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Assessing Spiradoline-Like Discriminative Effects of DuP 747: Influence of Route of Administration

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HOLTZMAN, S. G., G. F. STEINFELS AND W. K. SCHMIDT. *Assessing spiradoline-like discriminative effects of DuP 747: Influence of route of administration.* PHARMACOL BIOCHEM BEHAV 47(3) 487–491, 1994.—DuP 747, *trans*-3,4-dichloro-*N*-methyl-*N*-[2-(pyrrolidin-1-yl)-1,2,3,4-tetrahydronaphthalen-1-yl]benzeacetamide methanesufonate, is a recently synthesized analgesic drug that binds with high affinity and selectivity to the κ-opioid receptor. In order to determine if DuP 747 has κ-like discriminative effects it was tested for stimulus generalization in rats trained to discriminate between SC injections of saline and 3.0 mg/kg of spiradoline, a potent κ-opioid agonist. A range of drug doses was administered by each of several routes 30 min before a test session. Spiradoline occasioned orderly dose-dependent increases in spiradoline-appropriate lever selection after SC or IP administration, with ED_{50}s of 0.65 and 1.75 mg/kg, respectively. In contrast, DuP 747 (1.0–30 mg/kg) occasioned little spiradoline-appropriate lever selection when administered SC, but was generalized from spiradoline partially when administered IP (ED_{50} = 5.9 mg/kg) or PO (ED_{50} = 59 mg/kg). The 5-hydroxy-desmethoxy metabolite of DuP 747, administered SC (0.3–10 mg/kg), occasioned selection of the saline-appropriate lever only. That DuP 747 had little spiradoline-like activity after SC administration suggests that a metabolite of DuP 747 was responsible for the spiradoline-appropriate responding that followed IP and PO administration of the drug, apparently a metabolite other than 5-hydroxy-desmethoxy-DuP 747.

Spiradoline Drug discrimination κ-Opioid analgesic Route of administration DuP 747

μ-OPIOID agonists, such as morphine, are highly effective in treating various types of pain, but have a number of undesirable characteristics. Among these are side effects, such as depression of respiration and, with repeated administration, tolerance to their analgesic effect and physical dependence (9). In addition, μ-opioid agonists have a high potential for abuse, due largely to the pleasurable subjective effects that they can produce (10). Agonists at κ-opioid receptors represent a more recently discovered class of drugs (13). Like the μ-opioid agonists, κ-opioid agonists are effective in a variety of animal models that are used to assess analgesic activity. However, in contrast to the μ-opioid agonists, their effects on respiration are more limited, and with repeated administration they produce less tolerance and physical dependence (9,13,14,19).

κ-Opioid agonists have not been examined in humans as extensively as have the μ-opioid agonists. However, there are several reports indicating that κ opioids produce a syndrome of unpleasant subjective effects, characterized by dysphoria and, occasionally, psychotomimetic symptomology (11,15). Although these effects minimize potential for abuse, they also constitute a bar to acceptance by patients. It is not known if agonist activity at κ-opioid receptors is invariably associated with unpleasant subjective symptomology, particularly if there are subtypes of these receptors (2,22).

DuP 747, *trans*-3,4-dichloro-*N*-methyl-*N*-[2-(pyrrolidin-1-yl)-1,2,3,4-tetrahydronaphthalen-1-yl]benzeacetamide methanesufonate, is a new compound that was derived from the fusion of a benzene ring to the cyclohexane ring of U50,488, a prototypic κ-opioid agonist [(18), Fig. 1]. DuP 747 has approximately twice the affinity for κ-opioid receptors compared to U50,488 and a 50-fold binding selectivity for κ versus μ receptors (19). It is active in antinociceptive tests in rodents follow-

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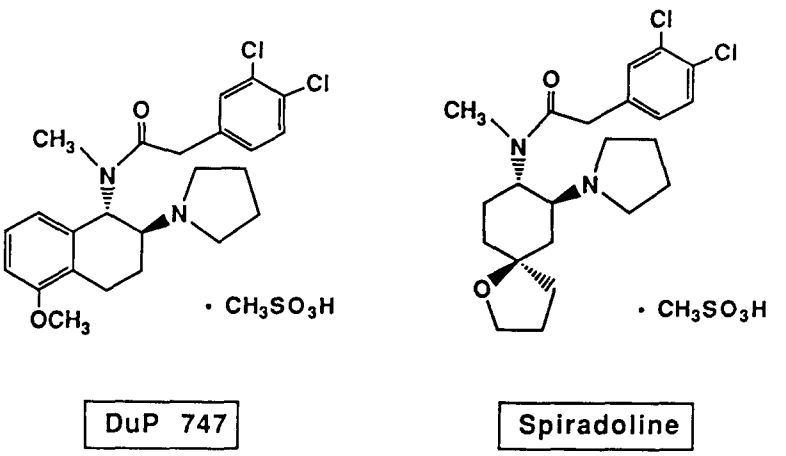

FIG. 1. Structures of DuP 747, *trans*-3,4-dichloro-*N*-methyl-*N*-[2-(pyrrolidin-1-yl)-1,2,3,4-tetrahydronaphthalen-1-yl]benzeacetamide methanesufonate, and spiradoline, U62,066.

ing both parenteral and oral administration (19). The purpose of the present study was to compare the discriminative stimulus effects of DuP 747 with those of spiradoline (Fig. 1), a potent κ-opioid agonist and congener of U50,488 (23). The discriminative effects of opioid drugs in animals have been shown to be predictive of their subjective effects in humans (6,7). Rats trained to discriminate between injections of saline and spiradoline (8) were tested for stimulus generalization with a range of doses of spiradoline and DuP 747. Several different routes of administration were examined. One known metabolite of DuP 747, 5-hydroxy-desmethoxy-DuP 747 (Y.-N. Wong, Du Pont Merck, personal communication), also was tested for spiradoline-like discriminative effects.

METHODS

Subjects

The subjects were 15 male rats of Sprague-Dawley descent (Charles River Laboratories, Wilmington, MA). Seven were experimentally naive at the start of the study and 8 had been used in a previously reported study on the discriminative effects of spiradoline (8). Between experiments the rats were housed in a colony room two per cage under a 12-h light/dark cycle, with food and water available continuously.

Procedure

The animals either had been trained or were newly trained to discriminate between SC injections of saline and 3.0 mg/kg of spiradoline, as described previously (8). A discrete-trial avoidance/escape procedure was used. The beginning of a trial was signalled by concurrently illuminating the houselight of the operant test chamber and turning on 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 animal completed the following two-response chain: a response on an "observing" lever mounted in one wall of the operant chamber, which turned off the white noise, and a response on one of two "choice" levers mounted in the opposite wall of the chamber, which extinguished the houselight and ended the trial if the choice lever was the appropriate one for what the rat had been injected with before the session. Trials were separated by 50 s, during which time the operant chamber was illuminated with a red stimulus lamp. A session ended after 20 trials had been completed or after 30 min had elapsed.

Rats were given an SC injection 30 min before a training session: 3.0 mg/kg of spiradoline on one day and saline on alternate days. Approximately 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 an injection of saline; the remaining rats had the opposite choice lever assignments. Stimulus control of behavior was achieved when a rat completed 18 or more trials out of 20 on the correct choice lever for the current drug state (i.e., spiradoline or saline) in six consecutive sessions, four training sessions, and two test sessions. During training sessions, only a response on the correct choice lever ended a trial (i.e., observing lever-correct choice lever [correct trial] or observing lever-incorrect choice lever (incorrect trial). During tests of stimulus generalization a trial was ended by the first response on either choice lever following a response on the observing lever. Generalization tests usually were conducted twice each week, three to four days apart, provided that discrimination performance remained at or above 90% correct in training sessions conducted on at least three days of each week.

Drugs

Spiradoline (U-62,066, The Upjohn Company, Kalamazoo, MI) and DuP 747 [synthesized as described in (18)] as methane sulfonate salts were dissolved in a 0.9% saline solution for parenteral administration; DuP 747 was dissolved in distilled water for administration by oral gavage. The vehicle for 5-hydroxy-desmethoxy-DuP 747 was dimethyl sulfoxide (Me_2SO). Drugs were administered in a volume of 1.0–2.0 ml per kg of body weight. The doses in each series of tests (drug × route) were administered in a random sequence that also included a separate test of the drug vehicle. All drugs were administered 30 min before a session.

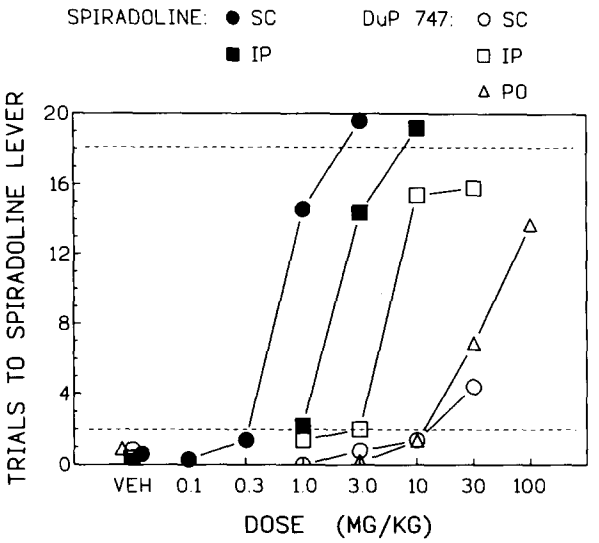

FIG. 2. Comparison of the discriminative effects of spiradoline administered SC or IP and DuP 747 administered SC, IP, or PO in rats trained to discriminate between saline and 3.0 mg/kg (SC) of spiradoline. Each point is the mean number of trials completed on the spiradoline-appropriate choice lever 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 five to seven rats. Points above VEH indicate the mean number of trials completed on the spiradoline-appropriate lever in test sessions that followed the administration of drug vehicle (i.e., saline or distilled water). 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.

Data

Discrimination data are shown as the 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. ED_{50} values (dose resulting in selection of the spiradoline-appropriate choice levers in 10 trials per session) were estimated for individual animals by linear regression of the ascending limb of the stimulus-generalization curve, using at least three points; in cases where only two points were available, the ED_{50} was estimated by simple extrapolation. From these values, average ED_{50}s and 95% confidence limits were calculated. The latency from the onset of a trial to the first observing response was recorded and summed over the 20 trials of each test session. Cumulative observing response latencies are presented as a group mean ± SE. The response latency data for each drug series were evaluated by one-factor analysis of variance with repeated measures followed, where appropriate, by the Student-Newman-Keuls test (4).

RESULTS

Spiradoline administered SC produced dose-related increases in the number of trials completed on the spiradoline-appropriate choice lever (Fig. 2), as reported previously (8). The ED_{50} for spiradoline-appropriate lever selection was 0.65 (0.43–0.98) mg/kg. SC spiradoline also produced dose-dependent increases in the latency to emit the observing response, $F(4, 24) = 8.25, p < 0.001$ (Table 1), possibly reflecting mild behavioral depression (20). The training dose of spiradoline (3.0 mg/kg) increased the average observing response latency by 130% compared to drug vehicle (Table 1). When administered IP, spiradoline again produced dose-dependent increases in trials completed on the spiradoline-appropriate lever (Fig. 2). However, with an ED_{50} of 1.75 (1.09–2.79) mg/kg the potency of spiradoline by the IP route was 2.7-fold lower than it was following SC administration. This almost threefold decrease in potency by the IP route of administration was also evident in the effects of spiradoline on observing response latency. Latencies were increased over the range of doses tested, $F(3, 12) = 4.42, p = 0.026$, but now it took 10 mg/kg of spiradoline to more than double the latency to respond (Table 1).

DuP 747 was largely inactive following SC administration of 1.0–30 mg/kg. It occasioned a maximum of only 4.4 trials to the spiradoline-appropriate lever at 30 mg/kg (Fig. 2) and had no effect on response latency, $F(4, 16) = 0.62, p > 0.1$ (Table 1). In contrast, the same range of doses administered IP produced a dose-dependent increase in spiradoline-appropriate responding, with a maximum of 15.8 trials to the spiradoline-appropriate choice lever at 30 mg/kg (Fig. 2) and an ED_{50} of 5.9 (2.8–12.5) mg/kg. Observing response latencies were not affected consistently by IP DuP 747, $F(4, 16) =$

TABLE 1

CUMULATIVE OBSERVING RESPONSE LATENCY (mean ± SE) IN RATS TRAINED TO DISCRIMINATE BETWEEN SALINE AND 3.0 mg/kg OF SPIRADOLINE (SC)

		Drug Dose (mg/kg)						
Drug (Route)	Vehicle	0.1	0.3	1.0	3.0	10	30	100
Spiradoline (SC)	182 ± 16	172 ± 16	171 ± 13	291 ± 58	420 ± 98*			
Spiradoline (IP)	167 ± 21			217 ± 65	258 ± 92	388 ± 119*		
DuP 747 (SC)	182 ± 14			174 ± 12	186 ± 19	167 ± 21	182 ± 17	
DuP 747 (IP)	168 ± 22			190 ± 26	187 ± 39	252 ± 79	340 ± 145	
DuP 747 (PO)	190 ± 29				198 ± 20	248 ± 36	258 ± 37*	328 ± 27*
5-Hydroxy-des-methoxy-DuP 747 (SC)	170 ± 34		159 ± 27	164 ± 29	164 ± 28	172 ± 37		

*Higher than latency after drug vehicle, $p < 0.05$ (n = 5–7).

1.62, $p > 0.1$ (Table 1), although the latency of one rat increased in an orderly manner across doses, more than tripling at 30 mg/kg. DuP 747 also was active following administration by oral gavage, but was less potent than it was by the IP route (Fig. 2). At 100 mg/kg, five rats selected the spiradoline-appropriate lever in 10 or more trials and a sixth selected it in only 2 trials. Assigning that latter animal an ED_{50} of 200 mg/kg, the average ED_{50} for the group was 59 (23–153) mg/kg. Response latencies were increased modestly, but uniformly and significantly, $F(4, 20) = 9.02$, $p < 0.001$, over the range of PO doses (Table 1).

The 5-hydroxy-desmethoxy metabolite of DuP 747 was tested by the SC route over the dose range of 0.3–10 mg/kg. It occasioned responding only on the saline-appropriate choice lever at each dose (data not shown) and did not affect response latencies, $F(4, 16) = 0.49$, $p > 0.1$ (Table 1).

The Me_2SO vehicle alone resulted in an average of 1.2 trials being completed on the drug-appropriate choice lever, similar to the average after saline, and an average response latency (170 ± 34) that was within the range of latencies recorded after saline or distilled water (Table 1).

DISCUSSION

The stimulus control of behavior by spiradoline in the present study was similar to that described previously (8). In fact, the ED_{50} for spiradoline administered SC in this study, 0.65 mg/kg, was almost an exact replication of the 0.66-mg/kg ED_{50} in that earlier study. Thus, the discriminative stimulus effects of spiradoline in rats are reproducible.

The ED_{50} of spiradoline was increased by a factor of almost threefold following administration by the IP route. Presumably this was because a significant amount of the dose of spiradoline that was administered entered the hepatic portal circulation and was exposed to metabolizing enzymes in the liver before reaching the brain. The opposite occurred with DuP 747. It had little behavioral activity following SC administration of up to 30 mg/kg. However, following IP and PO administration it engendered dose-related partial generalization with spiradoline. In cats, DuP 747 induced a higher incidence of "psychotomimetic-like" behaviors, such as backward walking and chasing unseen objects, when administered PO than when it was administered SC (19). These results suggest that it was not the parent compound, DuP 747, that occasioned the spiradoline-appropriate lever selection, but instead it was a metabolite of DuP 747. That the higher doses of DuP 747 elevated response latencies after IP and PO administration (albeit not significantly in the case of IP) but not after SC administration, is consistent with the formation of a behaviorally active metabolite. We tested one known metabolite of DuP 747, 5-hydroxy-desmethoxy-DuP 747. It had no activity in the drug discrimination procedure over a dose range that spanned the ED_{50} for IP DuP 747 and that should have been adequate to see effects if this compound was the metabolite with spiradoline-like discriminative properties.

Opioid drugs that have κ-like discriminative effects in animals have the potential to produce unpleasant subjective effects in humans (6,7,17). Although partial generalizations can be difficult to interpret unambiguously, the orderliness of the data suggests that DuP 747 has significant κ-like discriminative effects in the rat. The ED_{50} of DuP 747 in the rat phenylbenzoquinone (PBQ)-induced stretching test of analgesia was 1.1 mg/kg PO, well below the ED_{50} for spiradoline-appropriate lever selection (Table 2). DuP 747 also was active in this analgesic assay after SC administration, with an ED_{50} of 0.046 mg/kg, a full two orders of magnitude lower than its ED_{50} for spiradoline-appropriate lever selection (Table 2). In contrast, the analgesic potency of SC spiradoline in the PBQ analgesic assay was only 14-fold greater than its potency as a discriminative stimulus (Table 2). This raises the possibility that the parent compound, DuP 747, has analgesic activity but not spiradoline-like discriminative properties, which would reside in the metabolite.

TABLE 2

COMPARISON OF ED_{50} VALUES IN DRUG DISCRIMINATION AND ANALGESIC ASSAYS IN THE RAT

Drug (Route)	ED_{50} (mg/kg) Spiradoline Discrimination	ED_{50} (mg/kg) PBQ Analgesic Assay*	Ratio (Discrimination/ Analgesia) Assay
Spiradoline (SC)	0.65	0.046†	14×
Spiradoline (IP)	1.75	0.25‡	7×
DuP 747 (SC)	>30.0	0.30†	>100×
DuP 747 (IP)	5.9	0.19‡	31×
DuP 747 (PO)	59.0	1.1†	54×
5-Hydroxy-desmethoxy-DuP 747 (SC)	>10.0	0.22‡	>45×

*Modified from Blumberg, H.; Wolf, P. S.; Dayton, H. B. Use of writhing test for evaluating analgesic activity of narcotic antagonists. Proc. Soc. Exp. Biol. Med. 118:763–767; 1965. †Data from Schmidt et al. Dup 747: Review of the pharmacology of a κ-selective analgesic. Pharmacol. Rev.; in press. ‡W. K. Schmidt, unpublished data.

There are several other possible explanations for why a ligand of the κ-opioid receptor might be fully active in the PBQ analgesic assay but have only limited spiradoline-like discriminative effects. For example, DuP 747 could have a component of action other than or in addition to κ-opioid that contributes to its analgesic activity, analogous to the situation with novel derivatives of the μ-opioid agonist fentanyl (3). A second component of action, if sufficiently prominent, also might mask less prominent κ-opioid-like discriminative stimulus properties. Last, the PBQ test has one of the lowest efficacy requirements of the commonly used analgesic assays (5). The relatively high training dose of spiradoline suggests that the discrimination procedure has a relatively high efficacy requirement (16,21). Thus, if DuP 747 has low to intermediate intrinsic efficacy at the κ-opioid receptor, it could have more activity in the analgesic assay than in the discrimination procedure.

All of these explanations are plausible theoretically and are deserving of further investigative attention. However, they do not readily account for the observed route-dependency of the spiradoline-like discriminative effects of DuP 747. On the other hand, if DuP 747 does have a behaviorally active metabolite that can be identified, it might be possible to modify the structure of DuP 747 to prevent conversion to the unwanted metabolite while preserving the analgesic properties of the parent compound. The drug discrimination paradigm is well suited for this type of preclinical evaluation of new opioid analgesic drugs.

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