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Modulation of Morphine Antinociception by the Brain-Penetrating H_2 Antagonist Zolantidine: Detailed Characterization in Five Nociceptive Test Systems

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NALWALK, J. W., J. E. KOCH, K. E. BARKE, R. J. BODNAR AND L. B. HOUGH. *Modulation of morphine antinociception by the brain-penetrating H_2 antagonist zolantidine: Detailed characterization in five nociceptive test systems.* PHARMACOL BIOCHEM BEHAV **50**(3) 421–429, 1995.—Because histamine (HA) in the CNS may be a mediator of antinociception, a detailed investigation of the effects of the brain-penetrating H_2 antagonist zolantidine (ZOL) was performed on five nociceptive tests in the presence and absence of morphine (MOR) in rats. ZOL inhibited MOR antinociception on the tail flick test, although a diurnal difference (inhibition in the dark cycle ➤ light cycle) was found. Similar results were found with the hot plate test, although details of the test procedure were significant. In contrast, ZOL induced opposing effects on MOR antinociception on two nonthermal tests (jump test and tail pinch test); ZOL alone induced moderate antinociception on the former test and mild antinociception on the latter test. Thus, ZOL exerts differential effects on baseline nociception and on MOR antinociception that vary depending on the nociceptive test employed, the light–dark cycle of the subjects, and the degree of stress associated with the nociceptive testing. These complex effects reveal the heterogeneous nature of opiate-induced modulation of nociception, and show that ZOL is a powerful tool for studying the relationships between opiates, HA, and nociceptive mechanisms.

Histamine Brain H_2 receptor Antinociception Morphine Nociceptive test Tail pinch test Tail flick test Hot plate test Jump test Diurnal cycle

THE HYPOTHESIS that histamine (HA) functions as a mediator of antinociception in the CNS is supported by several recent findings: 1) intracerebral injections of HA induce a reversible antinociceptive response that is inhibited by H_2 antagonists (40); 2) H_2 antagonists inhibit antinociceptive responses induced by either morphine (MOR) (20,23,24) or inescapable foot shock (19); and 3) systemically administered MOR selectively releases HA from the midbrain periaqueductal grey (PAG) (4,5), an important site for the regulation of pain transmission [see review (7)].

Although recent work has utilized intracerebral and microdialysis techniques to study HA in the PAG, earlier observations were made with systemically administered zolantidine (ZOL), the first brain-penetrating H_2 antagonist (18,20). When given subcutaneously, ZOL attenuated both a naloxone-sensitive (opiate) form of foot shock-induced antinociception (20), and a naloxone-insensitive (nonopiate) form (18). The nonopiate response was unchanged in MOR-tolerant animals (42). Nevertheless, not all forms of environmental antinociception were inhibited by ZOL, because this drug potentiated 2-deoxy-D-glucose-induced antinociception (28), a response that is reduced by MOR tolerance but unaffected by opiate antagonists (12,36).

A role for brain HA in MOR antinociception was also inferred from studies with systemically administered ZOL. Thus, this compound inhibited MOR responses in rats on both

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the hot plate (HP) and tail flick (TF) tests without altering baseline nociceptive responses (20). In primates, ZOL effectively antagonized MOR antinociception on the tail immersion test (25). The antiopiate action of ZOL was not due to pharmacological antagonism at opiate or other CNS receptor sites, nor due to changes in brain MOR levels (20). The conclusion that the effect resulted from antagonism of brain H_2 receptors is supported by extensive structure-activity studies (20,23).

In subsequent unpublished studies, we sometimes found considerable variation in the ability of systemically administered ZOL to attenuate MOR antinociception as assessed on thermal nociceptive tests. In addition, ZOL-MOR interactions have not been assessed on nonthermal nociceptive tests. In the present report, two different laboratories have collaborated to perform detailed pharmacological studies under a variety of nociceptive test conditions aimed at more thoroughly understanding the interactions between MOR, brain H_2 receptors, and nociception. Three goals were identified for these studies: 1) to characterize ZOL-MOR interactions in pharmacological detail on a variety of thermal and nonthermal nociceptive tests, 2) to determine the reproducibility of findings in one laboratory by another laboratory, and 3) to characterize methodological and biological variables that might account for apparently inconsistent findings. The latter included the study of animals from different suppliers, as well as comparisons of the same experiments performed during two different portions of the diurnal cycle.

METHOD

Animals

Male Sprague-Dawley rats were obtained from either Taconic Farms [(TAC), Germantown, NY] or Charles River Laboratories [(CR), Wilmington, MA]. Weights at the time of testing were 200-360 g (TAC) or 300-650 g (CR). Subjects were housed either individually (TF/jump protocol) or three per cage (all other tests) with food and water freely available. Experiments were performed during two different portions of the animals' diurnal cycle. Light-cycled animals were maintained on a normal 12L : 12D cycle (lights on 0700 h, lights off 1900 h) for at least 3 days prior to testing. Dark-cycled animals were maintained on a reverse 12L : 12D cycle (lights on 1900 h, lights off 0700 h) for at least 6 days before testing. In both groups, testing occurred between 0900 and 1700 h under normal laboratory illumination. Procedures were reviewed and approved by the Institutional Animal Care and Use Committees of either Albany Medical College or Queens College, City University of New York.

Drug Solutions

Zolantidine dimaleate (ZOL, SmithKline Beecham, Herts, UK), sodium maleate vehicle (Sigma, St. Louis, MO), morphine sulfate (MOR, Sigma), and naltrexone hydrochloride (Sigma) were dissolved in saline. Doses are specified as the salt for all drugs. ZOL and its vehicle were injected SC (flank); MOR, naltrexone and saline were administered SC (neck). With the exception of the TF/jump experiments (as explained below), all animals were used for only one experiment.

Nociceptive Testing

Five different nociceptive test protocols were used.

Combined HP/TF test. This test was performed as described previously (24). A constant-temperature water bath was used to maintain a hot plate surface temperature of 52°C, verified by routine measurements of the surface with a thermistor. Animals were placed on the surface and the latency for a hind paw lift or lick was recorded as a baseline response, with a cutoff of 60 s. Nearly all animals either lick the hind paw as part of this response or produce a multiple "stamping" response. Those few subjects that merely lift the paw hold the limb off of the heated surface near the abdomen, inconsistent with a simple spinal reflex action. The animals were removed from the surface as soon as a baseline response occurred. Immediately following this test, animals were gently restrained with a laboratory bench pad, and the ventral surface of the tail was exposed to a radiant heat source (2-5 cm from the tip), adjusted to produce baseline latencies of 3 to 4 s. Adjustments were not made for individual animals. A response was recorded when the animal removed the tail from the heat source, with a cutoff of 15 s. Three baseline TF tests were performed at 1-min intervals, followed by drug administration. Animals received combinations of MOR, ZOL, vehicle, or saline; single HP and single TF tests were repeated 20, 40, and 60 min later.

HP test (normal baseline). Rats were tested by the HP procedure exactly as described above, with no concurrent TF test. A normal HP test consisted of removing the subject from the heated surface as soon as a response was observed during the baseline period. Animals then received combinations of MOR, ZOL, vehicle, or saline, and the test was repeated 30 min later.

HP test (prolonged baseline). Rats were tested exactly as described for the normal HP test, except that at the time of baseline testing, each subject remained on the heated surface for 60 s regardless of the latency of their baseline response. Animals then received combinations of MOR, ZOL, vehicle, or saline, and the test was repeated 30 min later. The prolonged HP test has been used by several laboratory groups (20,30), although the normal test has been more widely used (15,24).

Combined TF/Jump test. This procedure has been extensively used and documented [e.g., (10)]. TF testing was performed with radiant heat applied to the dorsal surface of the tail 3-8 cm from the tip (IITC Analgesia Meter). Jump tests were determined in a chamber (30 × 24 × 26.5 cm) with 14 grid bars 1.9 cm apart. A shock generator (BRS/LVE) and scrambler (Campden Instruments) delivered electric shocks (0.3 s) to the animals through the grids. To perform these tests, three baseline TF latencies were measured at 10-s intervals (with a cutoff of 10 s). Animals were then placed in the shock chamber, and were tested by an ascending method of limits procedure. Shock was initially delivered at 0.1 mA with incremental 0.05-mA increases at 5-s intervals (with a cutoff of 1.3 mA) for each trial. The jump threshold (mA) was defined over six trials as the lowest of two consecutive intensities at which the rat simultaneously removed both hind paws from the grid.

Following 4 days of baseline TF and jump testing (to ensure stability of response), groups of six animals received drug injections at weekly intervals according to an incompletely counterbalanced design. Animals were tested 20, 40, and 60 min following drug administration. Seven groups of animals from the two suppliers were given various doses of vehicle, naltrexone, MOR, and ZOL.

Tail pinch. This test was performed by a modification of Haffner's method similar to that described in Bianchi and Franceschini (9). An alligator clip (4 × 0.4 cm) with plastic tubing on the teeth was used to pinch the rat's tail approximately 2.5 cm from the tip. The force exerted by the clip was determined with a spring balance to be 679 g. The latency (s)

for the rat to respond to the clip by turning towards the clip and biting it, or vocalizing, was recorded. The clip was removed immediately after an appropriate response, or after the 90-s cutoff. Following a single baseline measurement, drugs were administered, and the test was repeated 20, 40, and 60 min later.

Data Analysis

Repeated-measures analyses of variance (ANOVA, CSS Statistica, Tulsa, OK, or BMDP Statistical Software, Los Angeles, CA) were performed on all latency scores. Post hoc analyses were performed with either the Dunnett or Dunn test (TF/jump data) or the LSD test (all other data). An alpha level of 0.05 or less was chosen to indicate a significant difference between groups. Results are expressed as latencies (mean ± SEM).

RESULTS

HP Tests

When TAC animals were tested during the dark cycle with the HP/TF protocol, ZOL (0.03–30 mg/kg) failed to alter HP latencies in the absence (20) or presence (Fig. 1) of MOR. Furthermore, identical results were found in animals tested during the light cycle (data not shown). Because these results were in contrast to previous findings showing ZOL-induced inhibition of MOR antinociception on the HP test, additional experiments were conducted with two variations of the HP test on dark-cycled TAC animals. These HP experiments were designed to more closely reproduce the conditions used previously (20), in which animals were tested 30 min after drug administration (vs. 20 and 40 min, Fig. 1) with no concurrent TF test. Results confirmed the absence of an effect of ZOL on MOR antinociception (normal group, Fig. 2). However, when the animals were left on the surface for 60 s during baseline testing [prolonged baseline protocol, used in the previous ZOL–MOR study (20)], ZOL significantly reduced MOR antinociception (60 sec group, Fig. 2). As above, ZOL had no effect of HP latencies in either protocol in the absence of MOR.

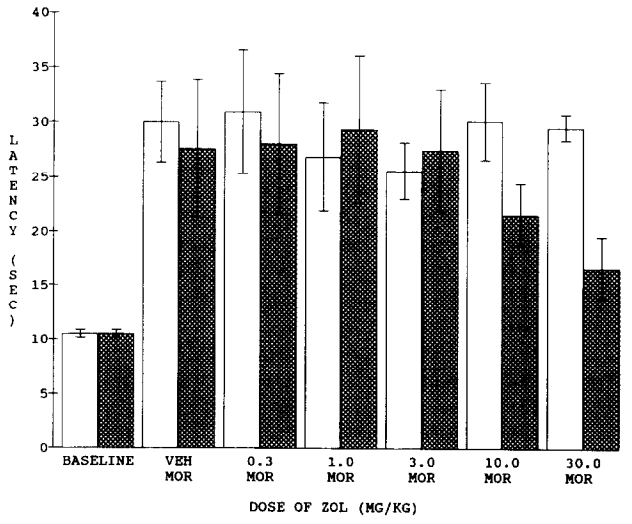

FIG. 1. Effect of ZOL on HP nociception in the presence of MOR. HP tests were performed as part of the HP/TF protocol. Dark-cycled TAC animals were tested for baseline nociception (Baseline, n = 36), received MOR (4 mg/kg, SC) along with either ZOL (doses on abscissa, SC) or ZOL vehicle (VEH, 15.3 mg/kg), and were tested 20 (open bars), 40 (hatched bars), and 60 min (not shown) later. Nociceptive latencies (s, mean + SEM, ordinate, n = 6) are given for each group. A one-factor (ZOL) ANOVA with repeated measures (time) showed no significant effect of drug or time.

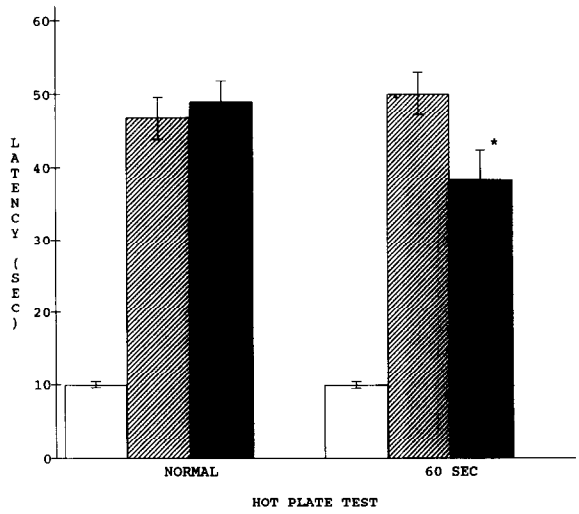

FIG. 2. Effects of ZOL on MOR antinociception as assessed by two HP methods. HP tests were not combined with other nociceptive tests. Dark-cycled TAC animals were tested for baseline nociception (open bars) and were either immediately removed from the surface (Normal group, n = 43) or left on the surface for 60 s regardless of their response (60 Sec group, n = 30), as described. Subsequently, animals received MOR (8 mg/kg, SC) along with either ZOL (1 mg/kg, SC solid bars, n = 15) or ZOL vehicle (0.51 mg/kg, hatched bars, n = 21–22) and were retested 30 min later. Nociceptive latencies (s, mean ± SEM, ordinate) are given for each group. A two-factor (test, ZOL) ANOVA with repeated measures (time) showed a significant effect of time ($p < 0.001$) with a significant test by ZOL interaction ($p < 0.04$) as well as a significant three-way interaction ($p < 0.03$). *Significantly different ($p < 0.002$) from MOR vehicle in same test group.

TF Tests

TF responses were assessed in two different laboratories as parts of two different test protocols (HP/TF and TF/jump). The effects of ZOL in the absence of MOR on both types of TF responses are summarized in Table 1. ZOL had no effect on TF latencies in the HP/TF protocol, although it exerted slight nociceptive or antinociceptive effects on TF latencies from the TF/jump protocol; the direction of these effects depended on the animal supplier (Table 1).

When ZOL was tested against MOR on the TF tests, differences were found between the two TF procedures and also between light- and dark-cycled subjects. In the HP/TF protocol, ZOL inhibited MOR (TF) antinociception (Fig. 3). In light-cycled subjects, ZOL induced slight but significant inhibition (3 mg/kg) as well as potentiation (30 mg/kg) of MOR responses (Fig. 3). When the same experiments were performed during the subjects' dark cycle, ZOL clearly attenuated MOR (TF) antinociception over a range of doses (0.03–3 mg/kg, Fig. 3). In contrast to these findings with the HP/TF protocol, ZOL (0.03–3 mg/kg) failed to significantly affect MOR (TF) antinociception on the TF/jump protocol in light-

TABLE 1

EFFECT OF ZOL ON TAIL FLICK LATENCY

Test	Time (min)	Supplier	Vehicle	Dose of Zolantidine (mg/kg) 0.03	0.3	1.0	3.0	10.0	30.0
HP/TF	20	TAC	5.1 ± 0.2		5.2 ± 0.8	5.2 ± 0.1	5.0 ± 0.5	4.7 ± 0.4	5.0 ± 0.7
HP/TF	40	TAC	4.5 ± 0.3		5.0 ± 0.3	5.1 ± 0.4	4.9 ± 0.4	5.7 ± 0.6	4.9 ± 0.1
HP/TF	60	TAC	4.7 ± 0.2		4.6 ± 0.4	4.9 ± 0.2	4.8 ± 0.3	4.7 ± 0.4	4.5 ± 0.2
TF/Jump	20	CR	3.6 ± 0.2	3.4 ± 0.3	3.0 ± 0.1*	2.9 ± 0.2*	2.6 ± 0.1*		2.6 ± 0.1*
TF/Jump	40	CR	3.3 ± 0.2	3.0 ± 0.1	2.9 ± 0.1	2.7 ± 0.2*	2.7 ± 0.1*		2.7 ± 0.1*
TF/Jump	60	CR	3.2 ± 0.1	3.2 ± 0.1	3.0 ± 0.1	2.7 ± 0.2	2.7 ± 0.1*		2.7 ± 0.1*
TF/Jump	20	TAC	3.5 ± 0.2				4.2 ± 0.2*		
TF/Jump	40	TAC	3.3 ± 0.1				3.3 ± 0.1		
TF/Jump	60	TAC	3.2 ± 0.1				3.5 ± 0.1		

Effect of the H_2 antagonist ZOL on TF nociception in the absence of MOR. TF latencies were measured in light-cycled animals with either the HP/TF or the TF/jump method. Animals from two suppliers (CR or TAC) received vehicle (1.0 or 15.3 mg/kg, SC) or the specified dose of ZOL (SC) and were tested at the times shown. Nociceptive scores (sec) are shown as mean ± SEM (n = 6). For each test method and supplier, a 1-factor (ZOL) repeated measures (time) ANOVA showed significant effects ($p < 0.05$) of ZOL in CR and TAC animals tested with the TF/jump protocol, but not the HP/TF protocol.

*Significantly different ($p < 0.05$) from vehicle control.

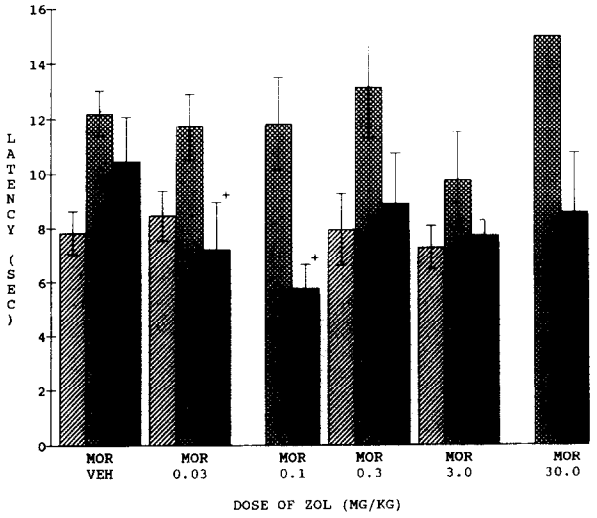

FIG. 3. Effect of ZOL on MOR antinociception as assessed by two types of TF tests. Animals received ZOL (SC, doses on abscissa) or vehicle (VEH, 1.0 or 15.3 mg/kg) in combination with MOR (4 mg/kg, SC) and were tested with either the TF/jump protocol (hatched bars, light cycle only) or the HP/TF method (light cycle: cross-hatched bars; dark cycle: solid bars). Results from the TF/jump protocol are pooled from animals of both suppliers (TAC and CR), and were found not to be significantly different from each other; HP/TF results are from TAC animals only. Nociceptive scores (s, mean latency ± SEM, ordinate, 60 min, n = 6–14) are given for each group. A one-factor (ZOL) repeated-measures (time) ANOVA showed no significant effect of drug on MOR antinociception in the TF/jump results. A two-factor (light–dark cycle, ZOL) with repeated measures (time) ANOVA on the HP/TF results showed significant main effects of diurnal cycle and time ($p < 0.001$) with significant ($p < 0.001$) cycle by time and drug by time ($p < 0.01$) interactions. *, + Significantly different ($p < 0.05$) from light- and dark-cycled VEH/MOR group, respectively.

cycled animals (Fig. 3). Because 1) TF/jump experiments were performed in CR rats, 2) HP/TF experiments were performed in TAC rats, and 3) TF/jump results were the opposite of those found with the HP/TF protocol, the TF/jump experiments were repeated in TAC animals. The results in TAC animals were identical to those of CR rats (TF/jump data of Fig. 3 show combined data from both suppliers). Thus, differences in TF results from the two protocols are not due to the source of the animals. This conclusion, in turn, shows that ZOL inhibits MOR antinociception on the TF test when this test is combined with the HP test, but not when it is combined with the jump test. Dark-cycled animals were not studied with the TF/jump test.

Jump Test

In the absence of MOR, ZOL induced a significant antinociceptive response on the jump test in both CR and TAC rats. In the CR animals (Fig. 4A), the response was dose dependent (0.3–30 mg/kg) and occurred at all times tested (20 and 60 min, data not shown). Peak antinociceptive effects of ZOL were equivalent to those produced by small doses (1–2 mg/kg) of MOR (Fig. 4B). A significant but smaller effect was found in TAC animals (3 mg/kg, Fig. 4C).

ZOL (0.3, 3.0 mg/kg) was also an effective inhibitor of MOR antinociception (2–6 mg/kg) on the jump test in animals from both suppliers (Fig. 4B,C). When tested against the lower doses of MOR (2 and 4 mg/kg), ZOL reduced MOR-induced nociceptive thresholds to those induced by ZOL alone (Fig. 4B). The inhibition was at least partially surmounted by the larger dose of MOR (6 mg/kg, Fig. 4B). ZOL-induced inhibition of MOR antinociception on the jump test also appeared to be dependent on the dose of ZOL studied (Fig. 4C). Similar effects were seen at 20 and 60 min after drug administration (not shown).

Tail Pinch Test

TAC animals only were studied with the tail pinch test. In the absence of MOR, ZOL significantly increased tail pinch

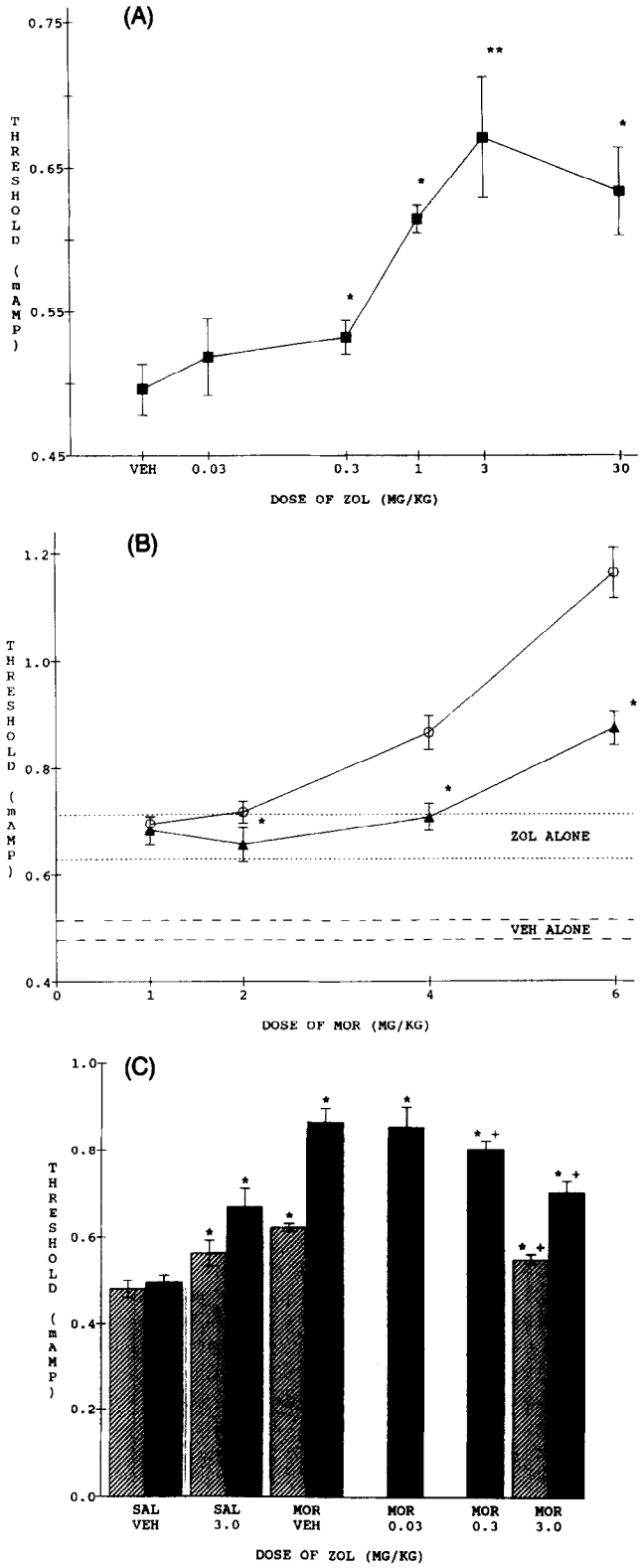

FIG. 4. Effect of ZOL on the jump test in the absence and presence of MOR. Light-cycled rats received combinations of ZOL, ZOL vehicle (VEH, 1.0 mg/kg), MOR (4 mg/kg, SC), or saline (SAL) and were tested with the TF/jump protocol. Data are nociceptive thresholds (mA, mean ± SEM, ordinate, 40 min, $n = 6$) plotted against dose of drug (mg/kg, SC) on the abscissa. (A) ZOL dose-response curve in the absence of MOR in CR rats. Animals received the treatments shown along with saline. A one-factor (drug) with repeated-measures (time) ANOVA showed a significant effect of ZOL in the absence of MOR ($p < 0.001$). *,**Significantly different ($p < 0.05$ and 0.01, respectively) from VEH. (B) MOR dose-response curve in the absence (open circle) and presence (triangle) of ZOL (3 mg/kg) in CR rats. Animals received the indicated dose of MOR along with either ZOL or VEH. The labelled zones show the upper and lower ranges (mean ± SEM) for VEH ALONE or ZOL ALONE (3 mg/kg) in the absence of MOR [from (A)]. A separate one-factor (drug) with repeated-measures (time) ANOVA was performed for each dose of MOR and the corresponding doses of ZOL ($p < 0.001$ for 2, 4, and 6 mg/kg MOR). A combined ANOVA with multiple doses of MOR could not be performed because all doses of ZOL were not tested with all doses of MOR. *Significantly different ($p < 0.05$) from corresponding VEH group at the same dose of MOR. (C) ZOL dose-response curve in the presence of a fixed dose of MOR (4 mg/kg) in TAC (hatched bars) and CR (solid bars) rats. SAL/VEH and SAL/3 mg ZOL groups are the same as displayed in (A) and (B) for CR rats. In the presence of MOR, a one-factor (ZOL) with repeated-measures (time) ANOVA showed significant attenuation of MOR antinociception ($p < 0.05$) for both CR and TAC animals. *, + Significantly different ($p < 0.01$, $p < 0.05$) from SAL/VEH and MOR/VEH, respectively.

latencies in dark-cycled, but not light-cycled, animals (Fig. 5A vs. 5B). Nevertheless, ZOL antinociception on this test was mild, and was not related to either dose or time parameters (Fig. 5A).

In dark-cycled animals, however, ZOL significantly enhanced MOR antinociception on the tail pinch test in a time- and dose-dependent manner (Fig. 5A). The same trends were evident in light-cycled animals, but the effects seemed less related to either time or dose (Fig. 5B).

DISCUSSION

The present results show that the brain-penetrating H_2 antagonist ZOL has diverse effects upon baseline nociception and MOR antinociception, depending upon the nociceptive test employed, the particular parameters of some of the nociceptive tests, and the portion of the subject's light-dark cycle. When tested in the absence of MOR, ZOL alone had little or no effect in thermal nociceptive tests (Table 1), confirming previous findings when ZOL was tested on separately performed HP or TF tests (18-20). When the TF test was performed within the TF/jump protocol, ZOL had slight effects in opposite directions, depending on the supplier of animals (Table 1). In nonthermal tests, ZOL induced slight (tail pinch) or moderate (jump test) antinociception. Thus, ZOL alters nonthermal nociceptive latencies considerably more than thermally evoked responses. The possibility that ZOL induces alterations in skin temperature or limb perfusion seems to be ruled out by the negligible or opposing effects of this agent on thermal baseline responses (Table 1).

ZOL inhibited MOR antinociception in both the TF and HP tests, although it is clear that methodological variables are important. ZOL also exerted a consistent inhibition of MOR antinociception on the jump test. In contrast, ZOL consistently potentiated MOR antinociception on the tail pinch test. In all cases where the light-dark cycles were compared, ZOL had either more pronounced or more consistent effects in dark-cycled subjects. The findings with each nociceptive test are considered in more detail below.

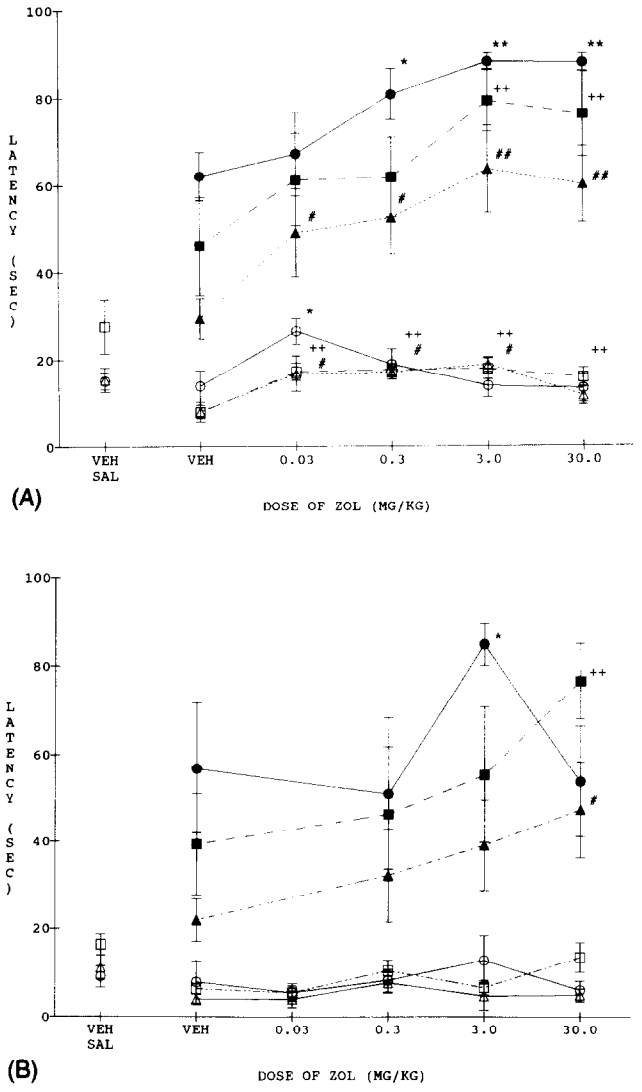

FIG. 5. ZOL dose-response curves on tail pinch nociception in the absence and presence of MOR. ZOL was administered alone (dose on abscissa, SC, open symbols) or with MOR (closed symbols) and subjects were retested at 20 (circle), 40 (square), and 60 (triangle) min. Animals received either: 1) vehicle and saline (15.3 mg/kg, VEH SAL, two injections SC), 2) VEH (with or without MOR, 4 mg/kg, SC), 3) ZOL alone, or 4) ZOL in combination with MOR. Nociceptive latencies (s, mean ± SEM, ordinate, $n = 6$) are shown in dark-cycled (A) and light-cycled (B) animals. A one-factor (drug) with repeated measures (time) ANOVA of the dark-cycled data (A) showed a significant effect of ZOL alone (drug, $p < 0.001$; time, $p < 0.001$); no such effect was seen with light-cycled animals (B) at any dose or time tested. In the presence of MOR, one-factor (drug) with repeated-measures (time) ANOVAs for both cycles showed that ZOL increased MOR antinociception in both light- and dark-cycled animals (dark cycle: significant effects of drug ($p < 0.01$), time ($p < 0.001$), and interaction ($p < 0.001$); light cycle: significant effects of drug ($p < 0.001$), time ($p < 0.001$), and interaction ($p < 0.02$)). A separate ANOVA comparing light vs. dark cycle with all common data showed a highly significant ($p < 0.001$) difference between the portions of the diurnal cycle. *,+,#Significantly different ($p < 0.05$) from VEH control group at 20, 40, or 60 min, respectively. **,++,##Significantly different ($p < 0.01$) from VEH control group at 20, 40, or 60 min, respectively.

HP Test

HP results from the HP/TF experiments showed that ZOL had no effect on MOR antinociception over a range of doses in either light- or dark-cycled subjects (Fig. 1 and the Results section). This result seemed in contrast to a previous study that reported ZOL-induced inhibition of MOR antinociception with the HP test in dark-cycled animals (20). Because the previous study used the prolonged HP method (in which animals were left on the plate for 1 min independent of their baseline response with no concurrent TF testing), whereas the present TF/HP experiments used the normal HP test (in which subjects were removed as soon as the baseline response was observed), the two HP methods were compared. These experiments (Fig. 2) also were performed without concurrent TF testing (unlike those of Fig. 1). The results confirmed that ZOL inhibited MOR antinociception only when the prolonged protocol was used (Fig. 2). Although the degree of MOR antagonism in these experiments seems small, the dose of MOR used was large (8 mg/kg, SC); a larger antagonism of MOR was previously found when the dose of MOR was smaller, and this effect was surmountable by larger doses of MOR (20).

The most parsimonious explanation for the different effects of ZOL in the two HP procedures appears to be the amount of stress associated with each protocol during the baseline testing period. Thus, animals tested by the normal method terminate their exposure by making the nociceptive response, whereas those in the prolonged group endure additional exposure to the heated surface. It is well established that supramaximal exposure to nociceptive stimuli induces antinociception [see reviews (11,39)]. Furthermore, depending on the characteristics of the stressor, pairing of various types of stressors with MOR can result in either increased (2,12) or decreased (37) nociceptive responses. Lesion studies suggest that even when a stressor does not alter the magnitude of a MOR-induced antinociceptive response, it can still change the mechanism by which the response is produced (27). The results of Fig. 2 seem consistent with this idea, suggesting that the stressful additional exposure to the hot plate enhances the histaminergic character of the MOR response. A number of studies have shown that stressful stimuli can affect histaminergic mechanisms (3,21,44,45), and strong evidence supports a role for brain HA as a mediator of stress-induced antinociception (19,20). Furthermore, recent in vivo microdialysis studies showed that the opiate-induced release of HA in the PAG was altered by introduction of the tail pinch nociceptive test, undoubtedly a stressor [(5) and see below].

Even though systemic ZOL did not alter MOR antinociception on the normal HP test (Fig. 2), this same test procedure showed clear antagonism of systemic MOR antinociception by ZOL and other H_2 blockers when the latter were administered into the lateral ventricle (23) or into the ventrolateral PAG (24). The prolonged HP procedure may not be necessary to show effects of H_2 antagonists in such microinjection studies, where considerable stress may be associated with either the surgery or the intracerebral injection procedure.

Although ZOL inhibits MOR antinociception on both the HP (Fig. 2) and TF tests [Fig. 3, (20)], the mechanisms for these effects can be distinguished. Thus, microinjections of the H_2 antagonist tiotidine into the PAG of dark-cycled rats attenuated systemic MOR antinociception on the HP but not on the TF test (24). This finding suggests that the PAG mediates a portion of the antiopiate effect on the HP, but that other CNS areas are involved in attenuating the effect of MOR in the TF test [see (24) for discussion].

TF Test

ZOL inhibited MOR antinociception in dark-cycled animals on the TF test when it was performed as part of the HP/TF procedure (Fig. 3). Gogas et al. (20) reported the same results when the TF was not combined with the HP test. In both cases, the inhibition was induced by several doses of ZOL, with no effects on baseline nociceptive latencies [Table 1, (20)]. In both the present (Fig. 3) and previous experiments (20), there was a tendency for larger doses of ZOL to be less effective than smaller doses. The mechanism for this inverted U-shaped dose–response curve is not certain, but is not due to independent effects on baseline nociception nor to ZOL-induced inhibition of brain HA metabolism (22). Other results support the suggestion that the attenuation of MOR antinociception by low doses of ZOL and the reversal of this effect by higher doses of ZOL might result from actions on anatomically distinct populations of brain H_2 receptors (20,24).

The present results with the TF test (HP/TF protocol) show that ZOL inhibits MOR antinociception more consistently in dark-cycled than in light-cycled animals (Fig. 3). In the latter subjects, the reduction in MOR antinociception by a single dose of ZOL (3 mg/kg, Fig. 3) is similar to previous work (20) in which ZOL (1 mg/kg) was effective against MOR in the TF test in light-cycled rats. However, the conclusion from that result, that the diurnal cycle is unimportant for the H_2–opiate interaction, was based on the results from a single dose of ZOL. The present, more comprehensive study, utilizing several doses of ZOL in both light- and dark-cycled animals, supports the existence of a diurnal variation in the ZOL-induced inhibition of MOR antinociception. In previous studies, we found that dark-cycled animals demonstrated a more reliable antagonism of naltrexone-resistant foot shock-induced antinociception by ZOL when compared to light-cycled subjects [(19,20), unpublished observations]. The present findings show that this same tendency is true for MOR antinociception.

CR rats failed to exhibit ZOL-induced inhibition of MOR on the TF test (TF/jump protocol) in the light cycle (Fig. 3). However, in addition to the use of the light-cycled animals in this experiment, reasons for the failure to observe an effect of ZOL could be the use of a different animal supplier (CR vs. TAC) or the use of combined nociceptive testing with the jump test. Because the same results were found when the experiments of Fig. 3 were repeated in TAC rats, the source of the animals does not explain the difference. The possible contribution of combined jump testing to the TF results is unclear. The jump test is administered after the TF test to minimize carry-over effects, and the combined procedure has no effect on the size of subsequent TF or jump latencies when compared to separately performed test results (26). However, it should also be noted that in the same light-cycled subjects in which ZOL had no effect on MOR responses in the TF test (Fig. 3), the drug inhibited MOR antinociception on the jump test (Fig. 4, discussed below). Taken together, these findings suggest that in light-cycled animals, either ZOL is much more effective against MOR in the jump test when compared to the TF test or that the foot shock delivered as part of the jump test reduces the effectiveness of ZOL on the TF test. The importance of repeated testing of the same subject (TF/jump protocol only) has also not been comprehensively addressed with respect to the action of ZOL.

The finding that MOR antinociception is more dependent on histaminergic mechanisms during the dark cycle (Fig. 3) is consistent with the known diurnal variation in brain histaminergic activity. Thus, increased brain HA turnover and enhanced brain HA release occur during the dark cycle (31,32). In rats, which are nocturnal animals, the dark cycle coincides with the period of enhanced motor activity and electrographic arousal, both of which may be maintained in part by increases in histaminergic activity (41). In addition, it is well known that nociceptive thresholds exhibit diurnal fluctuations, with lowest sensitivity in the light cycle and highest sensitivity at the end of the dark cycle, variations that are abolished by opiate antagonists (6,16). We have recently suggested that the histaminergic modulation of nociception may also depend on endogenous opiates (40). Therefore, the enhanced sensitivity of MOR antinociception to H_2 antagonists during the dark cycle may depend on the interactions between endogenous opioid and histaminergic systems.

The fact that ZOL-induced inhibition of MOR antinociception is more easily demonstrated in dark-cycled animals does not mean that histaminergic mechanisms are unimportant during the light cycle. As mentioned, selected doses of ZOL are effective against MOR in the light cycle (discussed above). In addition, systemic MOR releases HA in the PAG of light-cycled rats (4), and centrally administered HA induces antinociception during this period (17) as well. It is rather more likely that MOR activates histaminergic antinociceptive mechanisms during both periods, but that these mechanisms are more apparent during the dark period.

Jump Test

Although jump tests were limited to light-cycled subjects, ZOL produced a dose-related increase in jump thresholds (Fig. 4A), confirming and extending previous results (28). ZOL-induced antinociception has not been commonly observed, although large doses induced a slight antinociceptive effect in mice with the writhing test (33). In preliminary unpublished studies, we found ZOL-induced antinociception on the jump test to be blocked by naltrexone (5 mg/kg, TAC animals), suggesting the involvement of endogenous opiates. ZOL also exerted a dose-dependent inhibition of MOR antinociception in the same test (Fig. 4C). Interestingly, the combination of ZOL and MOR never yielded thresholds that were below the effects of ZOL alone, a pattern typical of the interaction between a partial and a full agonist (38). Although ZOL lacks appreciable affinity for mu, delta, or kappa opiate receptors (20), the present results could be explained by a ZOL-induced release of an endogenous opiate that could function as a partial opiate agonist. The antagonism was partially surmounted by larger doses of MOR (Fig. 4B), also consistent with, but not proof of, such a model. Antagonism of MOR antinociception by supraspinally administered ethylketocyclazocine may be an example of such an interaction (13). Whether or not ZOL induces opiate release, further studies are needed to determine the mechanism of ZOL-induced antinociception in the jump test. For example, studies with other H_2 blockers could determine if ZOL is acting as an antagonist or partial agonist at H_2 receptors. The ZOL-induced inhibition of MOR antinociception in the jump test also demonstrates that the antiopiate activity of this compound is demonstrable with nonthermal as well as thermal nociceptive tests.

ZOL's contrasting effects on the jump and TF tests in the same subjects are noteworthy. Thus, the compound had appreciable antinociceptive activity on the jump, but not the TF, test. Furthermore, because ZOL inhibited MOR antinociception on the jump test (Fig. 4B,C), but not on the TF test (Fig. 3) in the same subjects, and yet ZOL inhibits MOR on the TF

test when it is performed in conjunction with the HP test (Fig. 3), it seems likely that the mechanisms by which ZOL modulates MOR antinociception in these two nociceptive tests (TF and jump tests) are distinct. The importance of repeated testing and light-dark cycle differences needs to be further examined to resolve these inconsistencies.

Tail Pinch Test

As assessed by the tail pinch method, MOR antinociception was unexpectedly potentiated by ZOL. The effect was evident at several times after MOR, was dose-dependent in dark-cycled animals (Fig. 5A), and occurred in the absence of pronounced effects on baseline tail pinch latencies. The ability of ZOL to antagonize MOR antinociception on the hot plate (Fig. 2), TF (Fig. 3), and jump tests (Fig. 4), while potentiating MOR antinociception on the tail pinch test (Fig. 5), argues strongly that MOR modulates these nociceptive responses by different mechanisms. Previous studies also support this conclusion. For example, intracerebral mapping studies found that MOR is active against mechanical stimuli in portions of the PAG in which it has no effect on thermal nociception (43). Depletion of spinal 5-HT was also found to dissociate the actions of MOR on mechanical vs. thermal nociceptive stimuli (29).

If, as argued previously (20), ZOL modulates MOR action by blockade of brain H_2 receptors, then the present results suggest that brain histaminergic mechanisms can modulate different kinds of nociceptive transmission in different ways. Taken at face value, the results imply that, in the presence of MOR, HA contributes *antinociceptive* effects upon exposure to thermal and electrical noxious stimuli, but *nociceptive* effects upon exposure to mechanical stimuli. Although more direct studies are needed to test such a hypothesis, tail pinch is known to induce a complex combination of neurochemical changes (1,8) and to also affect opiate antinociception (14, 34,35). As mentioned, tail pinch testing also modified the opiate-induced release of HA in the PAG (5).

In conclusion, ZOL exerts differential effects on baseline nociception and on MOR antinociception that vary as functions of the nociceptive test employed, the light-dark cycle of the subjects, and the degree of stress associated with the details of nociceptive testing. These diverse and sometimes opposing effects of ZOL reveal the heterogeneous nature of opiate-induced modulation of nociception, and offer clues to the relationship between brain histaminergic systems and nociceptive mechanisms.

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