Pergamon

Pharmacology Biochemistry and Behavior, Vol. 47, No. 3, pp. 575–578, 1994

0091-3057/94 $6.00 + .00

Monoamine Systems in the Discriminative Effects of Spiradoline, a Kappa-Opioid Agonist

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Received 20 April 1993

HOLTZMAN, S. G. AND G. F. STEINFELS. *Monoamine systems in the discriminative effects of spiradoline, a kappa-opioid agonist.* PHARMACOL BIOCHEM BEHAV 47(3) 575–578, 1994.—The results of studies on mice indicate that the antinociceptive effects of kappa-opioid agonists are due, in part, to activation of the 5-HT_2 type of serotonin receptor. One objective of this study was to determine if the discriminative effects of spiradoline, a kappa-opioid agonist, are mediated by 5-HT_2 receptors in rats also. A second objective was to confirm findings that dopamine receptor antagonists produce spiradoline-like discriminative effects (Ohno et al., 1992). Rats were trained to discriminate between spiradoline (3.0 mg/kg) and saline in a discrete-trial avoidance/escape procedure. In subsequent tests of stimulus generalization, the discriminative effects of spiradoline were not mimicked by fenfluramine (0.3–10 mg/kg) or fluoxetine (1.0–10 mg/kg), drugs that enhance serotonergically mediated neurotransmission, nor were they blocked by the 5-HT_2 antagonists pirenperone (0.01–1.0 mg/kg) and ketanserin (0.1–10 mg/kg), or potentiated by fluoxetine pretreatment. Neither the dopamine receptor antagonists haloperidol (0.01–0.3 mg/kg) and sulpiride (3.0–100 mg/kg) nor the agonists apomorphine (0.03–0.3 mg/kg) and *d*-amphetamine (0.1–3.0 mg/kg) engendered spiradoline-like discriminative effects. These results demonstrate further the pharmacological specificity of the discriminative effects of spiradoline, but provide no evidence for mediation by serotonergic or dopaminergic systems.

Drug discrimination Kappa-opioid agonist Spiradoline Serotonin receptor antagonists Dopamine receptor antagonists Fenfluramine Fluoxetine

THE interactions of kappa-opioid agonists with monoamine neurotransmitter systems in the brain appear to underlie some of the analgesic and other behavioral effects of these drugs. For example, U50,488, a prototypical kappa-opioid agonist, enhanced the release of serotonin from cortical and striatal brain slices of mice (10). This facilitatory effect on serotonergically mediated neurotransmission appears to contribute to the antinociceptive effect of U50,488. In the tail-flick and hot-plate tests in mice, the antinociceptive effect of U50,488 was reduced substantially by the administration of ketanserin or pirenpirone, selective antagonists at the 5-HT_2 receptor, or by treatment with reserpine or para-chlorophenylalanine to lower the amount of serotonin in the brain (10,19).

On the other hand, several kappa-opioid agonists have been shown to decrease the release of dopamine from dopaminergic terminal areas in rat brain (8,14,18). The reduction of dopaminergically mediated neurotransmission appears to contribute to the discriminative stimulus effects of this class of compounds. Rats trained to discriminate the selective kappa-opioid agonist spiradoline generalized completely or almost completely to the dopamine receptor antagonists haloperidol and sulpiride (15).

One objective of the current study was to determine whether or not serotonergic systems contribute to the discriminative stimulus effects of kappa-opioid agonists in the rat. Rats trained to discriminate spiradoline from saline (13) were tested for stimulus generalization to fenfluramine and fluoxetine, drugs that enhance serotonergically mediated neurotransmission by, respectively, releasing serotonin from neurons (16)

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and inhibiting its neuronal reuptake (3). In addition, ketanserin and pirenpirone were tested for their ability to block the discriminative effects of the spiradoline training dose. A second objective of the study was to confirm the findings of Ohno et al. (15) that dopamine receptor antagonists produce spiradoline-like discriminative effects.

METHOD

Subjects

Fourteen male rats of Sprague–Dawley descent (Charles River Laboratories, Wilmington, MA) were used. Three were experimentally naive at the start of the study; the others had been trained previously to discriminate spiradoline and had been tested for stimulus generalization with a range of doses of one or more opioid drugs (13).

Procedure

The rats were trained in a discrete-trial avoidance/escape procedure to discriminate between SC injections of saline and 3.0 mg/kg of spiradoline, which were injected on alternate days, 30 min before a 20-trial session (13). Trial onset was signalled by concurrently illuminating the house light of the experimental chamber and turning on a white noise. Five seconds later a 1.0–1.5-mA current was distributed to the grid floor of the chamber in 1.0-s pulses every 3.0 s until the animal completed a two-response chain, consisting of pressing an observing lever mounted in one wall of the chamber and then pressing one of two choice levers in the opposite wall. The observing response turned off the white noise, and the choice response, if appropriate for what the rat had been injected with before the session, turned off the house light and ended the trial. The next trial began 50 s later. Half of the rats were trained to press the left choice lever in sessions that followed an injection of spiradoline and the right choice lever in sessions that followed saline administration; choice lever assignments were reversed for the other half of the rats. The behavior of a rat was considered to be under the stimulus control of spiradoline and saline when the animal could reliably complete 18 or more trials out of 20 on the choice lever that was appropriate for the substance injected before the session. Once the criterion for stimulus control of behavior was met, tests of stimulus generalization to novel drug conditions were conducted, usually twice each week. During test sessions, which also consisted of 20 trials, a trial was ended by the first response on either choice lever following a response on the observing lever regardless of what the animal had been injected with before the session. Successive test sessions were separated by at least 3 days. Training sessions with saline and 3.0 mg/kg of spiradoline were conducted on at least 3 days of each week to maintain stable discriminative performance.

Drugs

Spiradoline methane sulfonate (U62,066) was obtained from The Upjohn Company (Kalamazoo, MI) and fluoxetine hydrochloride from Lilly Research Laboratories (Eli Lilly and Company, Indianapolis, IN). The other drugs were purchased commercially (Research Biochemicals, Inc., Natick, MA or Sigma Chemical Company, St. Louis, MO): *d*-amphetamine sulfate, apomorphine hydrochloride, fenfluramine hydrochloride, haloperidol, ketanserin hydrochloride, pirenperone hydrochloride, sulpiride hydrochloride. Haloperidol was dissolved in three parts of 8.5% lactic acid and two parts of 1.0 N sodium hydroxide. All of the other drugs were dissolved in 0.9% saline solution (apomorphine solutions were prepared immediately before use). Drugs were injected SC in a volume of 1.0 ml/kg body weight (2.0 ml/kg for high doses of sulpiride). The doses of spiradoline refer to the salt; all other doses are expressed as the free base of the drug. For each drug, doses were administered to five to six rats in a random sequence that also included the drug vehicle alone.

Data

Discrimination data are presented as the average number of trials completed on the choice lever appropriate for spiradoline; the remaining trials of the 20-trial session were completed on the saline-appropriate choice lever unless stated otherwise. The dose of spiradoline resulting in selection of the spiradoline-appropriate lever in 10 trials per session (ED_{50}) was estimated for individual animals by linear regression of the ascending limb of the stimulus generalization curve, using 3–4 points. Average ED_{50}s and 95% confidence limits were then calculated.

RESULTS

All of the drugs tested for stimulus generalization with spiradoline occasioned responding almost exclusively on the choice lever appropriate for saline: *d*-amphetamine (0.1–3.0 mg/kg), apomorphine (0.03–0.3 mg/kg, fenfluramine (0.3–10 mg/kg), fluoxetine (1.0–10 mg/kg), haloperidol (0.01–0.3 mg/kg), and sulpiride (3.0–100 mg/kg). The most trials completed on the spiradoline-appropriate choice lever with any of these drugs was an average of 1.2 (n = 5) after 0.1 mg/kg of haloperidol. No individual animal completed more than four trials on the spiradoline-appropriate choice lever at any dose of these six drugs. Some of the drugs disrupted behavior at high doses, setting a ceiling on the upper dose that could be tested. Four out of five rats completed less than 20 trials (average: 11 trials) at 3.0 mg/kg of *d*-amphetamine, five out of five at 0.3 mg/kg of haloperidol (average: 10.4 trials), and one out of five at 10 mg/kg of fluoxetine.

Figure 1 shows that the discriminative effects of spiradoline were largely unaffected by drugs that either increase or decrease serotonergically mediated neurotransmission. The average ED_{50} for spiradoline was 0.74 (0.33–0.94) mg/kg when the drug was tested alone and 0.93 (0.36–2.09) mg/kg following pretreatment with 10 mg/kg of fluoxetine (Fig. 1A). Neither ketanserin (0.1–10 mg/kg) nor pirenperone (0.01–1.0 mg/kg) had a consistent or large effect on the stimulus control of behavior by the training dose of spiradoline (Fig. 1B). The combination of pirenperone and 3.0 mg/kg of spiradoline was disruptive to behavioral performance. Two out of six animals did not complete all 20 trials of the session that followed the administration of 0.1 mg/kg of pirenperone and spiradoline, and five out of six completed less than 20 trials (average: 18 trials) at 1.0 mg/kg of pirenperone and spiradoline.

DISCUSSION

The results of this study provide no evidence that the discriminative effects of spiradoline in the rat are mediated prominently by either enhanced release of serotonin and activation of 5-HT_2 receptors or by inhibition of dopamine release. The 5-HT_2 antagonists pirenperone and ketanserin, which blocked the analgesic effects of U50,488 in mice (20), had no consistent effect on the stimulus control of behavior by spiradoline. The antagonists were tested over a range of

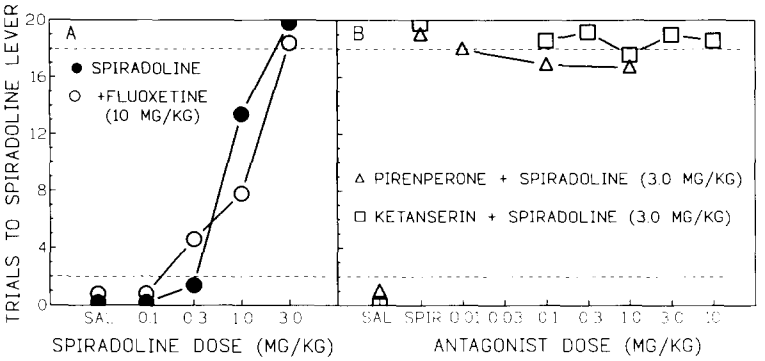

FIG. 1. The discriminative effects of spiradoline are largely unaffected by pretreatment with: (A) fluoxetine (10 mg/kg), an inhibitor of the neuronal reuptake of serotonin; (B) pirenperone (0.01–1.0 mg/kg) or ketanserin (0.1–10 mg/kg), 5-HT_2 receptor antagonists. Fluoxetine (n = 5), pirenperone (n = 6), or ketanserin (n = 5) were injected 60 min before a session and either 0.1–3.0 mg/kg of spiradoline (A) or 3.0 mg/kg of spiradoline (B) was injected 30 min before a session. Each point is the mean number of trials completed on the spiradoline-appropriate choice lever in a 20-trial session; the remaining trials of the session were completed on the choice lever appropriate for saline, except after 0.1 and 1.0 mg/kg of pirenperone, when not all of the animals completed all of the trials (see text). Points above SAL and SPIR indicate the number of trials completed on the spiradoline-appropriate lever in that sessions that followed the administration of saline or 3.0 mg/kg of spiradoline alone, respectively. The upper and lower horizontal dashed lines indicate the minimum levels at which discrimination performance of the rats was maintained in training sessions with 3.0 mg/kg of spiradoline or saline, respectively.

doses adequate to block serotonergically mediated discriminative effects. Those of LSD were blocked completely by 0.08–0.16 mg/kg of pirenperone and by 3.2 mg/kg of ketanserin (6,7), and those of mescaline by as little as 0.04 mg/kg of pirenperone and 0.25 mg/kg of ketanserin (1), doses well below the highest ones that we tested. In a study published recently (5), the discriminative effects of a 5.6 mg/kg training dose of U50,488 in pigeons were reduced 50–60% by 0.32 and 1.0 mg/kg of ketanserin (but only 20–30% by 3.2 and 10 mg/kg). In addition, fenfluramine and fluoxetine neither mimicked the discriminative effects of spiradoline nor, in the case of fluoxetine, potentiated them. Fenfluramine is discriminable in rats at doses of 2.0–3.0 mg/kg, and the discriminative effects of it and its active metabolite, norfenfluramine, are blocked by pirenperone (4,9,17). In rats discriminating the serotonin precursor 5-hydroxytryptophan, discriminative effects are potentiated by pretreatment with 5.0 mg/kg of fluoxetine (2). Therefore, fenfluramine and fluoxetine were tested at doses sufficient to enhance serotonergically mediated neurotransmission.

Studies on the role of serotonin in the antinociceptive effects of kappa-opioid agonists were performed with U50,488 (see the Introduction), whereas we used spiradoline. Therefore, our negative results could be a consequence of the particular kappa-opioid agonist that was tested. This seems unlikely, however. Spiradoline is closely related structurally to U50,488 and both drugs have similar profiles of antinociceptive (19) and discriminative effects (13) in the rat, and of discriminative effects in the pigeon (5). Perhaps of greater importance, the studies on the role of serotonin in the antinociceptive effects of U50,488 were performed on mice. Species differences in the discriminative effects of kappa-opioid agonists are well documented (12). Indeed, in pigeons trained to discriminate 5.6 mg/kg of U50,488 from saline, U50,488-appropriate lever selection occasioned by the training dose was blocked completely by 8-hydroxy (di-*N*-propylamino)-tetralin (8-OH-DPAT), a selective 5-HT_{1A} receptor agonist, as was the U50,488-appropriate lever selection occasioned by 3.2 mg/kg of spiradoline (5). In addition, the discriminative effects of the U50,488 training dose in the pigeon were blocked partially by several other drugs that reduce serotonergically mediated neurotransmission: 3-tropanyl-3,5-dichlorobenzoate (MDL72222), a 5-HT_3 antagonist, para-chlorophenylalanine, which depletes neuronal stores of serotonin, and ketanserin. Therefore, in contrast to the situation in the rat, the serotonergic system appears to have a prominent role in the discriminative effects of kappa-opioid agonists in the pigeon.

That neither haloperidol nor sulpiride engendered spiradoline-like discriminative effects is at variance with the results of a study by Ohno et al. (15). There are a number of methodological differences between the two studies, one or more of which could account for the seemingly discrepant findings. Ohno et al. used Wistar rats, a food reinforcement procedure, and a training dose of 1.0 mg/kg, IP. We used a Sprague-Dawley line of rats, an avoidance/escape procedure, and a 3.0 mg/kg, SC, training dose of spiradoline. More notable, however, is that Ohno et al. were unable to block the discriminative effects of spiradoline with naloxone. In contrast, we have found the discriminative effects of spiradoline to be blocked surmountably by naltrexone (13) as well as by naloxone and other opioid antagonists (unpublished observations). Therefore, in our studies, the discriminative effects of spiradoline have the characteristics of mediation by the kappa-opioid receptor. In the study by Ohno et al. (15), the discriminative effects of spiradoline must have derived largely from a nonopioid (i.e., naloxone-insensitive) component of drug action.

Negative results are rarely definitive; the present study is no exception. For example, it is possible that the discriminative effects of kappa-opioid agonists in the rat are mediated by serotonin receptors other than or in addition to 5-HT_2, as they seem to be in the pigeon (5). It is also possible that more extreme manipulation of serotonergic or dopaminergic function would reveal a role for one of these neurotransmitter systems in kappa-opioid-induced stimulus control of behavior. These possibilities remain to be tested. Nevertheless, our results suggest that it would be most productive for future studies on the mechanisms of stimulus control of behavior by kappa-opioid drugs in the rat to focus on neurochemical systems other than the serotonergic and dopaminergic systems. That readily discriminable doses of *d*-amphetamine, apomorphine, and fenfluramine lacked spiradoline-like discriminative effects demonstrates further the pharmacological specificity of spiradoline-induced stimulus control of behavior.

ACKNOWLEDGEMENTS

Technical assistance was provided by Christina Schad. We thank The Upjohn Company and Eli Lilly and Company for contributing drugs. This research was supported, in part, by grant DA00541 and Research Scientist Award K05 DA00008, both from the National Institute on Drug Abuse.

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