.

Training days were conducted as usual with either the training dose of morphine or saline being injected 15 min before the onset of the first trial with the monkey remaining restrained in the test chamber during this time.

Since this study used doses of morphine which would cause a fatal respiratory depression in the monkeys during the pretreatment interval, the test dose of naltrexone had to be administered simultaneous with morphine. However, a 30-min pretreatment time still allowed both drugs to be tested during their peak activity [8, 19, 20]. The order of determining the five dose-response curves was counterbalanced for the six monkeys with the exception of 0.1 mg/kg of naltrexone which was tested last in all animals. Control injections of saline + saline and 3.0 mg/kg or morphine + saline were included in every other drug series and the order of testing the various doses of morphine and the controls was randomized within each series.

Data analysis. Antagonism of the discriminative stimulus effects of morphine was considered to have occurred if the average number of trials completed on the morphine-appropriate lever during a test session was less than 22. Complete antagonism of the discriminative stimulus effects of morphine was defined as the completion of an average of 3 or less trials on the morphine-appropriate lever (i.e., responding appropriate for the saline condition).

Regression lines for the antagonism of morphine's discriminative stimulus effects by the two antagonists were calculated for each pretreatment time by the method of least-squares [27]. Since multiple observations were not made at the 100% response level or at the 0% response level, all points were used in the regression analyses. These lines were used to determine the antagonism dose-50 (AD_{50}) for both antagonists at each pretreatment time. The AD_{50} was defined as the dose of the antagonist which decreased the mean number of trials completed on the morphine appropriate lever to 12.5. The regression lines were also analyzed for a correlation coefficient (r) and a standard error of the estimate which was used to calculate the 95% confidence limits of the slope (b in the slope intercept equation) of the regression line [6]. Lines with slopes that had overlapping confidence limits were considered not to differ significantly from parallelism.

The apparent pA_2 for naltrexone was determined as described by Smits and Takemori [26]. Regression lines for each of the five morphine dose-response curves were determined and analyzed as described above. From these lines, the effective dose −50 (ED_{50}) was determined for morphine. The ED_{50} was defined as the dose of morphine required to engender a mean of 12.5 trials completed on the morphine-appropriate lever. The ED_{50} values were used to calculate the dose ratio for each dose of naltrexone by dividing the ED_{50} of morphine in the presence of a given dose of naltrexone by the ED_{50} of morphine in the presence of saline (i.e., in the absence of antagonist). The log of *the dose ratio minus one* was plotted on the ordinate and the negative log of *the dose of naltrexone in moles/kg* was plotted on the abscissa. A regression line was determined for these points and the slope and pA_2 value were calculated. The pA_2 value is defined as the value where the dose ratio equals 2 (i.e., the x-intercept. The standard error was determined by the small sample method [11].

The data are presented as the mean (n=3–6) number of trials completed on the morphine-appropriate lever. The remaining trials of the 25-trial session were completed on the saline-appropriate lever.

Drugs. The following drugs were used in this study: morphine sulfate (S. B. Penick and Company, Newark, NJ), naloxone hydrochloride and naltrexone hydrochloride (National Institute on Drug Abuse). The drugs were dissolved in 0.9% saline and injected into the thigh muscle in a volume of 0.5 ml/kg of body weight. Drug doses are expressed in terms of the free base.

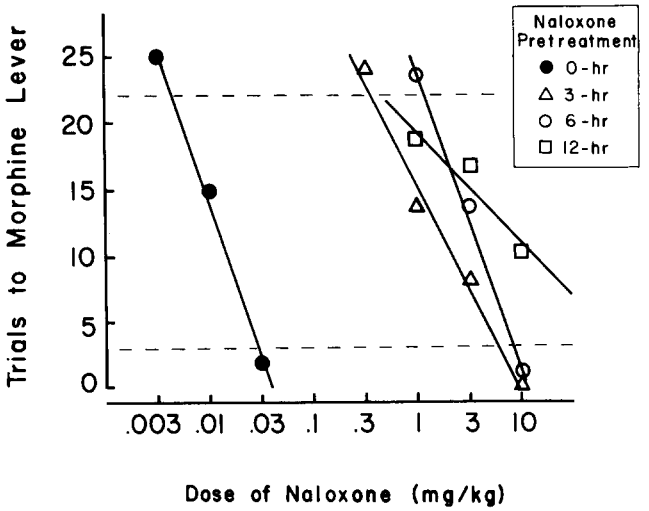

FIG. 1. Effects of graded doses of naloxone given in combination with the training dose of morphine in squirrel monkeys trained to discriminate 3.0 mg/kg of morphine from saline. Animals were pretreated with naloxone 0,3,6, or 12 hours before morphine was administered; 15 min after morphine was administered, the first trial of the test session started. Each point is the mean number of trials completed on the morphine-appropriate lever in a 25-trial session; the remaining trials of the session were completed on the saline-appropriate lever. Means are based upon one observation in each of 4 monkeys except at 0-hr where n=3. Linear regression lines connect the points for each pretreatment time. The correlation coefficients for the regression lines ranged from −0.958 to −0.999. The upper and lower horizontal dashed lines indicate the minimum levels of discriminative responding at which the performance of the monkeys was maintained with the morphine-training dose and saline, respectively.

RESULTS

Antagonism by Naloxone

Figure 1 shows the dose-response curve for antagonism of the stimulus effects of 3.0 mg/kg of morphine by graded doses of naloxone administered at 0, 3, 6 or 12 hr before morphine. When naloxone and morphine were administered concomitantly (i.e., zero hour pretreatment), 0.003 mg/kg of naloxone had no effect on the discriminative stimulus effects of morphine. However, the discriminative stimulus effects of morphine were partially blocked by 0.01 mg/kg of naloxone and completely blocked by 0.03 mg/kg. The potency of naloxone as an antagonist diminished rapidly over time. With a 3-hr pretreatment interval, 0.3 mg/kg of naloxone failed to reduce the stimulus effects of morphine, and only partial antagonism was achieved with 1.0 and 3.0 mg/kg. Complete antagonism of morphine's effects required 10 mg/kg of naloxone, a dose two orders of magnitude higher than that needed when the two drugs were administered together just before the session. The potency of naloxone at 6 hours was only slightly less than that at 3 hours, but by 12 hours, even 10 mg/kg of naloxone produced only a partial

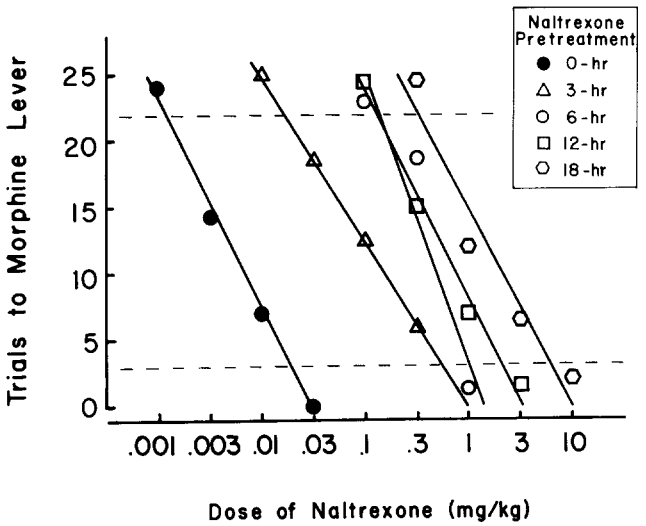

FIG. 2. Effects of graded doses of naltrexone given in combination with the training dose of morphine in squirrel monkeys trained to discriminate 3.0 mmg/kg of morphine from saline. Animals were pretreated with naltrexone 0,3,6,12 or 18 hours before morphine was administered; 15 min after morphine was administered, the first trial of the test session started. Means are based upon one observation in each of 4 monkeys except at 0-hr where n=3. The correlation coefficients for the regression lines ranged from −0.953 to −0.999. Other details are the same as in Fig. 1.

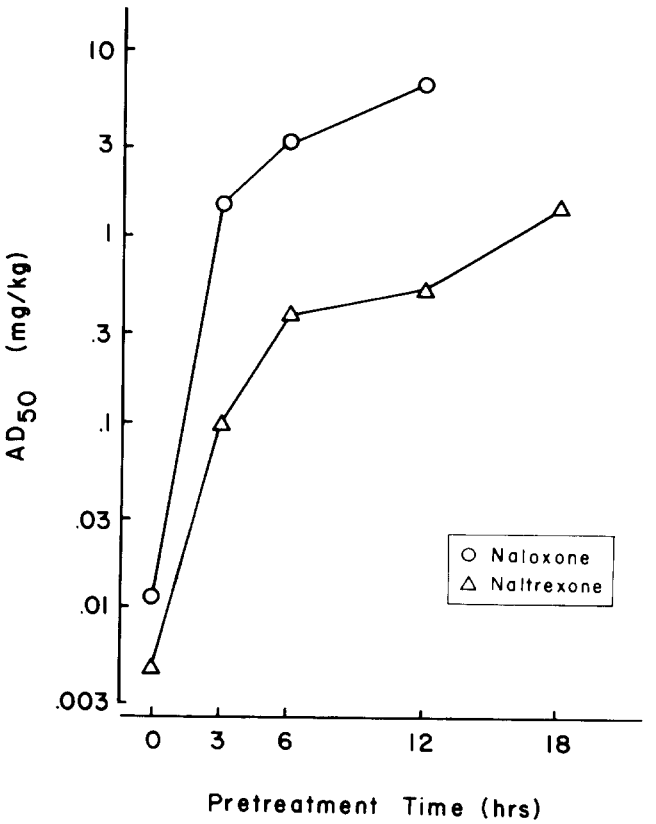

FIG. 3. The AD_{50} value for the antagonism of the discriminative effects of the training dose of morphine by naloxone and naltrexone as a function of the pretreatment time of the antagonist. The dose of naloxone or naltrexone which decreased the mean level of morphine-appropriate responding engendered by 3.0 mg/kg of morphine by 50% (i.e., 12.5 trials) was calculated for each pretreatment time from data in Figs. 1 and 2. Note that the AD_{50} values are plotted along a log scale on the ordinate.

blockade of the stimulus effects of morphine. Higher doses of naloxone produce nonspecific effects in the monkey such as excessive salivation and vomiting [10,12], and, consequently, were not tested. Two control tests were conducted for each pretreatment time interspersed with the test drugs; saline was administered instead of naloxone and after the appropriate pretreatment time either 3.0 mg/kg of morphine or saline was administered. The results of these tests indicated that the animals' behavior was under the stimulus control of the training drugs throughout these experiments (data not shown).

The slopes of the regression lines for the 0-, 3-, 6- and 12-hr pretreatment intervals were −23.25, −15.42, −22.52 and −8.366, respectively. Confidence limits of these slopes could not be determined by the statistical procedures described in the methods due to the small number of points in each line. However, based on the statistical analysis of data from the naltrexone time-course experiment (see below), the lines for the 0-, 3- and 6-hour pretreatments would appear to be parallel.

Antagonism by Naltrexone

Antagonism of the discriminative stimulus effects of morphine by naltrexone is shown in Fig. 2. With zero hour pretreatment, 0.001 mg/kg of naltrexone did not reduce the discriminative stimulus effects of morphine. The 0.003 mg/kg dose of naltrexone did partially antagonize the discriminative stimulus effects of morphine, whereas this same dose of naloxone had no effect. However, the dose of naltrexone needed to completely antagonize morphine's effects, 0.03 mg/kg, is comparable to the results obtained with naloxone. The morphine antagonist activity of naltrexone decreased over time much more slowly than did that of naloxone. Nearly complete antagonism of the stimulus effects of morphine was achieved with 0.3 mg/kg of naltrexone at the 3-hr pretreatment time, and complete antagonism of the stimulus effects of morphine was obtained with 1.0, 3.0, and 10 mg /kg of naltrexone at pretreatment times of 6, 12 and 18 hours, respectively. The slopes of the regression lines for the five pretreatment intervals did not significantly differ from each other with the slope for the zero-hour pretreatment being −15.98. Control tests in which saline + saline or saline + 3.0 mg/kg of morphine were administered were conducted for each pretreatment interval in a manner identical to those for the naloxone antagonism study.The results of these tests confirmed that the animals' behavior was under stimulus control throughout the experiments (data not shown).

AD_{50} comparisons. The dose of naloxone and naltrexone that produced a 50% antagonism of the discriminative stimulus effects of morphine (AD_{50}) was determined for each pretreatment time (Fig. 3). At time zero, naltrexone is only 2 times more potent than naloxone. However, at 3 hours the difference in potency becomes maximal with naltrexone being 16 times more potent than naloxone. At 6 and 12 hours naltrexone is 8–13 times more potent than naloxone in antagonizing the discriminative stimulus effects of morphine. Clearly, the most dramatic decreases in potencies of both

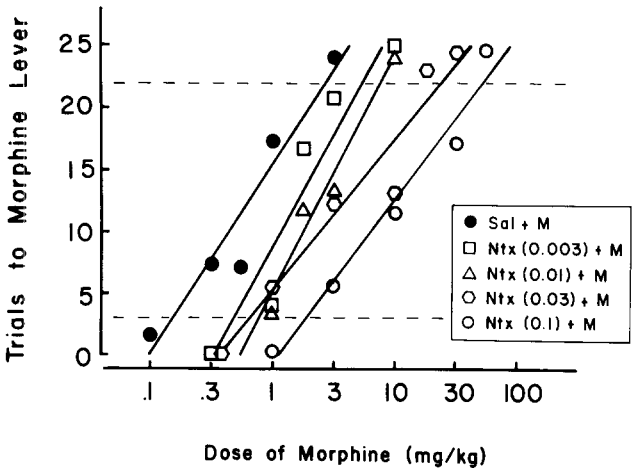

FIG. 4. Morphine dose-response curves in the presence of four different doses of naltrexone or saline in squirrel monkeys trained to discriminate saline from 3.0 mg/kg of morphine. Each point represents a mean based upon one observation in each of six monkeys; the same six monkeys were used for all of the determinations. The correlation co-efficients for the regression lines ranged from 0.945 to 0.986. Other details are the same as in Fig. 1.

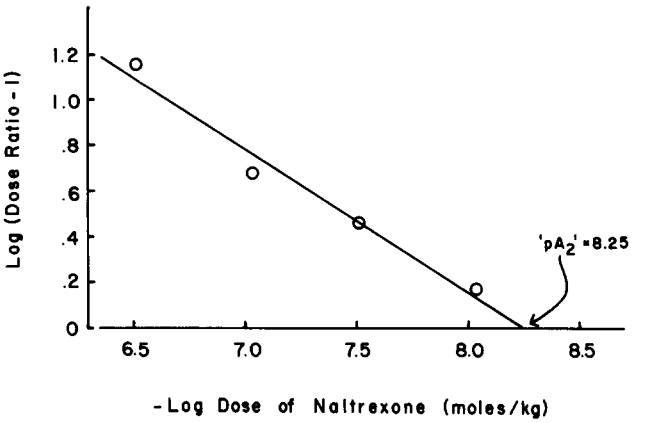

FIG. 5. Schild plot of antagonism of the discriminative stimulus effects of morphine by naltrexone. The negative log of the dose of naltrexone in moles/kg is plotted on the abscissa and the log of *the dose ratio-1* is plotted on the ordinate. Points were calculated from the AD_{50} values in Fig. 4. The slope of the linear regression line was -0.63 ± 0.2 and the apparent pA_2 value was calculated to be 8.25 ± 0.2.

naltrexone and naloxone occur within the first three hours. However, this decrease occurs much faster for naloxone.

Morphine dose-response curves in the presence of naltrexone. Graded doses of morphine were tested in the presence of saline and four different doses of naltrexone (0.003–0.1 mg/kg) to determine the extent to which naltrexone would shift the morphine dose-response curve to the right along the abscissa (Fig. 4). In all five determinations morphine engendered dose-related morphine-appropriate responding which resulted in complete substitution for the training dose. When morphine was tested in the presence of saline an ED_{50} value of 0.63 mg/kg was obtained; the slope of the regression line was 15.55. As the dose of naltrexone was increased, the morphine dose response curve was shifted in a parallel manner progressively further to the right along the abscissa. In the presence of 0.003 mg/kg of naltrexone, the ED_{50} for morphine and the lowest dose of morphine which substituted for the training dose of morphine in the absence of the antagonist were increased to 1.6 and 5.6 mg/kg, respectively. The morphine dose-response curve was shifted progressively further to the right by increasing the dose of naltrexone, with ED_{50} values of 2.5, 3.6, and 9.7 mg/kg, at naltrexone doses of 0.01, 0.03 and 0.1 mg/kg, respectively. In combination with the highest dose of naltrexone, 56 mg/kg of morphine was required to restore stimulus control of behavior to the level produced by morphine in the absence of the antagonist. Results from control tests with saline + saline and saline + 3.0 mg/kg of morphine administered 30 min before the session indicated that the animals' behavior was under stimulus control (data not shown).

Apparent pA_2 value for naltrexone. Using the ED_{50} values determined for morphine in the presence of saline or one of the four doses of naltrexone, a Schild plot [21,22] was constructed to determine the apparent pA_2 for naltrexone (Fig. 5). The negative log of the dose of naltrexone in moles/kg was plotted against the log of the *dose ratio-1*. The linear regression line calculated for these points intercepts the X-axis at 8.25 ± 0.2 which is the pA_2 value (i.e., where the dose ratio=2). The slope of the line was calculated by the slope intercept equation to be -0.63 ± 0.2.

DISCUSSION

The relative potency and time-course of naloxone and naltrexone for antagonizing the discriminative stimulus effects of morphine in the squirrel monkey were found to be similar to those determined in discrimination studies in the rat [23] and in the pigeon [13], as well as in other procedures in other species [7, 9, 17]. Naltrexone was found to be longer acting than naloxone with the most dramatic decrease in potency of the two antagonists occurring within the first three hours after administration. Naltrexone could still completely antagonize the training dose of morphine after 18 hours although the dosage had to be increased by a factor of 333 over the zero hour dose. In contrast, naloxone failed to completely block the same dose of morphine after only 12 hours.

The stimulus generalization curve for morphine was shifted progressively to the right by graded doses of naltrexone, indicating a competitive interaction. The highest dose of naltrexone, 0.1 mg/kg, shifted the curve for morphine to the right by a factor of 15, whereas this same dose of naloxone produced a 10-fold shift to the right of the morphine dose-response curve in a comparable study [20].

Another way to evaluate the interaction between an agonist and an antagonist is by the determination of an apparent pA_2 value. The apparent pA_2 value is believed to reflect the antagonist's affinity for the receptor mediating the effects of the agonist and has become a useful way of comparing different groups of opioid antagonists [26,38]. However, this determination is based on several assumptions the validity of which is not easily determined in vivo. Both the agonist and the antagonist must be tested at their peak activity, must be interacting in a completely competitive manner according to the Langmuir equation, and the concentration of the drugs at the receptor must be directly proportional to the doses administered [1,26]. If any of these assumptions are not met the slope of the Schild plot could vary from the theoretical slope of -1.0. This may have been the case in the

present study since the slope of the Schild plot was calculated to be -0.63 ± 0.2. However, it is not uncommon to find reports of Schild plots with slopes that vary significantly from -1.0; apparent pA_2 values derived from these plots appear to be readily reproducible across different species as well as from laboratory to laboratory (i.e., [26,38]). Nonetheless, a pA_2 value based on a Schild plot whose slope differs significantly from -1.0 needs to be interpreted with caution.

An apparent pA_2 of 8.25 ± 0.2 was calculated for antagonism of the discriminative effects of morphine by naltrexone. This value is higher than the apparent pA_2 value obtained for diprenorphine (7.73 ± 0.12) and naloxone (7.07 ± 0.09) to antagoize morphine's analgesic effects in mice [30] and for naloxone (7.16 ± 0.09) to antagonize morphine's effect on the shock titration task in rhesus monkeys [38]. Since the apparent pA_2 is believed to reflect the affinity of the antagonist for the receptor, the differences in the pA_2 values for these three antagonists should be due to their different affinities for the opiate receptor. This idea is supported in part by studies showing that the binding of diprenorphine and naltrexone in rat brain is 3 times greater than the binding of naloxone [4]. Further, both of these antagonists are more potent than naloxone in antagonizing the effects of morphine in the guinea pig ileum assay and in precipitating withdrawal in morphine dependent rhesus monkeys [34]. However, these studies do not provide data which would support the finding that naltrexone's apparent pA_2 value was higher than that observed for diprenorphine.

Several investigators [8, 33, 37] found that in the mouse morphine pretreatment caused the apparent pA_2 value of morphine and naloxone for analgesia to increase. This apparent increase in receptor affinity for naloxone is believed to be associated with the early stages of the development of tolerance and physical dependence [15]. Since the monkeys received morphine 2–4 times per week in training and test sessions during the course of the study, it is possible that a mild degree of tolerance had developed which could account for the higher pA_2 value. Naltrexone was administered following each morphine-session to prevent respiratory depression. The concurrent administration of a narcotic antagonist and morphine will usually prevent the development of tolerance to morphine [16]. However, since morphine has an extremely long duration of action in the squirrel monkey [20], residual amounts of morphine could still have been present after the effects of naltrexone had dissipated resulting in the development of a low level of tolerance which, in turn, would have inflated the apparent pA_2 value.

Species differences might also account for the high apparent pA_2 value in this study. To our knowledge, pA_2 values for opioids have not been reported for the squirrel monkey. Although apparent pA_2 values for other antagonists will have to be determined in the squirrel monkey in order to resolve some of these issues, this study has demonstrated the feasibility of quantitatively assessing the drug-receptor interactions that subserve the discriminative stimulus effects of morphine in a primate.

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