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Attenuation of Morphine Analgesia by the S_2 Antagonists, Pirenperone and Ketanserin

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PAUL, D., M. J. MANA, J. G. PFAUS AND J. P. J. PINEL. *Attenuation of morphine analgesia by the S_2 antagonists, pirenperone and ketanserin.* PHARMACOL BIOCHEM BEHAV 31(3) 641–647, 1988.—The involvement of serotonin type-2 (S_2) receptors in morphine-induced analgesia was assessed by challenging the effect of 10 mg/kg of morphine sulphate (IP) with the S_2 receptor blockers, pirenperone and ketanserin. Tail-flick latencies were assessed at 0, 30, 60, 90 and 120 min after injections by measuring the time that it took each rat to remove its tail from a 52°C water bath. Pirenperone, at 0.08, 0.16, and 0.24 mg/kg (SC) attenuated morphine-induced antinociception. In contrast, only the high 10 mg/kg (SC) dose of ketanserin attenuated the effect of morphine. Because pirenperone easily enters the central nervous system whereas ketanserin does not, these results indicate the involvement of central S_2 receptors in morphine-induced antinociception. The 10 mg/kg dose of ketanserin, however, did not attenuate the antinociception produced by 100 mg/kg of ketamine. Thus, the antianalgesic effect of S_2 receptor blockers may be specific to opioid-mediated analgesia.

Pirenperone Ketanserin Morphine Ketamine Antinociception Tail-flick Serotonin S_2 receptors

MORPHINE is generally thought to exert much of its antinociceptive effect through a brainstem-spinal-cord system [e.g., (13,22)]. According to this model, opioids inhibit afferent nociceptive discharges by binding to receptors in various brainstem nuclei, such as the periaqueductal grey (PAG), nucleus raphe magnus (NRM), nucleus reticularis gigantocellularis, nucleus reticularis paragigantocellularis, and locus ceoruleus, thus activating a pathway that descends the spinal cord and exerts an antinociceptive influence through inhibitory synapses in the spinal dorsal horn. Serotonin is thought to mediate transmission at these dorsal horn inhibitory synapses (2, 13, 22, 23).

This hypothesized role of serotonin has been one of the most contentious aspects of this model. Support for the hypothesis that serotonin mediates morphine-induced analgesia has been offered by studies in which: 1) the depletion of serotonin stores with para-chlorophenylalanine (4, 12, 14, 33, 40–42, 44); 2) the selective destruction of serotonin neurons with para-chloroamphetamine, fenfluramine, or 5,6-dihydroxytryptamine (4, 11, 37, 39, 42, 44); or 3) the blockade of serotonin receptors (8,14) have been found to attenuate the antinociceptive effect of morphine. However, there is also a substantial body of evidence inconsistent with this view. For example, despite claims to the contrary, some investigators have found no evidence that para-chlorophenylalanine disrupts morphine-induced analgesia (5, 12, 15, 37). Similarly, morphine-induced analgesia is not consistently disrupted by the selective serotonin receptor blockers, methysergide, mianserin, and metergoline (3, 12, 29). However, these selective serotonin receptor blockers have been found to attenuate the analgesia produced by injections of morphine into the PAG (45), or NRM (10), or intrathecally (32) in the rat, suggesting that central and peripheral opiate injections are not equivalent. Although these inconsistencies have received considerable formal discussion (3, 12, 41), they have yet to be convincingly resolved.

Recently, Peroutka, Lebovitz and Snyder (30) and Peroutka and Snyder (31) have shown that there are at least two types of serotonin receptors, which they called S_1 and S_2 receptors. Autoradiographic techniques have established that serotonin binding sites in the median raphe are primarily S_1 sites and that the sites in the dorsal raphe are primarily S_2 sites (9, 18, 21, 27, 28). Moreover, there are also S_1 binding sites in the spinal gray matter, particularly in the substantia gelatinosa of the dorsal horn. Thus, S_1 and S_2 binding sites are concentrated in areas of the central nervous system thought to mediate morphine-induced analgesia.

Because it has been hypothesized that dorsal horn spinal serotonin receptors mediate morphine-induced analgesia (2, 13, 23, 35) and because dorsal spinal cord serotonin recep-

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tors are almost exclusively S_1 receptors (24,27), many have assumed that spinal S_1 receptors mediate morphine-induced analgesia [e.g., (27)]. However, the data of Zemlan, Kow and Pfaff (46) suggest that stimulation of spinal S_1 receptors produces hyperalgesia rather than analgesia: they found that systemic administration of the serotonin agonists quipazine and 5-methoxy-N,N-diamethyltryptamine to rats with transected spinal cords increased their responsiveness to noxious stimuli. On the basis of this finding and the evidence supporting the existence of a spinal antinociceptive mechanism, Zemlan *et al.* suggested that there may be two spinal serotonergic systems involved in responding to painful stimuli: the first inhibiting ascending nociceptive fibers, and the second facilitating local spinal withdrawal reflexes.

Paul and Phillips (26) recently attempted to assess the effect of S_2 receptor blockade on the analgesia produced by systemic injection of morphine. When morphine administration was preceded by 60 min with an injection of 0.16 mg/kg of pirenperone, but not by 0.04 or 0.08 mg/kg, there was a significant attenuation of the morphine-induced analgesia. These results suggest that S_2 receptors, which have not been found in the dorsal horn of the spinal cord, mediate morphine-induced analgesia. The inconsistencies between the widely held view that the antinociceptive effects of morphine are mediated by S_1 receptors in the dorsal horn and the recent findings of Zemlan *et al.* (46) and Paul and Phillips (26), suggested that the role of S_2 receptors in morphine-induced analgesia required further study.

GENERAL METHOD

This section describes the methods common to all three of the experiments reported here. Any specific modifications or additions to this general methodology are described in the Method section of each experiment.

Subjects

Serving as subjects in each of these experiments were 300–450 g male rats (Charles River Canada, St. Constance, Quebec) housed individually with free access to Purina lab chow and water. All subjects were tamed prior to the start of baseline testing.

Apparatus

The hot-water immersion tail-flick test (7) was used to assess nociception. All tail-flick tests were conducted in a 6.5×6.5×20 cm chamber. Each rat's tail was drawn through a 2 cm wide slot at the rear of the chamber, and approximately 5 cm of the tail was submerged in a 52°C water bath. The time that it took each rat to remove its tail from the bath, that is the tail-flick latency, was recorded electronically. On the few trials that a subject did not respond within 10 sec, its tail was removed from the bath by the experimenter to prevent tissue damage, and a tail-flick latency of 10 sec was assigned. All testing occurred in the colony room during the last 5 hr of the light phase of the 12/12 hr light/dark cycle.

Procedure

Baseline tail-flick latencies were recorded on 5 or 6 consecutive days. Each daily test session consisted of five tail-flick tests administered one every 30 min. Each subject spent the 30-min intertest intervals in its home cage. The first of the four drug-test sessions occurred on the day after the last baseline session, and the remaining three occurred at 4-day intervals thereafter. Baseline sessions were conducted on each of the 3 days between consecutive drug-test days. The drug-test sessions were identical to the baseline sessions except that immediately after the first tail-flick test on each drug-test day, each rat was injected with either the appropriate dose of a serotonin antagonist or its vehicle, followed either by an analgesic or its saline vehicle. Thus, there were four basic conditions in each study: vehicle-vehicle, analgesic-vehicle, vehicle-antagonist, and analgesic-antagonist. In each study, each subject was tested under all four of these treatment combinations in a counterbalanced sequence.

Drugs

Morphine sulphate was dissolved to a concentration of 10 mg/ml in saline and injected in a volume of 1 ml/kg. Pirenperone and ketanserin HCl were dissolved in 0.001 M citric acid (pH adjusted to 6 with NaOH) and injected subcutaneously in a volume of 0.2 ml/kg. Ketamine HCl was purchased in liquid form at a concentration of 50 mg/ml and was injected in a volume of 2 ml/kg.

Statistical Analysis

For each dose of the antagonist an ANOVA was used to assess the significance of the within-group differences in tail-flick latencies for the four postinjection intervals. In all cases, the main effects of both test interval and treatment and the interval × treatment interaction were significant at the 0.05 alpha level. Newman-Keul's post-hoc comparisons were then used to assess the significance of overall treatment differences and differences at specific test intervals. The alpha level was 0.05 for all post-hoc comparisons.

EXPERIMENT 1: EFFECT OF PIRENPERONE ON MORPHINE ANALGESIA

The first experiment was designed to confirm and extend the preliminary finding (26) that the selective S_2 antagonist, pirenperone (6, 7, 19) blocks morphine-induced analgesia. Confirmation of this finding was deemed necessary because of its inconsistency with the current view of morphine-induced analgesia.

In the Paul and Phillips experiment, pirenperone was injected 60 min before morphine, and the rats were tested only once, 15 min after the morphine injection; whereas, in Experiment 1, pirenperone and morphine were injected concurrently, and the rats were tested repeatedly throughout the 2-hr session.

METHOD

Following 5 days of baseline testing the 39 rats serving as subjects were randomly divided into three groups. The analgesic effect of 10 mg/kg of morphine or saline injected intraperitoneally was challenged with 0.08 (n=12), 0.16 (n=14), or 0.24 mg/kg (n=13) of pirenperone or its citrate vehicle administered subcutaneously.

RESULTS

Figure 1 illustrates the results of Experiment 1. Injection of 10 mg/kg of morphine sulphate by itself produced potent analgesia, as indicated by substantially longer tail-flick latencies at the 30-, 60-, and 90-min test intervals in the morphine-vehicle condition in comparison to those in the vehicle-vehicle condition at all three doses of pirenperone. The major finding of this study was that when 0.08, 0.16 or

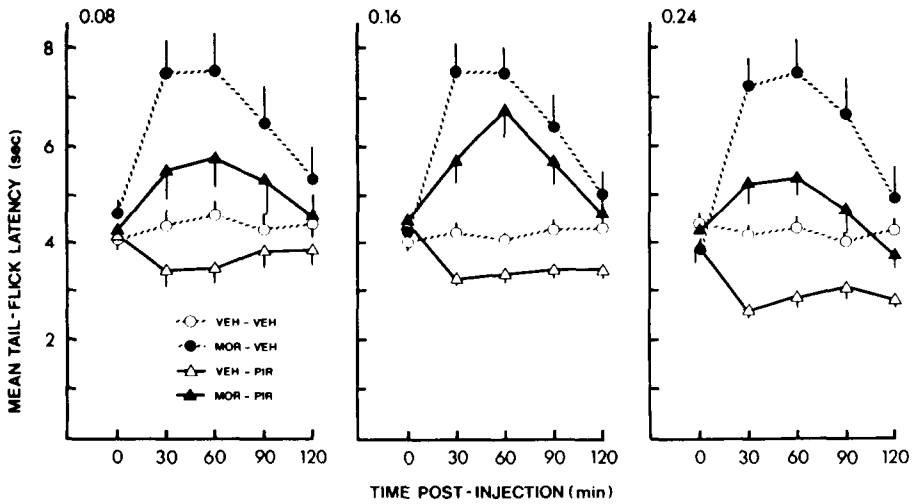

FIG. 1. The analgesic effect of 10 mg/kg of morphine sulphate challenged with three doses of pirenperone. Mean tail-flick latencies were assessed at 0, 30, 60, 90, and 120 min after the injections in each of the four treatments. The three graphs illustrate the effects of 0.08, 0.16, and 0.24 mg/kg doses of pirenperone (n=12, 14, and 13, respectively). The analgesic effect of morphine is illustrated by the difference between the VEH-VEH and MOR-VEH conditions. The effect of pirenperone by itself on mean tail-flick latency is illustrated by the difference between the VEH-VEH and VEH-PIR conditions. The attenuation of morphine-induced analgesia by pirenperone is illustrated by the difference between the MOR-VEH and MOR-PIR conditions.

0.24 mg/kg of pirenperone was injected with the morphine, the morphine-induced analgesia was significantly attenuated. A second important finding was that each of the three doses of pirenperone produced hyperalgesia when administered by themselves; that is, the tail-flick latencies in the vehicle-pirenperone condition were significantly shorter than those in the vehicle-vehicle control condition.

For all three groups, the overall ANOVAs revealed significant main effects for treatment [0.08 mg/kg group, $F(3,33)=15.1$, $p<0.0001$; 0.16 mg/kg group, $F(3,39)=17.0$, $p<0.0001$; 0.24 mg/kg group, $F(3,36)=31.7$ $p<0.0001$]. The analgesic effect of morphine was confirmed by the significance of the overall difference between the vehicle-vehicle treatment and the morphine-vehicle condition for each of the three groups (all three Newman-Keul's $p<0.05$). In each group, morphine produced a significant increase in mean tail-flick latency at the 30-, 60-, and 90-min test intervals (all nine Newman-Keul's $p<0.05$).

The tail-flick latencies of rats were significantly shorter at the 30-, 60-, and 90-min test intervals when they were injected with morphine and any of the three doses of pirenperone than when they were treated with morphine by itself (all nine Newman-Keul's $p<0.05$).

Although the mean tail-flick latencies of rats treated with each of the three doses of pirenperone by itself were shorter than when they received the vehicle-vehicle injections at every interval (excluding the 0-min interval), this effect reached statistical significance only at the 30- and 60-min test intervals after the 0.08 mg/kg dose of pirenperone and at all four intervals after the 0.24 mg/kg dose (Newman-Keul's $p<0.05$).

DISCUSSION

The results of Experiment 1 confirm the finding of Paul and Phillips (26) that the selective S_2 receptor blocker, pirenperone, attenuates the analgesic effect of morphine. Moreover, pirenperone, when administered by itself, produced hyperalgesia. Because pirenperone has a preferential affinity for S_2 receptors, this pattern of results provides further evidence that S_2 receptors play a significant role in nociception and in the analgesic effect of morphine.

Considering that S_2 receptors have not been found in the dorsal spinal cord (24,27), the fact that a selective S_2 blocker such as pirenperone can attenuate morphine-induced analgesia suggests that serotonin may be exerting its effect on nociception and analgesia through activation of serotonin receptors in the brain or peripheral nervous system. These results challenge the widely held view that serotonin exerts control over nociception and analgesia entirely through spinal cord receptors.

EXPERIMENT 2: EFFECT OF KETANSERIN ON MORPHINE ANALGESIA

Experiment 2 was designed to assess the effect of the selective S_2 antagonist ketanserin on morphine-induced analgesia. Although ketanserin and pirenperone have affinities for the same receptors (17) ketanserin does not readily penetrate the central nervous system (17,18). Janssen (17) has proposed that an effect seen at a wide range of doses of pirenperone but only at high doses of ketanserin can be attributed to the action of these drugs at central S_2 receptors. For example, in the sensitive LSD discrimination test, pirenperone is effective at 0.08 to 0.16 mg/kg, whereas an effect of ketanserin is not seen until a 5 to 10 mg/kg dose is used (7). Because S_2 receptors have been shown to exist in the periphery (43) as well as in the brain, it is not clear whether the antianalgesic effect of pirenperone demonstrated in Experiment 1 was mediated by central or peripheral receptors. Accordingly, blockade of morphine-induced analgesia by low doses of ketanserin would implicate periph-

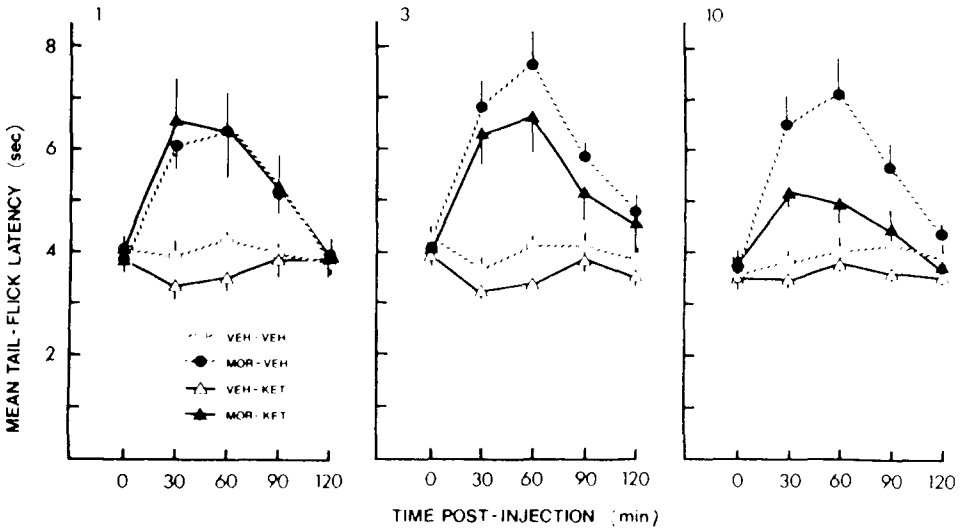

FIG. 2. The analgesic effect of 10 mg/kg of morphine sulphate challenged with three doses of ketanserin HCl. Mean tail-flick latencies were assessed at 0, 30, 60, 90, and 120 min after injections of rats in the four treatment conditions. The three graphs illustrate the 1, 3, and 10 mg/kg doses of ketanserin (n=10, 11, and 11, respectively). The analgesic effect of morphine is illustrated by the difference between the VEH-VEH and MOR-VEH conditions. The effect of ketanserin by itself on mean tail-flick latency is illustrated by the difference between the VEH-VEH and VEH-KET conditions. The attenuation of morphine-induced analgesia by ketanserin is illustrated by the difference between the MOR-VEH and MOR-KET conditions.

eral S_2 receptors, whereas blockade restricted to high doses would support a role for central receptors.

METHOD

Following 6 days of baseline testing, the 32 rats serving as subjects in Experiment 2 were randomly divided into three groups. The analgesic effect of 10 mg/kg of morphine or saline injected intraperitoneally was challenged with 1 (n=10), 3 (n=11), or 10 mg/kg (n=11) of ketanserin HCl or its citrate vehicle administered subcutaneously.

RESULTS

Figure 2 illustrates the results of Experiment 2. Injection of 10 mg/kg of morphine sulphate by itself produced potent analgesia, as seen by substantially greater tail-flick latencies at the 30-, 60-, and 90-min test intervals in the morphine-vehicle condition in comparison to the vehicle-vehicle condition at all three doses of ketanserin. The major finding of this study was that the 10 mg/kg dose of ketanserin, but not the 1 or 3 mg/kg doses, attenuated morphine-induced analgesia.

For all three groups, the overall ANOVAs revealed significant main effects for treatment [1 mg/kg group, $F(3,27)=10.6$, $p<0.0001$; 3 mg/kg group, $F(3,30)=17.5$, $p<0.0001$; 10 mg/kg group, $F(3,30)=19.3$, $p<0.0001$]. The analgesic effect of morphine was confirmed by the significance of the overall difference between the vehicle-vehicle condition and the morphine-vehicle treatment for all three groups (all three Newman-Keul's $p<0.05$). Morphine produced a significant increase in mean tail-flick latency above the vehicle-vehicle baseline at the 30-, 60-, and 90-min test intervals in both the 3 and 10 mg/kg groups; but it produced significant increases at the 30- and 60-min intervals in the 1 mg/kg group (all eight Newman-Keul's $p<0.05$).

The tail-flick latencies of rats at the 30-, 60-, and 90-min test intervals were significantly shorter when they were injected with morphine and 10 mg/kg of ketanserin than when they received morphine alone (all three Newman-Keul's $p<0.05$). In contrast, 1 or 3 mg/kg of ketanserin did not significantly reduce the tail-flick latencies of morphine-treated subjects at any of the test intervals.

Although the mean tail-flick latencies were consistently shorter when the rats were treated with each of the three doses of ketanserin and the vehicle than when they received the vehicle-vehicle injections, this effect never reached statistical significance.

DISCUSSION

The fact that ketanserin significantly attenuated morphine-induced analgesia at the high 10 mg/kg dose provides further evidence that S_2 receptors are important for the mediation of morphine-induced analgesia. In Experiment 1, pirenperone, a drug that is pharmacologically similar to ketanserin (17), blocked morphine-induced analgesia at a dose much lower than the doses of ketanserin that failed to block it in this experiment. Beacuse of differences in central absorbtion of these two S_2 antagonists, this pattern of results provides evidence that the S_2 receptors that are important for morphine-induced analgesia are in the central nervous system.

EXPERIMENT 3: EFFECT OF KETANSERIN ON KETAMINE ANALGESIA

Experiment 3 was designed to assess the effect of S_2 receptor blockade on analgesia that is not mediated by a descending inhibitory system activated by supraspinal opioid receptors. The analgesia produced by ketamine appeared to meet this requirement (25,36). Three findings suggest that its

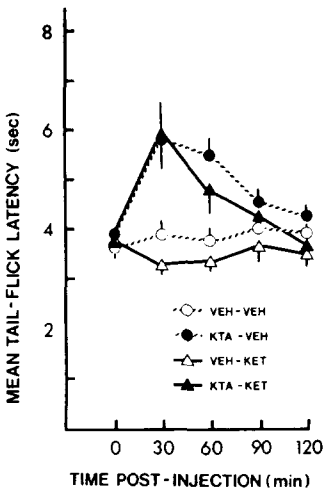

FIG. 3. The analgesic effect of 100 mg/kg of ketamine HCl challenged by 10 mg/kg of ketanserin HCl. Mean tail-flick latencies (N=15) were assessed 0, 30, 60, 90, and 120 min after injections in the four treatment conditions. The analgesic effect of ketamine is illustrated by the difference between the VEH-VEH and KTA-VEH conditions. The effect of ketanserin by itself on mean tail-flick latency is illustrated by the difference between the VEH-VEH and VEH-KET conditions. The lack of an effect of ketanserin on ketamine-induced analgesia is illustrated by the difference between the KTA-VEH and KTA-KET conditions.

analgesic effects are produced by direct action on spinal receptors rather than by the activation in the brainstem of a descending inhibitory pathway: First, although transection of the spinal cord attenuates morphine-induced analgesia (16,38), spinal cord transection produces a 9-fold increase in the potency of ketamine-induced analgesia (29); second, spinal cord transection enhances the inhibitory effect of ketamine on dorsal horn nociceptive neurons (25); and third, naloxone injected into the PAG attenuates morphine-induced but not ketamine-induced analgesia (36).

METHOD

The 15 rats serving as subjects in Experiment 3 received 5 days of baseline testing. Then the analgesic effect of 100 mg/kg ketamine hydrochloride was challenged with 10 mg/kg ketanserin, the dose of ketanserin that was found in Experiment 2 to block morphine-induced analgesia.

RESULTS

Figure 3 illustrates the results of Experiment 3. Injection of 100 mg/kg of ketamine produced a potent analgesia at the 30- and 60-min test intervals. The major finding of Experiment 3 was that the dose of ketanserin that attenuated morphine-induced analgesia in Experiment 2 did not significantly attenuate ketamine-induced analgesia.

The overall ANOVA revealed a significant main effect of treatment, $F(3,45)=13.8$, $p<0.0001$. The analgesic effect of ketamine was confirmed by the significance of the overall difference between the vehicle-vehicle and the ketamine-vehicle conditions (Newman-Keul's $p<0.05$). Ketamine produced a significant increase in tail-flick latency above the vehicle-vehicle baseline at both the 30- and 60-min test intervals (both Newman-Keul's $p<0.05$).

The lack of significant effect of ketanserin on ketamine-induced analgesia was indicated by the lack of significant differences between the mean tail-flick latencies of the rats in the ketamine-ketanserin condition and the ketamine-vehicle condition (all four Newman-Keul's $p>0.05$).

As in Experiment 2 the mean tail-flick latencies of rats in the vehicle-ketanserin condition were shorter than those in the vehicle-vehicle condition at each of the four intervals although none of these differences was statistically significant (all five Newman-Keul's $p>0.05$).

DISCUSSION

The results of Experiment 3 show that blockade of S_2 receptors with ketanserin does not attenuate ketamine-induced analgesia. This finding, when considered in combination with the results of Experiment 2, establishes that the antianalgesic effect of ketanserin is not completely general and suggests that it might be specific to morphine-induced analgesia.

GENERAL DISCUSSION

The general purpose of these experiments was to assess the role of S_2 receptors in the mediation of morphine-induced analgesia. Experiment 1 assessed the effect of the S_2-selective receptor blocker, pirenperone, on morphine-induced analgesia. The results of this experiment confirmed and extended the finding of Paul and Phillips (26) that pirenperone attenuates the analgesic effect of morphine. However, in contrast to the findings of Paul and Phillips, pirenperone by itself was found to produce significant hyperalgesia. The reason for this inconsistency is not clear, but it could be attributable to methodological differences. Paul and Phillips assessed the effect of pirenperone on tail-flick latencies in a single test, 75 min after injection of pirenperone, whereas in Experiment 1 this effect was assessed four times, once every 30 min.

Because S_2 receptors have been shown to exist in the periphery (43) as well as in the brain, it was not clear from Experiment 1 whether the antianalgesic effect of pirenperone was mediated by central or peripheral receptors. Thus, in Experiment 2, the analgesic effect of morphine was challenged with the S_2 receptor blocker, ketanserin. This drug is pharmacologically similar to pirenperone, however, it does not readily enter the central nervous system (17,18). Because ketanserin attenuated morphine-induced analgesia only at a very high dose (10 mg/kg), Experiment 2 provided evidence that the antianalgesic effect of S_2 receptor blockade is mediated by receptors within the central nervous system. Although ketanserin administered by itself slightly reduced mean tail-flick latencies after drug injection, this hyperalgesic effect did not reach statistical significance.

There is no way of telling from the first two experiments whether the antianalgesic effect of S_2 receptor blockers is specific to morphine-induced analgesia. Can S_2 receptor blockers affect analgesia produced by agents thought to act through different circuits? Morphine is thought to produce most of its analgesic effect through a descending PAG-to-NRM-to-spinal cord inhibitory system (1, 2, 13, 22). In contrast, ketamine is thought to produce its analgesic effect through a direct action on spinal cord receptors (25,36). Accordingly, in Experiment 3, ketamine-induced analgesia was challenged with a dose of ketanserin that had attenuated morphine-induced analgesia in Experiment 2 (10 mg/kg). The finding that ketanserin did not attenuate ketamine-induced analgesia established that the antianalgesic effect of S_2 recep-

tor blockade is not general and that it may be specific to analgesia produced by morphine or other drugs that act on the same system. This result appears to conflict with those of Pekoe and Smith's (29) finding that the nonspecific serotonin receptor blocker, methysergide, attenuates ketamine-induced analgesia. However, these authors also found that methysergide antagonized ketamine-induced antinociception in rats with spinal transections, suggesting a role for spinal serotonin receptors.

In each experiment, the S_2 blocker injected alone produced a reduction in mean tail-flick latencies at all postinjection test intervals. In Experiment 1, pirenperone, by itself, significantly reduced tail-flick latencies at all doses tested. In Experiments 2 and 3, tail-flick latencies were slightly reduced at all doses tested, however, these differences were not statistically significant. The mild hyperalgesia that seems to be produced by these S_2 antagonists suggests that they may block a tonic inhibition of nociception. However, it is not clear if this hyperalgesia is mediated by the same central S_2 receptors as the antianalgesic effect.

Despite the wide acceptance of the theory that morphine-induced analgesia is mediated by serotonergic synapses in the dorsal horn, the evidence for this view remains equivocal (4, 20, 34). The identification of serotonin receptor subtypes raises one possible explanation for conflicting results. The subsequent development of drugs that bind selectively to these subtypes provides a powerful methodology for testing the hypothesis that different serotonin receptor subtypes may serve different functions in the mediation of morphine-induced antinociception. Zemlan *et al.* (46) demonstrated that the selective activation of S_1 receptors in the spinal cord facilitates nociceptive reflexes, while inhibiting ascending nociceptive neurons. These authors propose that there are two different systems that use serotonin to modulate pain perception. The present findings and those of Paul and Phillips (26) suggest that a third system, mediated by S_2 receptors, may also have a role in morphine-induced antinociception.

Because S_2 receptors have not been found in the spinal cord, an effect of drugs that are selective for S_2 receptors can be seen as being mediated by supraspinal receptors. Thus the attenuation of morphine-induced analgesia by pirenperone and ketanserin seen in these experiments implicates supraspinal serotonin receptors. This view is supported by the finding that serotonin applied to NRM neurons produces a potent inhibition of tail-flick responding (20,34). The current view that serotonin mediates antinociception only via spinal cord receptors cannot account for these results. We must also consider the role of supraspinal serotonin receptors in morphine-induced analgesia.

A closing word of caution is in order. Pirenperone and ketanserin are selective in the sense that they are potent blockers of S_2 receptor sites but have virtually no activity at S_1 sites [6, 7, 19]. However, recently both pirenperone and ketanserin have been found to also be moderately potent blockers of alpha-adrenergic receptors (17). Thus, it is possible that the attenuation of morphine-induced analgesia by pirenperone and ketanserin observed in these experiments was mediated by their action at alpha-adrenergic receptors, or at other as yet unimplicated receptors. A recent study in which we rule out the possibility that the antianalgesic effects of pirenperone and ketanserin are mediated by alpha-adrenergic receptors is currently being prepared for publication.

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