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The Synergistic Effect of Concurrent Spinal and Supraspinal Opiate Agonisms Is Reduced by Both Nociceptive and Morphine Pretreatment[1]

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SIUCIAK, J. A. AND C. ADVOKAT. *The synergistic effect of concurrent spinal and supraspinal opiate agonisms is reduced by both nociceptive and morphine pretreatment.* PHARMACOL BIOCHEM BEHAV **34**(2) 265–273, 1989.—The antinociceptive effect of morphine administered into the periaqueductal gray (PAG), the intrathecal space (ITH) and concurrently, into both sites (in a 1:1 dose ratio), was assessed in 1) nontolerant rats, 2) rats made tolerant to the effect of morphine on the tail-flick (TF) test and 3) rats that were tested on the TF during chronic saline administration. In nontolerant rats, concurrent morphine injections produced a multiplicative antinociceptive effect ($ED_{50} = 0.392$ μg, total dose) relative to that obtained after separate PAG ($ED_{50} = 2.8$ μg) or ITH ($ED_{50} = 6.7$ μg) injections. The multiplicative effect of concurrent morphine administration was significantly reduced in rats made tolerant to morphine (one 3 mg/kg SC injection and TF test per day for six days). Opiate synergy was also reduced but to a smaller extent in rats that were repeatedly tested on the TF during chronic saline administration (one SC injection and TF test per day for six days). Neither chronic morphine nor saline pretreatment altered the dose-response function to intrathecal morphine. However, both morphine and saline pretreatment significantly reduced the antinociceptive effect of morphine administered into the PAG. The data indicate that concurrent morphine administration into the PAG and ITH space results in a synergistic antinociceptive action which is reduced by performance of the nociceptive response, even in the absence of opiate administration. We suggest that the decrease in opiate synergism produced by nociceptive assessment (behavioral tolerance) is mediated supraspinally, while the additional decline resulting from morphine administered in conjunction with the nociceptive tests (opiate tolerance) is mediated by a combined action at spinal and supraspinal sites.

Opiate analgesic synergy Morphine analgesic tolerance Periaqueductal gray Intrathecal morphine Tail flick

IT is well established that morphine can induce analgesia through both a direct action at the spinal cord (55, 57, 60) and an action at supraspinal sites (59). However, the complex relationship between these two sites, with respect to analgesia and tolerance produced by systemically administered opiates, is not well understood. In their attempt to clarify this relationship, Yeung and Rudy (62) found that concurrent injection of morphine onto the spinal cord and into the third cerebral ventricle of rats produced a multiplicative antinociceptive effect, which was maximal when equivalent doses of morphine were injected into both sites (1:1 ratio). These investigators proposed that the synergistic effect was responsible for the analgesia observed following systemic morphine administration.

In subsequent studies, Fujimoto and associates adapted this paradigm to assess opiate tolerance in mice (40). They demonstrated that tolerance, induced by morphine pellet implants, reduced the multiplicative interaction to an additive relationship. They suggested that the expression of tolerance to systemic morphine involved a decrease in the multiplicative interaction between the spinal cord and the brain.

Although the mechanism responsible for opiate synergy is unknown, there is support for the observation that tolerance to systemic morphine does not always confer tolerance to either spinal or supraspinal morphine administration. Prior studies showed that rats made dependent by scheduled access to a morphine solution (13) and mice made dependent by morphine pellet implants (28, 29, 36) are not tolerant on the tail withdrawal test to intracerebroventricular (ICV) morphine injections. Similarly, it has been demonstrated that tolerance to systemic morphine administration does not alter the antinociceptive effect of spinal morphine on the TF in mice or rats [(40, 45, 51), see also (5)].

There is at present no satisfactory explanation for the lack of tolerance to morphine administered to spinal or supraspinal sites in animals made tolerant to systemic morphine. To examine this question, we incorporated the multiplicative paradigm into our ongoing investigations of opiate tolerance. In designing these experiments, we noted that in previous studies, cited above, animals were made dependent on, as well as tolerant to systemic

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morphine prior to the evaluation of tolerance at a specific site. As a result, it is possible that tolerance to a spinal or supraspinally administered challenge was not observed because of the elevated concentration of morphine in the brains and spinal cords of dependent animals (13). This interpretation is consistent with the fact that tolerance to ICV or spinal morphine is obtained when animals are assessed after the termination of chronic opiates when levels are presumably declining (29, 36, 38, 41). The expression of tolerance at central sites under these conditions has been termed "withdrawal tolerance" (29) and may reflect processes that differ from tolerance elicited when animals are in the dependent state. Moreover, in their analysis of opiate synergism, Yeung and Rudy (62) acknowledged that, at sufficiently high doses of systemic morphine, pure spinal or supraspinal agonisms may mediate analgesia independently of the multiplicative effect. For these reasons, a moderate dose regimen was used to induce tolerance in the present experiments, and the final assessment occurred 24 hours after the last tolerance session.

However, tolerance induced by moderate doses of morphine can be profoundly altered by environmental contingencies (1, 8, 27). Numerous investigations have shown that tolerance to opiate antinociception is greater when animals are tested while under the influence of the drug, relative to animals who receive chronic drug exposure without being tested [for reviews see (7, 18, 34)]. Although the majority of these studies have used supraspinally-mediated responses such as the hot-plate assay, facilitation of tolerance has also been demonstrated with the spinally-mediated tail withdrawal reflex (2). Therefore, in order to determine the possible contribution of the nociceptive procedure on opiate synergy, the present studies included additional groups that received saline instead of morphine during the tolerance-inducing test sessions.

Finally, the generality of the multiplicative phenomenon was extended to another supraspinal site, the periaqueductal gray (PAG). This site, rather than the third ventricle, was chosen because numerous investigations have supported the role of the PAG in opiate antinociception (20, 22, 59, 61). Most significant for the present studies is the fact that injection of morphine into this site produces a total blockade of the tail-flick reflex, whereas injection into other brain loci does not (26), although this specificity has recently been questioned (12).

The results obtained in the present experiments demonstrate that opiate synergy can be elicited by concurrent administration into the PAG and onto the spinal cord. The results further suggest that this synergism is reduced by repeated performance of the nociceptive response, even in the absence of morphine, and that the decline in opiate synergy may be greater when morphine was administered in conjunction with the nociceptive tests. Additional studies showed that neither opiate nor nociceptive pretreatment altered the antinociceptive effect of intrathecal morphine. However, both of these treatments produced tolerance to a subsequent PAG challenge. The results are discussed in terms of the possible mechanisms responsible for opiate synergy and the respective effects of pharmacological and behavioral tolerance on these processes.

METHOD

Subjects

Male albino Sprague-Dawley derived rats (King Labs, Oregon, WI) weighing 300–350 grams were used as subjects. After surgery they were housed individually so that cannulae and catheters would not be damaged by cagemates. All animals had continuous access to food and water throughout the experiments.

Spinal Catheterization and Intrathecal Injections

The surgical technique was adapted from the method of Yaksh and Rudy (58). Rats were anesthetized with ether and placed in a stereotaxic frame. An incision was made behind the ears and the neck muscles were scraped to expose the back of the skull. An incision of the atlanto-occipital membrane allowed insertion of an 8-cm catheter of PE-10 polyethylene tubing filled with sterile saline into the spinal subarachnoid space. The catheter was held in place against the skull with adhesive. The incision was closed and the exposed tip of the catheter was heat sealed. Any animal that was crippled after surgery was immediately sacrificed.

For intrathecal administration, morphine sulfate was injected in a volume of 10 μl of sterile saline followed by a 10 μl wash of saline. When spinal injections were made the tip of the catheter was cut and then resealed after drug administration.

Cannulae Implantation and Microinjection

Rats were anesthetized with ketamine (100 mg/kg), placed in a stereotaxic frame, and implanted with a single guide cannula aimed at the periaqueductal gray (PAG). Stereotaxic coordinates were 5.0–6.0 mm posterior to bregma, 0.80 mm lateral to the sagittal suture and 4.0 mm below the dura (37, 45, 61). Each guide cannula was made of 24-gauge stainless steel tubing, 15 mm in length, and was kept patent with a stainless steel stylet. The guide assembly was attached to the skull with stainless steel screws and dental cement. Seven days were allowed for recovery from surgery before microinjections and testing were initiated.

Drug solutions were microinjected through a 32-gauge stainless steel injection cannula inserted through and projecting 2 mm past the guide tube. The injection cannulae were attached, through a length of PE-10 polyethylene tubing, to a gear-driven 10 μl Hamilton syringe. Fluid delivery was monitored by following the movement of a small air bubble placed in the polyethylene tubing. Injection volume was 1.0 μl to minimize diffusion and tissue damage. The injections were performed over an interval of about 60 sec, and the injector cannula was retained in place for another 60 sec so as to prevent fluid from being drawn back into the shaft of the cannula.

Drugs

Morphine sulfate (Merck, Rahway, NJ) was dissolved in sterile saline. For subcutaneous administration, concentrations were adjusted so that the injection volume was 1 ml/kg. For intracerebral and intrathecal administration, solutions were made such that the respective injection volumes contained the appropriate concentration.

Analgesiometric Test

The nociceptive procedure is derived from the method of D'Amour and Smith (15). Noxious stimulation was produced by a beam of high intensity light focused on the tail. The response time was measured automatically and was defined as the interval between the onset of the heat stimulus and the movement of the tail out of the light beam. The average of three consecutive determinations, taken in immediate succession, was assigned as the response latency. In order to minimize tissue damage, a different patch of skin was stimulated on each trial. The trial was terminated if the animal failed to respond within 14 sec, and the animal was assigned a 14-sec latency. Baseline TF latencies among the groups ranged from 3.88 to 4.98 sec. All tail-flick responses were converted to percent maximal measurable effect (%M.M.E.) for graphic representation and statistical analysis:

$$\text{MME} = \frac{\text{postdrug latency} - \text{predrug latency}}{\text{maximum latency (14 sec)} - \text{predrug latency}} \times 100$$

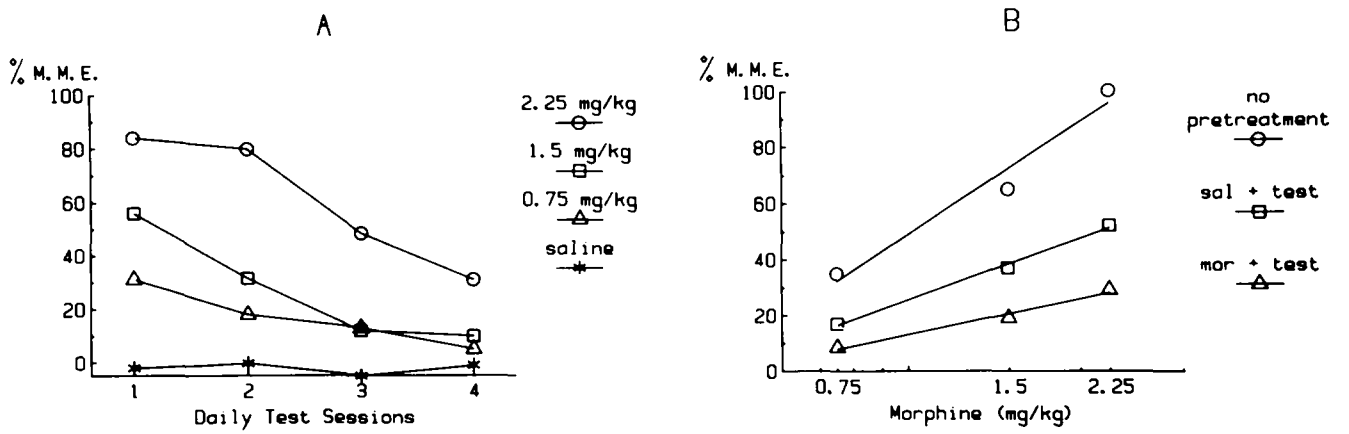

FIG. 1. (A) The development of tolerance to the antinociceptive effect of morphine on the tail-flick withdrawal reflex. Separate groups of rats received one of the indicated doses of morphine or saline. Each rat was tested before and 40 min after the respective injection on each of four successive days. (B) Effect of prior morphine administration plus tail-flick tests and tail-flick tests alone, on the antinociceptive response to systemic morphine. Dose-response lines for separate groups of nontolerant (no pretreatment, n = 15), morphine-tolerant (mor + test, n = 15) and saline-pretreated (sal + test, n = 15) rats to subcutaneous morphine injections.

Autopsies

Each rat was used in one experiment only. At the end of each experiment the animals were sacrificed by decapitation under ether anesthesia. The spinal column and the back of the skull were exposed and any rat with an improperly placed catheter, i.e., one that was inside rather than outside the spinal cord, was excluded from the experiment and the data from the animal was omitted from the analyses. The brains were removed and fixed in a solution of 30% sucrose and 10% formalin in buffer. Brains were sectioned in the coronal plane and stained with cresyl violet. Microinjection sites were located microscopically to verify accurate placement. Data from rats with improperly placed cannulae (i.e., outside of the periaqueductal gray) were excluded from the analyses.

Procedures

The purpose of the first experiment was to determine whether repeated TF tests alone would produce tolerance to systemically administered morphine on the TF test. Separate groups of rats were tested on the TF before and 40 min after a single daily injection of either morphine (0.75, 1.5 or 2.25 mg/kg, SC) or saline. This procedure was repeated for four days, at which time the response of all morphine-treated rats had declined by at least 50%. On the fifth day all groups received a final test session. Morphine-treated rats (mor + test) were injected with the same dose they had received on each of the four previous days. Saline-treated rats (sal + test) were divided into three groups, and each group was injected with one of the three morphine doses. An additional three groups of rats that had no prior exposure to morphine or the tail-flick test (no pretreatment) were also injected with one of the three morphine doses. There were five rats in each group, for a total of 45 subjects.

The second experiment was designed first, to determine whether concurrent PAG:ITH morphine administration would produce a synergistic effect on the TF response, second, to assess the effect of chronic systemic morphine on synergy and third, to assess the effect of chronic TF tests on synergy. Following recovery from surgery, all animals were tested on the TF. Separate groups of rats were then injected with equal doses of morphine at both spinal and supraspinal sites, or morphine at one site and saline at the alternate site, to yield PAG:ITH dose ratios of 1:1, 1:0 and 0:1. Tail-flick latencies were again determined forty minutes after the injections. On the following days, approximately half of the rats were injected with either morphine (3 mg/kg, SC) or saline. Tail-flick latencies were obtained both before and forty minutes after each injection, for six consecutive sessions, at which time the response of morphine treated (mor + test) rats had declined by at least 50%. On the next day, all rats were tested on the TF before and 40 min after a second concurrent injection of morphine into both the PAG and ITH space, or morphine at one site and saline at the alternate site, using the same dose ratio combinations administered prior to tolerance. There were 4 or 5 animals in each group, for a total of 100 rats, and all testing was conducted in the morning, between 9:00 a.m. and 12:00 p.m.

All analyses of variance, *t*-tests and regressions were performed with the IBM Statistical Analysis System General Linear Model program (SAS, Cary, NC) provided by the University of Illinois Biostatistics Facility, or a commercial statistical program, The Portable Statistician. The ED_{50} doses were determined by calculating the values of x, from the regression lines, for $y = 50$. These calculations were derived from the scores of all the rats. However, the regression lines shown in the figures were drawn by a computer program (Graphwriter) and are derived from the mean group scores. As a result, ED_{50} values obtained by visual inspection of the figures may differ from those obtained by statistical calculation. Unless otherwise indicated, all results were considered significant at $p<0.01$. Multiplicative interactions were assessed with isobolographic analyses using the calculated ED_{50} values, and the slope of the regression lines, with a computer program (CLIM) that was used to calculate the 95% confidence limits.

RESULTS

Effect of Nociceptive Assessment on Tolerance to Subcutaneous Morphine

Figure 1A summarizes the response of each of the four groups in the first experiment after their respective morphine or saline injections on each of four successive days. The scores of all morphine-treated rats declined from the first to the fourth session, indicating that tolerance developed to the antinociceptive effect of morphine on the TF. Figure 1B summarizes the dose response functions of the morphine pretreated (mor + test), saline pre-

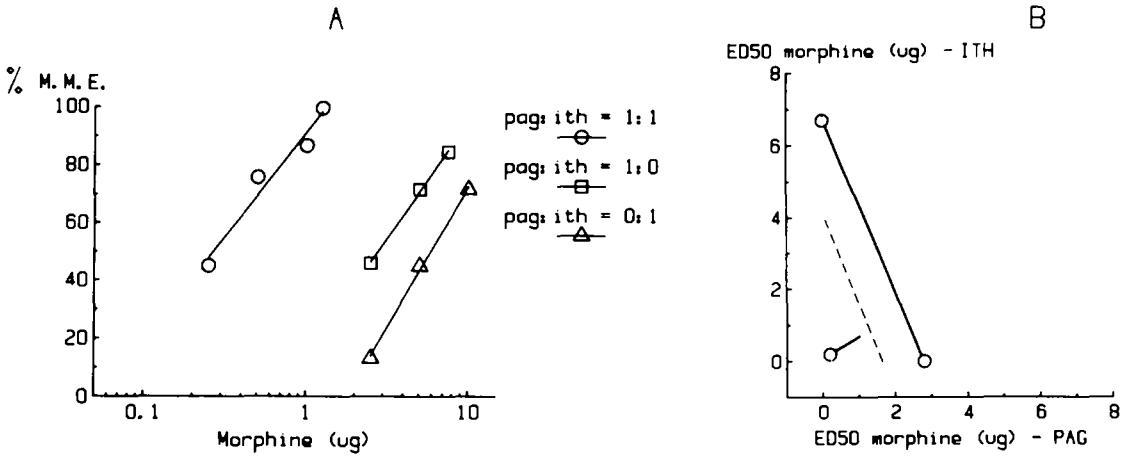

FIG. 2. (A) Synergistic effect of concurrent morphine injections into the periaqueductal gray (PAG) and the intrathecal space (ITH). Dose-response lines to morphine administered into the PAG (PAG:ITH = 1:0, n = 30), the intrathecal space (PAG:ITH = 0:1, n = 30) and concurrently to both sites (PAG:ITH = 1:1, n = 40) in nontolerant rats. (B) Isobolographic representation of antinociceptive ED_{50} values for morphine administered into the PAG (abscissa), the ITH space (ordinate), or concurrently to both sites in a 1:1 dose ratio (lower left). Each of the points represents an ED_{50} value obtained from nontolerant rats, whose dose-response curves are shown in Part A. The straight line indicates the theoretical ED_{50} values that would be observed if the effect at the two central sites were additive. The dashed line connects the 95% confidence limits of the ED_{50} values for the PAG and ITH dose-response lines. The figure shows that the 95% confidence limit of the ED_{50} value obtained after concurrent morphine injections into both sites does not cross the line connecting the 95% confidence limits of the ED_{50} values for the two separate lines. This demonstrates that concurrent injections produced a multiplicative effect.

treated (sal + test) and nonpretreated (no pretreatment) groups on the final test session. A two-way analysis of variance indicated first, that mor + test rats were tolerant, relative to those that received no pretreatment, $F(1,24) = 19.9$ and second, that a dose-response relationship was maintained, $F(2,24) = 5.22$, $p<0.02$, with no interaction, i.e., the lines were parallel. A second comparison showed that sal + test rats were also tolerant to morphine, relative to those that received no pretreatment, $F(1,24) = 10.43$, and that a dose-response relationship was retained, $F(2,24) = 7.35$, with no interaction. These results indicate that repeated performance of the TF response either in the presence or absence of morphine decreases the antinociceptive response to systemic morphine, and that the decrease is constant across the three test doses.

Additional comparisons indicated that the mor + test and sal + test animals did not differ in their antinociceptive response to morphine, $F(1,24) = 1.87$, NS, and that in the absence of the nonpretreated control groups there was no dose response relationship, $F(2,24) = 1.88$, NS, and no interaction. In other words, both treatments produced the same degree of tolerance of the TF response and eliminated the dose-dependent effect of the three test doses.

Antinociceptive Effect of PAG and ITH Morphine Administration in Nontolerant Rats

The antinociceptive effect of morphine or saline injections into the intrathecal space and PAG of nontolerant rats is summarized in Fig. 2A. The ED_{50} values of supraspinal morphine (1:0) and spinal morphine (0:1) were 2.8 and 6.7 μg respectively. These values are consistent with those of previous studies concerning PAG morphine administration (45, 59, 61), although the value for intrathecal administration is slightly higher than reported by others (55, 60, 62). It should be noted that the volume used for intrathecal injection in this study (10 μl) is greater than that used by Yeung and Rudy in their analysis of opiate synergy (4 μl), although it is the same volume used by Yaksh and many others to examine spinal opiate antinociception. Yaksh presented extensive evidence that the effect of an acute ITH injection was limited to an action at the spinal cord. More recently, Loomis and colleagues (32) reported that there was "no evidence of cervical or supraspinal staining" after seven days of continuous intrathecal infusion of methylene blue dye. In contrast, Yeung and Rudy found that volumes of dye greater than 4 μl appeared to reach rostral sites after a single ITH injection. It is possible that the volume of the ITH injections in the present studies promoted the rostral movement of morphine. If that were the case, it might be predicted that the ED_{50} for ITH morphine would be less, not greater than, that reported by Yeung and Rudy (4.2 μg) because of a synergistic interaction between spinal and supraspinal sites. In the absence of data to the contrary, we cannot exclude the possibility that some of the intrathecally administered morphine reached supraspinal sites during the 40-min postinjection interval. In agreement with previous studies (22,43), we also found that doses of 10 μg produced a hyperactive response when injected into the PAG which prevented an accurate determination of antinociception at this dose.

Equivalent injections of morphine (PAG:ITH = 1.1) yielded a dose-response line that was shifted to the left of the lines obtained with the 1:0 and 0:1 dose ratios (ED_{50} of 0.196 μg at each site).

Part B of Fig. 2 is an isobolographic representation of the results shown in Part A. The ED_{50} values for morphine administered to the PAG and the spinal cord are plotted on the abscissa and ordinate, respectively. The straight line connecting the spinal and supraspinal values designates the ED_{50} values that would theoretically indicate an additive interaction between the two sites. Points which lie below this line indicate a supra-additive or synergistic relationship, while points falling above the line represent an infra-additive or antagonistic interaction. The dashed line connects the 95% confidence limits of the ED_{50} values obtained from each of the two separate sites. The ED_{50} value for the concurrent injections clearly lies below the additive line, suggesting that a multiplicative interaction was obtained. This is supported by the fact that the sum of the ED_{50} ratios for the nontolerant groups

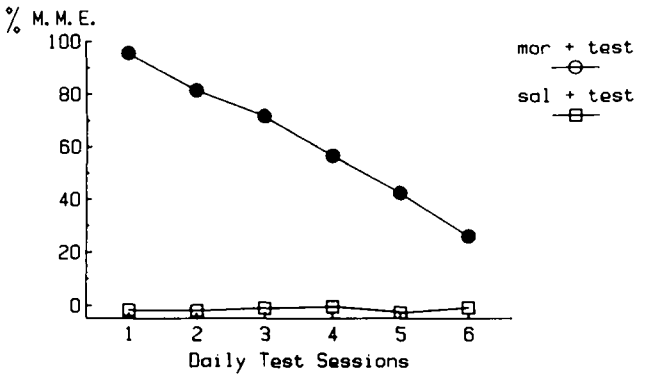

FIG. 3. The development of tolerance to the antinociceptive effect of systemic morphine on the TF withdrawal reflex. Separate groups of rats were tested on the TF before and 40 min after an injection of morphine (3.0 mg/kg, SC, n = 51) or saline (n = 49) on each of six successive days.

corresponds to 0.07 parts (0.196/2.8) and 0.03 parts (0.196/6.7) of the ED_{50} values obtained at the two separate sites. The sum of these parts (0.10) is much smaller than one. A value near one would be expected if the effect of the drug at the two separate sites were additive, whereas a value less than one indicates a synergistic interaction (40,52).

Additional support for the synergistic effect of concurrent PAG and ITH morphine injections is provided by a comparison of the 95% confidence intervals of the ED_{50} values for the three groups. As shown in Fig. 2B, the 95% confidence value of the ED_{50} obtained after concurrent morphine administration does not cross the line connecting the 95% confidence limits of the ED_{50} values obtained after morphine administration into each of the two separate sites. This confirms the conclusion that concurrent morphine administration into both sites produces a multiplicative analgesic effect (40,52).

Antinociceptive Effect of PAG and ITH Morphine Administration in Tolerant Rats

As shown in Fig. 3, the antinociceptive effect of subcutaneously administered morphine declined significantly during the daily test sessions, and mor + test rats became tolerant within six days [Day 1, mean M.M.E. = 95.5; Day 6, mean M.M.E. = 25.8; $t(50) = 29.2$]. Because the dose of 3.0 mg/kg used to induce tolerance in this study was greater than those used in the previous experiment, the number of sessions was increased from four to six. The additional sessions were conducted to be sure that a stable, tolerant condition was attained.

The effect of tolerance on the antinociceptive response to concurrent injections of morphine or saline into the PAG and the ITH space (PAG:ITH = 1:1) is summarized in Fig. 4A, B and C. In each part of the figure, the respective dose-response curve for the nontolerant (no pretreatment) group is the same as that shown in Fig. 2A. The other two functions represent the dose-response curves obtained from those rats that received either morphine (mor + test) or saline (sal + test) during the tolerance procedure.

Concurrent Spinal and Supraspinal Morphine

The results of this study are shown in Fig. 4A (PAG:ITH = 1:1). A two-way analysis of variance indicated first, that rats made tolerant to systemic morphine were also tolerant to concurrent PAG:ITH morphine injections [mor + test vs. no pretreatment, $F(1,32) = 71.23$]. A significant dose-response relationship was retained, $F(3,32) = 9.38$, with no interaction. Second, the analysis showed that saline-injected groups, which performed the TF response during tolerance sessions, were also tolerant [sal + test vs. no pretreatment, $F(1,32) = 27.23$] and that a dose-response relationship was retained, $F(3,32) = 13.75$. There was a statistically nonsignificant trend toward interaction, $F(3,32) = 2.89$, NS. A third comparison indicated that the reduction in the response of the morphine tolerant rats was significantly greater than that of the saline-pretreated rats, $F(1,32) = 11.12$.

These data suggest that opiate synergy is reduced when animals are repeatedly exposed to the nociceptive TF test and that such a decrease might be greater when morphine is administered in conjunction with the nociceptive test. The results are similar to the respective effects of opiate and behavioral pretreatment on the antinociceptive effect of systemic morphine (Fig. 1B).

The data are consistent with the conclusion that the multiplicative phenomenon is a reflection of processes which mediate tolerance to systemic morphine. However, the results do not indicate the source of these effects. In order to determine which of the two sites was responsible for mediating behavioral and pharmacological tolerance, the next experiments independently assessed morphine-induced antinociception elicited from the PAG and the spinal cord of morphine- and saline-pretreated animals.

Intrathecal Morphine

The effect of morphine and saline pretreatment on the TF response of animals injected with morphine in the ITH space (PAG:ITH = 0:1) is shown in Fig. 4B. The results of a two-way analysis of variance indicated that neither behavioral [no pretreatment vs. sal + test; $F(1,24) = 0.44$, NS] nor morphine [no pretreatment vs. mor + test; $F(1,24) = 0.89$, NS] pretreatment altered the dose-dependent effect of intrathecal morphine. These results are consistent with previous studies showing that the local effect of morphine at the spinal cord is unaltered in animals made tolerant to systemic morphine (40,51). These data are the first to demonstrate that nociceptive assessment alone does not alter spinal opiate analgesia.

Supraspinal Morphine

The effect of the morphine and saline pretreatments on the analgesic response of animals to morphine administered into the PAG (PAG:ITH = 1:0) is shown in Fig. 4C. In contrast to the results obtained with spinal morphine administration, statistical analysis indicated that both behavioral, $F(1,22) = 7.41$, and morphine pretreatment, $F(1,23) = 6.77$, reduced the analgesic effect of periaqueductal morphine administration. However, unlike the results obtained with concurrent morphine injections, there was no difference between the dose-response functions obtained after morphine and saline pretreatments, $F(1,11) = 0.12$, NS. These data indicate that pretreatment with the nociceptive TF test can induce tolerance to supraspinal morphine even in the absence of chronic morphine administration.

The data show that both morphine and saline pretreatment flattened the dose-response functions to PAG morphine. As a result, it was not possible to determine the ED_{50} for these groups. Because of the hyperactive reaction elicited by higher doses of morphine in the PAG of nontolerant rats, we did not assess additional doses in tolerant rats. Unfortunately, the fact that we could not determine the ED_{50} and corresponding 95% confidence limits for PAG morphine in tolerant rats meant that we could not perform an isobolographic analysis of opiate antinociception in tolerant rats.

DISCUSSION

Behavioral Tolerance to Systemic Morphine

Our results demonstrate first that the antinociceptive effect of

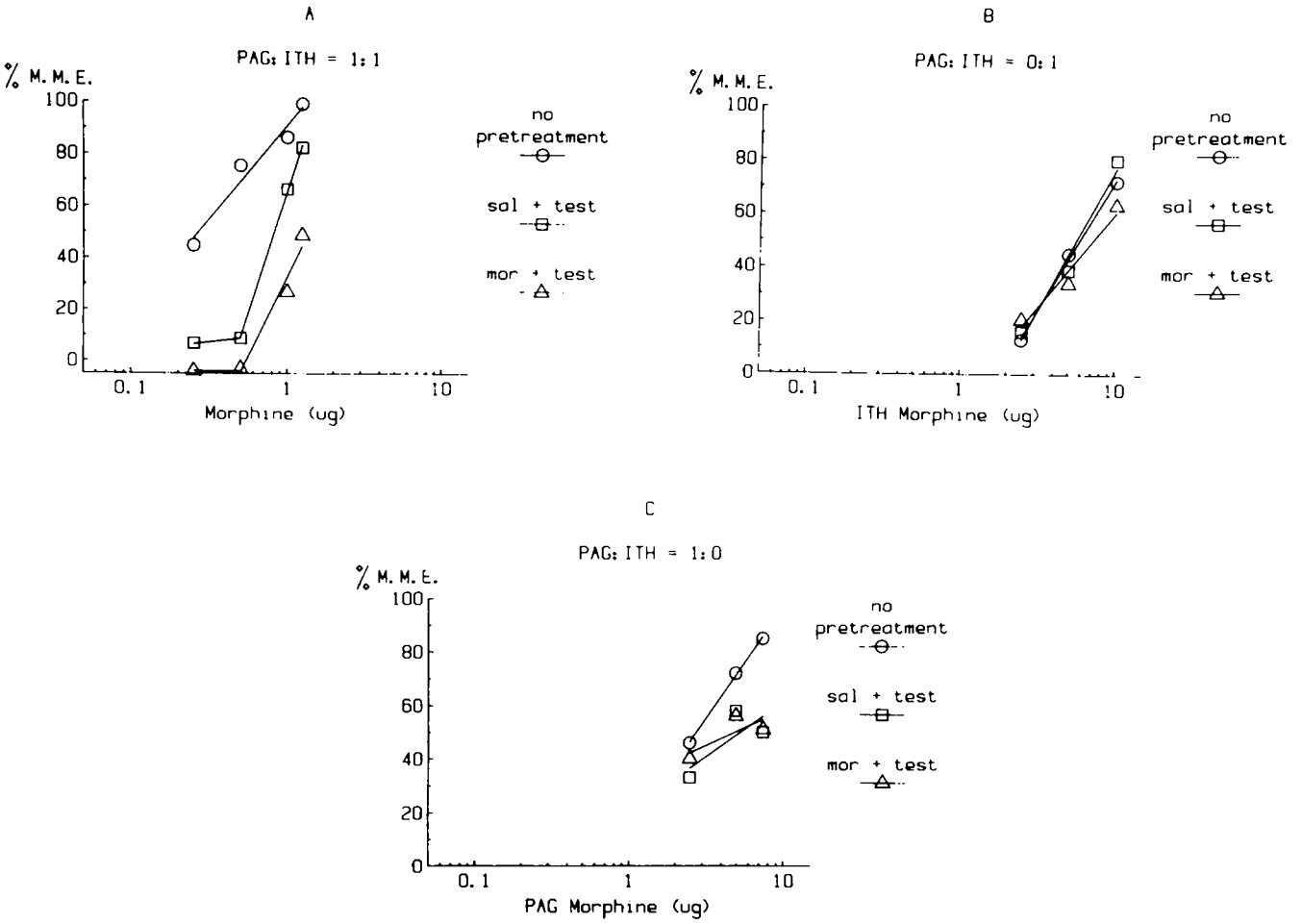

FIG. 4. (A) Effect of opiate and behavioral tolerance on the antinociceptive response to concurrent spinal and supraspinal morphine injections. Dose-response lines for nontolerant (no pretreatment, n = 40), morphine-tolerant (mor + test, n = 20), and saline-pretreated (sal + test, n = 20) rats to concurrent injections of morphine in the PAG and ITH space (1:1 dose ratio). (B) Effect of opiate and behavioral tolerance on spinal morphine-induced antinociception. Dose-response lines for nontolerant (no pretreatment, n = 30), morphine-tolerant (mor + test, n = 15) and saline-pretreated (sal + test, n = 15) rats to concurrent injections of morphine in the ITH space and saline in the PAG (0:1 dose ratio). (C) Effect of opiate and behavioral tolerance on supraspinal morphine-induced antinociception. Dose-response lines for nontolerant (no pretreatment, n = 30), morphine-tolerant (mor + test, n = 15) and saline-pretreated (sal + test, n = 13) rats to concurrent injections of morphine in the PAG and saline in the ITH space (1:0 dose ratio).

systemic morphine is reduced by repeated TF tests even in the absence of morphine administration (Fig. 1B). These data are in agreement with similar results obtained with the hot-plate procedure (8), and they are the first to show that nociceptive assessment can potentiate morphine tolerance of the spinally-mediated tail-flick test in a dose-dependent manner. This phenomenon has been termed behavioral tolerance, in distinction to pharmacological tolerance, which is induced by drug exposure per se (8).

It has been proposed that behavioral tolerance reflects the development of habituation to the experimental context as a result of repeated nociceptive assessment (8). This interpretation is derived from studies which have shown that repeated exposure to the context of morphine administration reduces opiate antinociception, whereas morphine administration in an unfamiliar context enhances it (7, 18, 34). Presumably, familiarity with the experimental environment allows the animal to adapt to the behavioral disturbance or stress produced by the test procedure and the physiological perturbation produced by the drug. When the organism is chronically exposed to both environmental and pharmacological stimuli, tolerance is maximal.

Multiplicative Phenomenon of Morphine-Induced Antinociception

At present, no attempts have been made to identify the neural mechanisms underlying behavioral tolerance. The present studies addressed this question by adapting a paradigm which describes the relationship between supraspinal and spinal sites mediating morphine-induced antinociception. In this procedure, morphine is administered concurrently into the brain and onto the spinal cord. In this situation, the total amount of morphine required to produce a given level of analgesia is reduced, relative to the dose required when morphine is administered to only one of the sites. This phenomenon, termed the multiplicative effect of morphine-induced antinociception (62), is presumed to mediate the analgesic effect of systemic morphine administration. As shown in Fig. 2A

and B, concurrent morphine injections into the PAG and the intrathecal space produced a synergistic effect. These data are the first to show that opiate synergy can be obtained by intracerebral, rather than intraventricular administration. Histological verification confirmed that the cannulae placements were located in the ventral and ventrolateral aspects of the mesencephalic periaqueductal gray. Except for a single placement in the reticular formation, the injection sites did not extend laterally beyond the bed nucleus of the posterior commissure, or dorsally into the dorsal fasciculus of Schutz (37).

Although these data demonstrate that opiate synergy can be obtained from concurrent PAG and intrathecal injections, they do not rule out the possibility that a multiplicative effect could also be mediated by other intracerebral sites. In addition, it is possible that the 1:1 dose ratio used in this study might not be the most effective combination for eliciting a synergistic effect from intracerebral sites, even though this dose ratio produced a maximal effect after intraventricular administration.

It also remains to be seen whether morphine injection into supraspinal sites, which do not independently elicit antinociception, would also induce a synergistic effect with concurrent intrathecal injections. If the supraspinal action of intracerebral morphine injections could be dissociated from the synergistic interaction produced by concurrent spinal administration, it might be possible to specify the origin of the descending pathways which mediate opiate synergy and determine whether they are the same as those which mediate antinociception produced by systemic morphine administration. Recent attempts to identify the locus of descending pathways responsible for either tonic inhibition of spinal nociceptive reflexes, or antinociception produced by electrical brain stimulation or supraspinal morphine, have produced conflicting results (16, 19, 23–25, 35, 42). Comparisons between descending pathways which support a multiplicative relationship and those responsible for other types of supraspinally-mediated inhibitions of spinal reflex pathways may be useful in resolving these issues.

Although the mechanism responsible for opiate synergy is unknown, recent results from our laboratory have suggested one hypothesis (3). We propose first that the antinociceptive effect of intrathecal morphine in intact animals is tonically suppressed by descending inhibitory input. Second, we propose that supraspinal morphine administration decreases this inhibitory input. The removal of descending inhibition allows the antinociceptive effect of spinal morphine to be expressed. The overt result is an increase in the potency of intrathecal morphine.

Although this interpretation of opiate synergy is similar to a previous suggestion made by LeBars and colleagues (30), it conflicts with the prevailing view that morphine acts in the brain in an opposite manner, i.e., to increase descending inhibition of spinal nociceptive processing (9). However, the model presented here postulates that it is the antinociceptive action of morphine at the spinal cord which is under inhibitory control, rather than nociceptive input per se. By taking this distinction into account, it may be possible to reconcile the present controversy regarding the effect of morphine on descending inhibitory pathways [see (3) for a more detailed discussion of this issue].

Effect of Tolerance on Opiate Synergy

In addition to replicating the multiplicative effect of morphine-induced antinociception, the present studies demonstrate that this interaction is reduced by both repeated morphine injections (3.0 mg/kg) accompanied by TF tests, or TF tests alone, administered once a day for six days (Fig. 4A).

We are aware of only one other report concerning the effect of opiate tolerance on opiate synergy (40). In that study, it was shown that the multiplicative relationship was reduced to an additive interaction in mice made tolerant and dependent as a result of morphine pellet implants. Our results suggest that opiate synergy was not reduced to an additive interaction in rats injected with a moderate dose of morphine for six days. Unfortunately, this conclusion could not be quantitatively verified because both morphine- and saline-pretreated rats were tolerant to PAG morphine injections. As a result, we could not obtain ED_{50}s from these groups. Therefore, we could not determine whether concurrent central morphine injections in tolerant rats induced a synergistic or additive effect.

However, the development of tolerance to PAG morphine administration was unexpected. First, it has been shown that tolerance to PAG morphine injections does not occur when they are separated by at least a week (45,61). Second, several previous investigators have shown that mice or rats (13, 28, 29, 36) made dependent on systemic morphine are not tolerant to ICV morphine. It has been suggested that the lack of tolerance in these studies is due to the fact that the local concentration of morphine in the brain, produced by an ICV morphine challenge, is much greater than that attained during the development of dependence to systemic morphine. As a result, tolerance is overcome and opiate antinociception is reinstated.

The present results do not support this interpretation. The data show that rats are tolerant to PAG morphine injections not only after exposure to moderate doses of systemic morphine, but even without prior opiate exposure, i.e., as a result of prior TF tests. Furthermore, it has been found that tolerance to ICV and ITH morphine also occurs during opiate withdrawal (28, 29, 36, 38, 41). In each of these situations tolerance was expressed even though the amount of morphine administered directly into the brain would also be greater than that present during withdrawal. It is possible that tolerance to PAG morphine occurred in the present studies because the animals were in a state of opiate withdrawal. This interpretation does not account for the fact that 1) pretreatment with the TF test alone produced tolerance to PAG morphine and 2) there was no tolerance to ITH morphine. These data suggest a pharmacodynamic, rather than a pharmacokinetic interpretation for the expression of tolerance to PAG morphine injections in the present studies.

We have proposed that opiate synergy occurs in nontolerant animals because the spinal action of morphine is blocked by descending inhibitory input, and that this input is reduced by an action of morphine in the brain. The remainder of this discussion will consider how this model might be applied to the results obtained in tolerant rats.

For this purpose, it is useful to assume that morphine produces analgesia by specific and independent actions in the brain, at the spinal cord and on descending supraspinal pathways. Presumably, each of these opiate actions could be modified during the development of tolerance. The fact that the antinociceptive effect of morphine at the spinal cord was not reduced in tolerant rats (Fig. 4B) suggests that the spinal cord does not become tolerant to morphine. Other evidence, however, indicates that this is not the case. Tolerance to the antinociceptive effect of systemic morphine on the TF reflex has been demonstrated in spinally-transected rats (4,10). By inference then, the lack of tolerance to spinal morphine in intact rats might be due to an intrinsic change in the level of activity of descending supraspinal pathways. This suggests that the postulated inhibitory control exerted by descending pathways on spinal opiate action might also decrease (become tolerant) during chronic morphine administration. The hypothesized decrease in supraspinally-mediated inhibition counteracts the tolerance that develops at the spinal cord. As a result, the effect of intrathecal morphine appears to be unaltered.

When morphine is injected systemically or concurrently into

the brain and onto the spinal cord of tolerant rats, tolerance is observed because the effect of the opiate is reduced in both the brain and the spinal cord and because the effect of morphine on descending inhibitory input is also reduced. As a result, more drug is required supraspinally to reduce descending inhibition in tolerant rats. However, once a sufficient dose is achieved at supraspinal levels to remove descending inhibition, the tolerant state of the spinal cord is expressed.

Although this analysis is purely speculative, it can account for a significant amount of the data concerning the antinociceptive effects of centrally-administered morphine in nontolerant and tolerant animals. This model also provides a role for the influence of behavior on tolerance. In this scheme, nociceptive assessment also modifies both of the supraspinal actions of morphine. It reduces the effect of intracerebrally administered morphine and reduces the ability of morphine to inhibit descending supraspinal inhibition. Because of the tolerance induced at these supraspinal sites by behavioral tests, the multiplicative effect of morphine is reduced, even in the absence of prior morphine exposure.

The fact that nociceptive assessment promotes tolerance is consistent with the postulated role of several endogenous substances, including norepinephrine, the enkephalins and beta-endorphin in the behavioral modulation of analgesia (6, 11, 14, 44–50). Furthermore, it has already been shown that norepinephrine and related adrenergic agonists interact in a synergistic manner with morphine at the spinal cord (21, 31, 33, 46, 53, 54, 56). Recent studies have also begun to characterize the opioid and adrenergic receptors involved in the multiplicative effect of morphine (17,39). Subsequent investigations of the neurochemical mechanisms of opiate synergy may ultimately determine whether the present speculations concerning the role of descending inhibitory pathways in opiate analgesia and tolerance will improve our understanding of these important phenomena.

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