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Further Characterization of the Discriminative Stimulus Effects of Spiradoline

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HOLTZMAN, S. G. *Further characterization of the discriminative stimulus effects of spiradoline.* PHARMACOL BIOCHEM BEHAV **66**(3) 517–522, 2000.—The results of a previous study in rats indicated that spiradoline has pharmacologically selective discriminative effects that are mediated by kappa-opioid receptors. However, the training dose, 3.0 mg/kg, increased response latencies, suggesting that it was relatively high. The current study was performed to characterize further the discriminative effects of spiradoline by using a lower training dose, 1.0 mg/kg, and testing a larger number of drugs for generalization with spiradoline. Rats were trained in a discrete-trial avoidance/escape procedure to discriminate 1.0 mg/kg spiradoline, SC, from saline in an average of 19.7 sessions; response latencies after saline and spiradoline were not different from each other. The rats generalized dose dependently and completely to other kappa-opioid agonists that have relatively high efficacy: ethylketocyclazocine, U69,593, and U50,488. They generalized partially to ketocyclazocine, (−)-N-allylnormetazocine, and DuP 747, and not at all to cyclazocine, butorphanol, nalorphine, and pentazocine, discriminable opioids that have relatively low efficacy at kappa-opioid receptors, or to morphine and dextromethorphan, discriminable drugs that do not act at kappa-opioid receptors. The discriminative effects of spiradoline were unaffected by the mu-opioid antagonist β-funaltrexamine, but were blocked completely for at least 4 weeks by the kappa-opioid antagonist nor-binaltorphimine. Thus, spiradoline-like stimulus control of behavior remains kappa-opioid selective, and continues to have a high efficacy requirement even at a training dose that does not impair performance. © 2000 Elsevier Science Inc.

Drug discrimination Kappa opioid Morphine Opioid antagonists Spiradoline

SPIRADOLINE is a structural congener of U50,488 and U69,593, opioid agonists selective for the kappa-opioid receptor (13,26). Like its two chemical relatives, spiradoline has analgesic effects in rodents, and these are blocked only by doses of naloxone that are significantly higher than doses that block the analgesic effects of the mu-opioid agonist morphine (27). Consistent with a profile as a kappa-selective opioid, spiradoline exhibits analgesic crosstolerance to U50,488 but not to morphine (27) and binds to kappa-opioid receptors with an affinity that is 50–100-fold higher than its affinity for mu-opioid receptors (3,15).

Drug discrimination procedures have afforded in vivo assays useful for distinguishing drug effects mediated at kappa-opioid receptors from those mediated at mu-opioid receptors and for characterizing and quantifying those drug-receptor interactions (5). In rats, spiradoline substitutes as a discriminative stimulus for the kappa-opioid agonist bremazocine (16) but not for morphine (8), and in rhesus monkeys it substitutes for the kappa-opioid agonist ethylketocyclazocine [EKC; (3)]. Similar to results obtained in assays of analgesic activity, opioid antagonists, such as naloxone and naltrexone, are less potent in blocking the discriminative effects of spiradoline than they are in blocking the discriminative effects of morphine (8,9,16).

In an early evaluation of the discriminative effects of spiradoline, rats were trained to discriminate between 3.0 mg/kg of the drug and saline. These rats generalized completely to a high dose of U50,488, 30 mg/kg, but did not generalize to morphine or EKC or to the mixed-action opioids butorphanol and nalorphine, which have a kappa-opioid component of action. The training dose of spiradoline was selected on the basis of potency in the rat tail-flick test of analgesia (27). It appears to have been relatively high, as it significantly slowed the response time of the animals during discrimination test sessions (8). Training dose is an important determinant of the pattern of stimulus generalization, especially in the case of opioids that act at more than one type of receptor and/or have intrinsic efficacy lower than that of the training drug (2,17,22,23). For example, low-efficacy agonists might generalize with a low training dose of a high-efficacy agonist but fail to generalize with a high training dose of that same agonist.

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This study was performed to characterize further the discriminative stimulus effects of spiradoline in rats by training the animals to discriminate a lower dose than was used previously and by testing a larger number of drugs for stimulus generalization. Among these drugs were the opioids that had been tested in rats trained with 3.0 mg/kg (8), the spiradoline congeners U69,593 and DuP 747 (10), and the benzomorphan derivatives ketocyclazocine, cyclazocine, pentazocine, and (−)-N-allyl-normetazocine. In addition, experiments were conducted to determine the influence of two receptor-selective opioid antagonists on the discriminative effects of spiradoline: β-funaltrexamine (β-FNA), an irrerversible antagonist at the mu-opioid receptor (28), and nor-binaltorphimine (nor-BNI), a pseudoirreversible antagonist at the kappa-opioid receptor (11,18). As a control, these antagonists also were tested in a separate group of rats that had been trained to discriminate between morphine and saline.

METHOD

Subjects

The subjects were male rats of Sprague–Dawley descent (Charles River Laboratories, Raleigh, NC). Twelve were experimentally naive at the beginning of discrimination training, and weighed 250–300 g. Six had been trained previously to discriminate between SC injections of 3.0 mg/kg morphine and saline in the same manner that the experimentally naive rats were trained to discriminate between spiradoline and saline [vida infra; also, see (20)]. They had been tested for stimulus generalization to a variety of opioid drugs; the last of those tests was approximately 2 weeks before the rats were used in the current study. The morphine-trained rats weighed 500–600 g at the time that this study was performed. Between experiments the rats were housed two per cage in a colony room that was maintained on a 12-h light/dark cycle. Food and water always were available in the home cage.

Drug Discrimination Training

The experimentally naive rats were trained to discriminate between SC injections of saline and 1.0 mg/kg spiradoline in a discrete-trial avoidance/escape procedure (8,20). Spiradoline and saline were injected on alternate days, 30 min before the start of a 20-trial session. The rats were trained and tested in standard operant chambers that contained a single "observing" lever in one wall and two "choice" levers in the opposite wall. The choice levers were separated by a Plexiglas partition that extended 5.0 cm into the chamber and ran from floor to ceiling. Trial onset was signaled by illuminating the house light of the operant chamber and turning on a white noise. Five seconds later a constant current of 1.0–1.5 mA was distributed to the grid floor of the chamber in 1.0-s pulses every 3.0 s until the rat completed the two-response chain of pressing the observing lever and then pressing one of the two-choice levers. The response on the observing lever turned off the white noise; the response on the choice lever appropriate for what the rat had been injected with before the session (i.e., saline or 1.0 mg/kg spiradoline) extinguished the house light and ended the trial. A trial was recorded as correct if the rat emitted the response sequence of observing lever-appropriate choice lever, and as incorrect if the rat emitted the response sequence of observing lever-inappropriate choice lever-appropriate choice lever. A trial also ended after 30 s had elapsed without the rat emitting one of those two response sequences, and was recorded as an incomplete trial; the interval between trials was 50 s later. Half of the rats were trained to press the left choice lever in sessions that followed an injection of the training drug, and the right choice lever in sessions that followed an injection of saline; the designation of choice levers was reversed for the other half of the rats. The behavior of a rat was considered to be under the stimulus control of the training drug and saline when the animal completed at least 18 out of 20 trials correctly in four consecutive training sessions followed by two consecutive test sessions (see below); half of these sessions were preceded by an injection of saline, and half by an injection of 1.0 mg/kg spiradoline.

Stimulus-Generalization Tests

Test sessions were similar to training sessions with the important exception that a trail ended after the response sequence of observing lever-choice lever, regardless of which of the two choice levers was pressed. With the exception of the experiments that involved cumulative dosing, test sessions usually were conducted twice each week, 3–4 days apart. Training sessions preceded by an injection of either saline or 1.0 mg/kg spiradoline on alternate days usually were conducted three times each week. If a rat failed to complete at least 18 trials correctly in any training session, test sessions were suspended until the animal once again met that performance criterion in four consecutive training sessions.

All of the rats trained with spiradoline were tested first with various doses of spiradoline. Doses were administered in a random sequence that also included saline. Other drugs were then tested in subgroups of rats in an unsystematic order. Drugs were administered SC 30 min before a test session in a volume of 1.0–2.0 ml of solution per kg of body weight.

In experiments in which spiradoline or morphine was administered by cumulative dosing, four to five test sessions were held on the same day. Injections were given SC 20 min before a session, and the interval between trials was reduced from 50 to 30 s. Saline was injected before the first test session. At the end of that session the first drug dose was given (e.g., 0.1 mg/kg spiradoline) and the next session commenced 20 min later. At the end of the session another dose was administered (e.g., 0.2 mg/kg), increasing the cumulative dose (0.3 mg/kg) by a half-log unit. This sequence of drug injections and test sessions continued until the desired dose range had been tested. The procedure of cumulative dosing was conducted no more often than once per week. Standard training sessions were held on the other weekdays with these exceptions: training sessions were suspended for 1 week after the morphine-trained rats received β-FNA, and no training sessions were conducted after the spiradoline-trained rats received nor-BNI. The experiments with cumulative dosing were performed at the end of the study and in following order of drug pretreatments: none, β-FNA, nor-BNI.

Drugs

The drugs used and their source were: spiradoline methanesulfonate (Pharmacia & Upjohn Company, Kalamazoo, MI), cyclazocine, ketocyclazocine, and ethylketocyclazocine (EKC; Sterling-Winthrop Research Institute, Rensselaer, NY), morphine sulfate (Penick Corp., Newark, NJ), nalorphine hydrochloride (Merck Sharp & Dohme, West Point, PA), *trans*-3,4-dichloro-*N*-methyl-N-[2-(pyrrolidin-1-yl)-1,2,3,4-tetrahydronaphthalen-1yl]benzeacetamide methanesulfonate (DuP 747; Du Pont Merck Pharmaceutical Company, Wilmington, DE), butorphanol tartarte (Bristol-Myers Squibb, Evansville, IN), dextromethorphan hydrobromide and penta-

zocine hydrochloride (Sigma, St. Louis, MO), diprenorphine hydrochloride, 6-β-funaltrexamine hydrochloride (β-FNA), nor-binaltorphimine dihydrochloride (nor-BNI), *trans*-3,4-dichloro-*N*-methyl-N[2-(1-pyrrolidinyl)cyclohexyl]benzeneacetamide methanesulfonate hydrate (U50,488), (−)-N-allylnormetazocine hydrochloride [(−)-NANM], and (5α,7α,8β)-(+)-*N*-methyl-N-[7-(1pyrrolidinyl)-1-oxaspiro(4,5)dec-8-yl]-benzeneacetamide (U69,593; National Institute on Drug Abuse, Rockville, MD). Spiradoline, (−)-NANM, and morphine were dissolved in 0.9% sodium chloride solution. The drugs that were not salts, U69,593, EKC, ketocylazocine, and cyclazocine, were dissolved in three parts of 8.5% lactic acid to which two parts of 1.0 N sodium hydroxide were then added. The remaining drugs were dissolved in distilled water. All of the drugs except β-FNA and nor-BNI were injected SC in a volume of 1.0–2.0 ml per kg of body wt. β-FNA and nor-BNI were injected intracisternally in a volume of 10 μl per rat while the animal was anesthetized briefly with methoxyflurane. All drug doses refer to the free base.

Data Analysis

Discrimination data are presented as the average number of trials completed on the drug-appropriate (spiradoline or morphine) choice lever in a 20-trial session; the remaining trials of the session were completed on the choice lever appropriate for saline. The dose of a drug that would occasion selection of the drug-appropriate choice lever in 10 trials (ED_{50}) was estimated for individual rats by linear regression of the ascending portion of the stimulus–generalization curve, using logarithm_{10} dose and at least three points. In those cases where only two points defined the ascending portion of the curve, ED_{50}s were estimated by simple interpolation. The ED_{50}s for each rat were averaged to obtain a group mean and 95% confidence limits. Comparisons of log ED_{50}s were accomplished with either a Student's *t*-test or analysis of variance, followed by a Student–Newman–Keuls test.

The latency from the onset of a trial to the first observing response was recorded and summed over the 20 trials of the test sessions. Cumulative observing response latencies are presented as group means ± SEM. Response latency data were evaluated by analysis of variance for repeated measures; this was followed by Dunnett's test to compare mean response latencies after each dose of a particular drug with the mean response latency after the vehicle for that drug. The alpha level was set at 0.05.

RESULTS

The 12 rats satisfied the performance criteria for discrimination of 1.0 mg/kg spiradoline from saline in an average of 19.7 (range: 11–38) training sessions. The rats exhibited an orderly stimulus–generalization gradient over the dose range of 0.1–3.0 mg/kg spiradoline, responding primarily on the saline-appropriate choice lever after 0.1 mg/kg spiradoline and primarily on the spiradoline-appropriate choice lever after 1.0 or 3.0 mg/kg (Fig. 1). The ED_{50} of spiradoline was 0.37 (0.26–0.52) mg/kg. In test sessions that followed, an injection of saline (or other drug vehicle), the number of trials completed on the spiradoline-appropriate choice lever never exceeded an average of 0.4; for the sake of clarity, those data are not shown in the figure.

There was a significant main effect of spiradoline dose on observing response latency, $F(4, 44) = 6.63$, $p < 0.001$, which was due to the 3.0-mg/kg dose. This dose increased the observing response latency to 293 ± 44 s from 179 ± 17 s after saline ($p < 0.01$). By comparison, the average response latency after an injection of training dose of spiradoline, 1.0 mg/kg, was 187 ± 25 s.

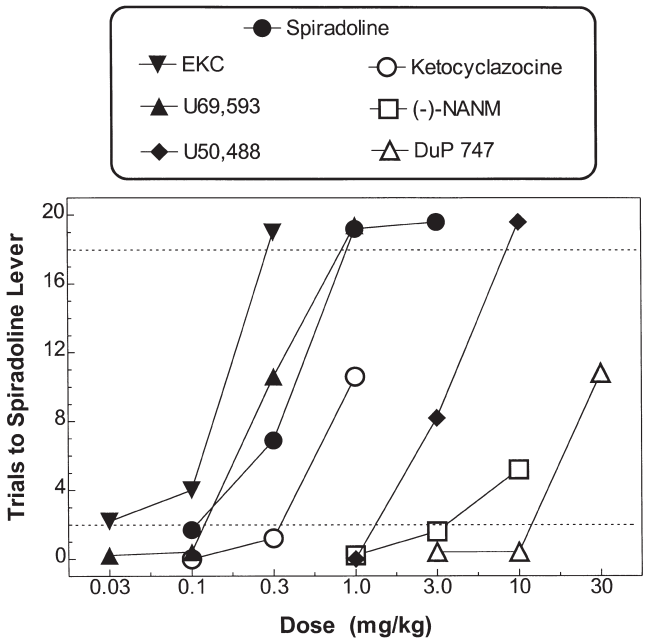

FIG. 1. Stimulus–generalization curves for drugs that rats trained to discriminate between SC injections of saline and 1.0 mg/kg spiradoline generalized to completely or partially. Each point is the mean number of trials completed on the spiradoline-appropriate choice lever in a 20-trial session; the remaining trials of the session were completed on the choice lever appropriate for saline. Means are based upon one observation in each of 12 (spiradoline) or 5 rats. The upper and lower dashed horizontal lines indicate the minimum levels at which discrimination performance was maintained in training sessions with spiradoline or saline, respectively.

The rats generalized dose dependently and completely to three drugs that were novel to them: EKC, which was almost three times more potent than spiradoline [ED_{50} = 0.13 (0.04–0.35 mg/kg, $p < 0.05$], U69,593, which was equipotent to spiradoline [ED_{50} = 0.31 (0.18–0.53) mg/kg, $p > 0.1$], and U50,488, which was one-ninth as potent as spiradoline [ED_{50} = 3.3 (1.58–6.93) mg/kg, $p < 0.01$]. None of the three drugs affected response latencies significantly over the dose ranges tested.

The rats generalized partially (i.e., an average of >2 but <18 trials to the spiradoline-appropriate lever) to three other drugs: ketocyclazocine, (−)-NANM, and DuP 747 (Fig. 1). Each of these drugs was tested up to the highest dose at which the animals were able to complete an entire test session; performance was impaired at doses 0.5 log units higher than the highest dose shown in Fig. 1. Out of these three drugs, over the dose ranges shown in Fig. 1, there was a significant main effect of dose on observing response latency only for ketocyclazocine, $F(4,16) = 10.64$, $p < 0.001$. The highest dose of ketocyclazocine increased the average response latency to 440 ± 52 s from 135 ± 7 s ($p < 0.01$) after the drug vehicle.

Seven drugs occasioned responding almost exclusively on the choice lever appropriate for saline over the dose ranges at which they were tested (Table 1). None occasioned an average of more than 1.6 trials to the spiradoline-appropriate choice lever at any dose. For five of the drugs, cyclazocine,

butorphanol, morphine, pentazocine, and dextromethorphan, the upper dose shown in the table was the highest at which the rats could complete an entire test session. The other two drugs were simply tested over the planned range of doses.

Antagonsm of the Discriminative Effects of Spiradoline and Morphine

The stimulus–generalization curve for spiradoline derived by administering cumulative doses (Fig. 2, Table 2) was similar to the curve derived previously by administering single doses (Fig. 1). Pretreatment of the spiradoline-trained rats with 10 μg β-FNA 24 h before testing had no significant effect on the stimulus–generalization curve for spiradoline. Nor did it prevent spiradoline from increasing latency to make the observing response. As before, there was a significant main effect of dose, $F(4, 12) = 3.31$, $p < 0.05$; the average observing response latency was 313 ± 89 s after 3.0 mg/kg spiradoline compared to 138 ± 13 s after saline ($p < 0.05$). In contrast to pretreatment with β-FNA, 24 h pretreatment with 10 μg nor-BNI almost completely blocked the discriminative effects of up to 10 mg/kg spiradoline, the highest dose tested, and 22 times the ED_{50} of spiradoline in the absence of pretreatment (Fig. 2, Table 2). In addition, nor-BNI blocked the effect of spiradoline on latency to make the observing response: the average response latency was 157 ± 27 s after saline and 165 ± 10 s after 10 mg/kg spiradoline. Spiradoline, 0.3–10 mg/kg, was tested at weekly intervals by cumulative dosing another three times. However, even at 4 weeks after treatment with nor-BNI, the rats continued to select only the saline-appropriate choice lever and response latencies remained unaffected by any dose of spiradoline. Saline was injected intracisternally into four other rats, which were tested 24 h later with 0.1–1.0 mg/kg spiradoline administered in cumulative doses, and then at weekly intervals with 0.1–3.0 mg/kg spiradoline for 3 weeks. These rats generalized completely to 1.0 mg/kg spiradoline in each test and had a significantly elevated response latency after 3.0 mg/kg on each of the three occasions that this dose was given (data not shown).

The results obtained with drug pretreatments of the rats trained to discriminate morphine were very different from those of the rats discriminating spiradoline. Twenty-four-hour pretreatment with 10 μg nor-BNI had no effect on the stimulus–generalization curve for morphine (Fig. 2, Table 2). In contrast, 24-hour pretreatment with 10 μg β-FNA blocked the discriminative effects of morphine surmountably, shifting the stimulus–generalization curve for morphine to the right by sevenfold (Fig. 2, Table 2). In experiments in which morphine was administered as single doses (vida supra), rats did not respond in a test session that followed 10 mg/kg (Table 1). However, rats pretreated with β-FNA completed all trials of test sessions that followed cumulative morphine doses of 10 and 30 mg/kg. Observing response latencies were not affected by any dose of morphine administered without pretreatment or after pretreatment with either nor-BNI or β-FNA.

TABLE 1

OPIOIDS THAT OCCASIONED SELECTION OF THE CHOICE LEVER APPROPRIATE FOR SALINE*

Drug	Dose Range Tested (mg/kg)
Cyclazocine	0.03–1.75
Butorphanol	0.1–3.0
Morphine	0.1–3.0
Diprenorphine	0.3–10
Pentazocine	1.0–17.5
Nalorphine	1.0–30
Dextromethorphan	1.0–30

*n = 5/drug.

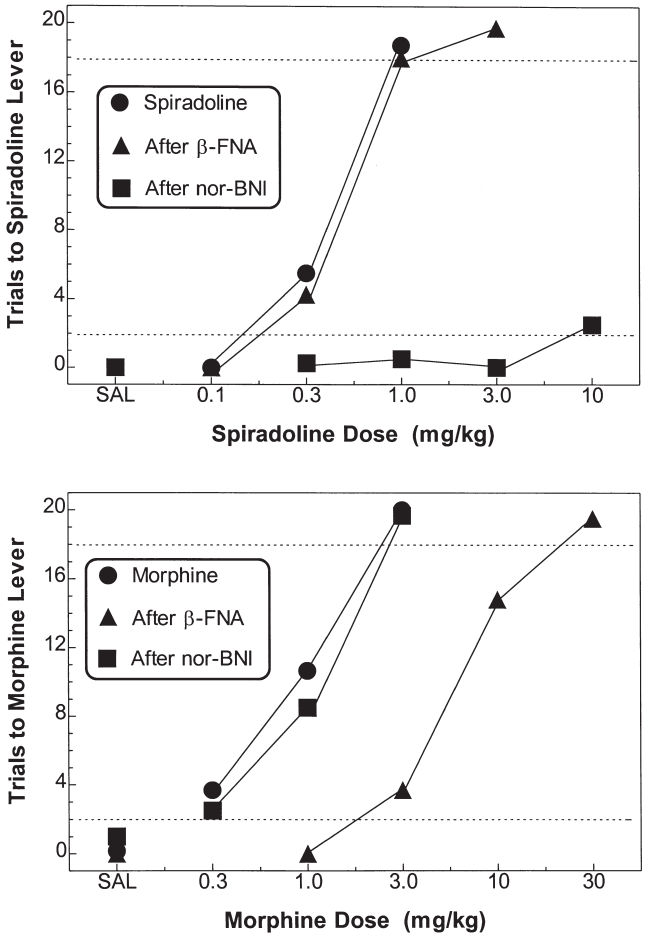

FIG. 2. Differential antagonism of the discriminative effects of spiradoline (top) and morphine (bottom) by β-FNA and nor-BNI in separate groups of rats trained to discriminate between SC injections of saline and either 1.0 mg/kg spiradoline (n = 4) or 3.0 mg/kg morphine (n = 6). Stimulus–generalization curves were generated by cumulative dosing, beginning with a session that followed an injection of saline (SAL). β-FNA or nor-BNI were administered intracisternally 24 h before testing began. The upper and lower dashed horizontal lines indicate the minimum levels at which discrimination performance was maintained in training sessions with drug or saline, respectively.

DISCUSSIONS

Rats were readily trained to discriminate 1.0 mg/kg spiradoline from saline without the increase in response latencies that were associated with a higher training dose, 3.0 mg/kg (8). Acquisition of the discrimination occurred more rapidly, in an average of 20 sessions vs. 30 sessions (8), presumably because the lower training dose did not impair performance. As would be expected, the ED_{50}s of spiradoline and U50,488 were lower in the animals in this study than they were in animals trained with 3.0 mg/kg (0.37 vs. 0.66 and 3.3 vs. 8.7 mg/

TABLE 2

DIFFERENTIAL ANTAGONISM OF THE DISCRIMINATIVE EFFECTS OF SPIRADOLINE AND MORPHINE BY 24-H PRETREATMENT WITH B-FNA OR NOR-BNI

	Spiradoline-Trained Rats ($n = 4$)		Morphine-Trained Rats ($n = 6$)	
Pretreatment	ED_{50} (95% C.L.)*	Ratio%	ED_{50} (95% C.L.)*	Ratio%
None	0.44 (0.22–0.88)	—	0.77 (0.36–1.67)	—
10 μg β-FNA	0.48 (0.23–1.00)	1.09	5.71 (1.36–12.7)†	7.42
10 μg nor-BNI	>10	>22	1.02 (0.49–1.73)	1.32

%Ratio of ED_{50} after pretreatment: ED_{50} after no pretreatment.

*Dose (mg/kg) occasioning completion of 10 trials on the drug-appropriate choice lever.

†Significantly different from the other two ED_{50}s of morphine, $p < 0.01$.

kg, respectively), commensurate with the difference in training dose. Rats in this study generalized completely to EKC, which rats trained with 3.0 mg/kg did not do (8), as well as to U69,593, a drug that was not tested in the earlier study. The order of potency of the drugs, EKC > U69,593 > spiradoline > U50,488, was similar to the order of potency in rats discriminating a low dose of the kappa-opioid agonist bremazocine (23) and in rhesus monkeys discriminating EKC (3).

The rats generalized only partially to three opioids that have agonist activity at the kappa-opioid receptor, ketocyclazocine, (−)-NANM, and DuP 747; DuP 747 occasioned more spiradoline-appropriate responding than it did when administered SC to rats discriminating 3.0 mg/kg spiradoline (10). The rats did not generalize at all to four other kappa-active opioids, cyclazocine, butorphanol, pentazocine, and nalorphine. All of these drugs appear to have lower efficacy at the kappa-opioid receptor than do the drugs that substituted fully for spiradoline, as measured by the ability to stimulate urine formation in rats (4,14) and/or binding of GTP in a cell line stably expressing the receptor (19). For example, the relative efficacy of U50,488, EKC, and U69,593 for stimulating GTP binding (spiradoline was not tested) ranged from 0.82 to 0.87, whereas the relative efficacy of butorphanol, nalorphine, and cyclazocine ranged from 0.22 to 0.39. Data on the relative efficacy of DuP 747 is not available. However, the fact that monkeys discriminating EKC generalized completely to nalorphine but only partially to DuP 747 (3) suggests that the latter drug is a relatively low-efficacy kappa-opioid agonist. Overall, the results of this study suggest that there is a high-efficacy requirement for full generalization with spiradoline, even at a training dose of 1.0 mg/kg.

Lowering the training dose of spiradoline from 3.0 to 1.0 mg/kg did not reduce the pharmacological selectivity of stimulus control of behavior. Rats responded only on the choice lever appropriate for saline in tests of generalization to otherwise discriminable doses of drugs that have a spectrum of activity that is different from that of spiradoline. These include morphine (20), pentazocine and butorphanol at doses that generalize with morphine in rats (20,21), cyclazocine (25), and the cough-suppressant dextromethorphan, which has discriminative effects in common with phencyclidine (6).

The discriminative effects of spiradoline and morphine were differentially affected by pretreatment with receptor-selective opioid antagonists in a manner consistent with the different opioid receptors that mediate the discriminative effects of these drugs. The discriminative effects of spiradoline were blocked by nor-BNI, which is selective for the kappa-opioid receptor (18), but not by β-FNA, which is selective for the mu-opioid receptor (28). The discriminative effects of morphine, on the other hand, were antagonized by β-FNA to an extent comparable to what has been described previously (7), but were unaffected by nor-BNI. Similar pharmacological selectivity of these antagonists has been observed in other bioassays in rats, such as diuresis induced by kappa-opioid agonists and inhibition of diuresis induced by mu-opioid agonists (4,24).

The dose of β-FNA that was used in this study, 10 μg intracisternally, antagonized the discriminative effects of morphine for 2 days, at which time 10 mg/kg morphine substituted completely for the 3.0-mg/kg training dose (7). In the present study, 10 μg nor-BNI blocked totally the discriminative effects of up to 10 mg/kg spiradoline for at least 4 weeks. This outcome almost certainly reflects a persisting blockade of kappa-opioid receptors rather than a nonspecific effect on behavior. The performance of rats discriminating morphine was unaffected by nor-BNI. In addition, the increase in response latencies that occurred reliably at 3.0 mg/kg spiradoline was never observed after pretreatment with nor-BNI, even at a dose of 10 mg/kg spiradoline. The kappa-opioid antagonist activity of nor-BNI has been shown to have a long time course in other types of in vivo bioassays, notably thermal tests of analgesia. Nor-BNI, in the same dose used in this study, blocked completely in rats the effects of up to 10 mg/kg spiradoline in the hot-plate test for at least 3 weeks (12). Given SC to rhesus monkeys, it attenuated the effects of U50,488 and U69,593 in a tail-withdrawal test for 2–3 weeks (1). A dose of less than 1.0 μg nor-BNI administered to mice intracerebroventricularly significantly attenuated the effect of U69,593 in a tail-withdrawal test for more than 4 weeks, and reduced the apparent affinity of the drug for kappa-opioid receptor for at least 8 weeks (11). The reason for the long duration of action of nor-BNI is obscure. Nevertheless, the results of the present study add spiradoline discrimination in rats to the list of drug effects that can be blocked for weeks or months by a single dose of nor-BNI.

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REFERENCES

1. Butelman, E. R.; Negus, S. S.; Ai, Y.; De Costa, B. R.; Woods, J. H.: Kappa opioid antagonist effects of systemically administered norbinaltorphimine in a thermal antinociception assay in rhesus monkeys. J. Pharmacol. Exp. Ther. 267:1269–1276; 1993.
2. Colpaert, F. C.; Niemegeers, C. J. E.; Janssen, P. F. M.: Factors regulating drug cue sensitivity: the effect of training dose in fentanyl-saline discrimination. Neuropharmacology 19:705–713; 1980.
3. France, C. P.; Medzihradsky, F.; Woods, J. H.: Comparison of

kappa opioids in rhesus monkeys: Behavioral effects and receptor binding affinities. J. Pharmacol. Exp. Ther. 268:47–58; 1994.
4. Hayes, A. G.; Skingle, M.; Tyers, M. B.: Evaluation of the receptor selectivities of opioid drugs by investigating the block of their effect on urine output by β-funaltrexamine. J. Pharmacol. Exp. Ther. 240:984–988; 1987.
5. Holtzman, S. G.: Discriminative stimulus properties of opioid agonists and antagonists. In: Cooper, S. J., ed. Theory in psychopharmacology. London: Academic Press; 1983:1–45.
6. Holtzman, S. G.: Discriminative stimulus effects of dextromethorphan in the rat. Psychopharmacology (Berlin) 116:249–254; 1994.
7. Holtzman, S. G.: Antagonism of morphine-like discriminative effects by β-funaltrexamine. Pharmacol. Biochem. Behav. 57: 771–777; 1997.
8. Holtzman, S. G.; Cook, L.; Steinfels, G. F.: Discriminative stimulus effects of spiradoline, a kappa-opioid agonist. Psychopharmacology (Berlin) 105:447–452; 1991.
9. Holtzman, S. G.; Steinfels, G. F.: Antagonism of the discriminative stimulus effects of the kappa-opioid agonist spiradoline. Psychopharmacology (Berlin) 116:243–248; 1994.
10. Holtzman, S. G.; Steinfels, G. F.; Schmidt, W. K.: Assessing spiradoline-like discriminative effects of DuP 747: Influence of route of administration. Pharmacol. Biochem. Behav. 47:487–491; 1994.
11. Horan, P.; Taylor, J.; Yamamura, H. I.; Porreca, F.: Extremely long-lasting antagonistic actions of nor-binaltorphimine (nor-BNI) in the mouse tail-flick test. J. Pharmacol. Exp. Ther. 260:1237–1243; 1992.
12. Jones, D. N.; Holtzman, S. G.: Long term kappa-opioid receptor blockade following nor-binaltorphimine. Eur. J. Pharmacol. 215:345–348; 1992.
13. Lahti, R. A.; Mickelson, M. M.; McCall, J. M.; VonVoigtlander, P. F.: [^{3}H]U-69593, a highly selective ligand for the opioid κ receptor. Eur. J. Pharmacol. 109:281–284; 1985.
14. Leander, J. D.: A kappa opioid effect: Increased urination in the rat. J. Pharmacol. Exp. Ther. 224:89–94; 1982.
15. Meecham, K. G.; Boyle, S. J.; Hunter, J. C.; Hughes, J.: An in vitro profile of the activity for the (+) and (−) enantiommers of spiradoline and PD117302. Eur. J. Pharmacol. 173:151–157; 1989.
16. Picker, M. J.: Discriminative stimulus and response rate-decreasing effects of kappa opioids: Antagonism by naloxone. Life Sci. 54:1767–1775; 1994.
17. Picker, M. J.; Doty, P.; Negus, S. S.; Mattox, S. R.; Dykstra, L. A.: Discriminative stimulus properties of U50,488 and morphine: Effects of training dose on stimulus substitution patterns produced by mu and kappa opioid agonists. J. Pharmacol. Exp. Ther. 254:13–22; 1990.
18. Portoghese, P. S.; Lipkowski, A. W.; Takemori, A. E.: Binaltorphimine and nor-binaltorphimine, potent and selective κ-opioid antagonists. Life Sci. 40:1287–1292; 1987.
19. Remmers, A. E.; Clark, M. J.; Mansour, A.; Akil, H.; Woods, J. H.; Medzihradsky, F.: Opioid efficacy in a C6 glioma cell line stably expressing the human kappa opioid receptor. J. Pharmacol. Exp. Ther. 288:827–833; 1999.
20. Shannon, H. E.; Holtzman, S. G.: Evaluation of the discriminative effects of morphine in the rat. J. Pharmacol. Exp. Ther. 198:54–65; 1976.
21. Shannon, H. E.; Holtzman, S. G.: Further evaluation of the discriminative effects of morphine in the rat. J. Pharmacol. Exp. Ther. 201:55–66; 1977.
22. Shannon, H. E.; Holtzman, S. G.: Morphine training dose: A determinant of stimulus generalization to narcotic antagonists in the rat. Psychopharmacology (Berlin) 61:239–244; 1979.
23. Smith, M. A.; Picker, M. J.: Examination of the kappa agonist and antagonist properties of opioids in the rat drug discrimination procedure: Influence of training dose and intrinsic efficacy. Behav. Pharmacol. 6:703–717; 1995.
24. Takemori, A. E.; Schwartz, M. M.; Portoghese, P. S.: Suppression by nor-binaltorphimine of kappa opioid-mediated diuresis in rats. J. Pharmacol. Exp. Ther. 247:974; 1988.
25. Teal, J. J.; Holtzman, S. G.: Discriminative stimulus effects of cyclazocine in the rat. J. Pharmacol. Exp. Ther. 212:368–376; 1980.
26. VonVoigtlander, P. F.; Lahti, R. A.; Ludens, J. H.: U-50488: A selective and structurally novel non-mu (kappa) opioid agonist. J. Pharmacol. Exp. Ther. 224:7–12; 1983.
27. VonVoigtlander, P. F.; Lewis, R. A.: Analgesic and mechanistic evaluation of spiradoline, a potent kappa opioid. J. Pharmacol. Exp. Ther. 246:259–262; 1988.
28. Ward, S. J.; Portoghese, P. S.; Takemori, A. E.: Pharmacological characterization in vivo of the novel opioid, β-funaltrexamine. J. Pharmacol. Exp. Ther. 220:494–498; 1982.