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Discriminative Stimulus Properties of the Mixed Agonist-Antagonist Pentazocine in the Rat Using Two-Choice Discrete-Trial Avoidance Paradigm

MAKOTO UKAI, SHINOBU NAKAYAMA, TAMI HAMADA AND TSUTOMU KAMEYAMA

Department of Chemical Pharmacology, Faculty of Pharmaceutical Sciences Meijo University, Nagoya 468, Japan

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UKAI, M., S. NAKAYAMA, T. HAMADA AND T. KAMEYAMA. *Discriminative stimulus properties of the mixed agonist-antagonist pentazocine in the rat using two-choice discrete-trial avoidance paradigm.* PHARMACOL BIOCHEM BEHAV 33(2) 355–359, 1989.—The discriminative stimulus effects of pentazocine were evaluated in the rat trained to discriminate 3.0 mg/kg of pentazocine from vehicle in a two-choice discrete-trial avoidance paradigm. The rats used could discriminate 3.0 mg/kg of pentazocine from vehicle within 20 sessions after the start of discrimination training. The stimulus effects lasted 30 to 90 min and disappeared thereafter except for one rat in which the discriminative effects lasted 30 to 150 min. Pentazocine produced dose-dependent stimulus effects at 0.3 to 3.0 mg/kg doses. The stimulus effects of pentazocine were fully reversed by higher doses of naltrexone. Butorphanol, nalorphine, morphine, levorphanol, SKF 10,047, and methamphetamine generalized to pentazocine cue. However, ethylketocyclazocine, dextrorphan or pentobarbital did not produce stimulus effects in common with pentazocine. These results suggest that the discriminative stimulus effects of pentazocine (3.0 mg/kg) are mediated through an opioid but non-mu and nonspecific mechanism.

Drug discrimination Pentazocine Opioid receptors Morphine Nalorphine Ethylketocyclazocine SKF 10,047 Naltrexone Butorphanol

WHITE and Holtzman (22) have investigated the properties of pentazocine (3.0 mg/kg) as a discriminative stimulus in the squirrel monkey using a discrete-trial avoidance paradigm. The cross-generalization tests indicate that pentazocine has both a morphine-like component of action and a component shared with nonmorphine-like opioids. Although rats are trained to discriminate a 10.0 mg/kg dose of pentazocine from vehicle using food reinforcement (6,11), the data on substitution tests reported for shock-avoidance in rats are obtained only with a training dose of 40.0 mg/kg (13). This dose is so high that most of the results of the substitution tests, although statistically significant, are partial substitutions. Moreover, this training dose itself produces an overt toxic effect such as rigidity. Therefore, a lower training dose of pentazocine should be appropriate for assessing the characteristics of pentazocine cue with a shock-avoidance schedule.

In view of the aforementioned, the present study was designed to examine the stimulus effects of pentazocine in the rat trained to discriminate 3.0 mg/kg of the drug from vehicle using two-choice discrete-trial avoidance paradigm.

METHOD

Subjects

The subjects were male Sprague-Dawley rats (Shizuoka Experimental Animal Agricultural Cooperative Association, Japan) weighing between 200 and 250 g at the start of discrimination training. The rats were housed individually in a ventilated colony room where they had continuous access to food and water. The lights in the room were illuminated between 8:00 a.m. and 8:00 p.m.

Apparatus

The apparatus (25 × 31 × 31 cm) has been described in detail elsewhere (15, 16, 20, 21). Briefly, it has one "starting" lever in one wall and two "choice" response levers on the wall opposite to the "starting" lever. The choice response levers were separated by a clear Plexiglas partition, 5.0 cm wide, that extended from the ceiling of the test chamber to 1.0 cm above the grid floor (Muro-

machi Kikai Co. Ltd., Japan). A constant current shock generator (model SGS-004, BRS/LVE, MD) delivered a scrambled electric shock to the grid floor of the chamber, which was housed within a ventilated, lightproof and sound-attenuating enclosure. Personal computers (PC-9801, UV21, NEC Corporation, Japan) were used to control the schedule contingencies.

Discrimination Training

Rats were trained to discriminate 3.0 mg/kg of pentazocine from vehicle in a two-choice discrete-trial avoidance paradigm. The onset of a trial was signalled by the simultaneous illumination of the house light and the presentation of white noise. At this time, the rat was required to press the "starting" lever mounted in one wall of the test chamber and then to press one of the two "choice" levers mounted in the opposite wall. The first starting response of the trial terminated the white noise and the appropriate choice response extinguished the house light and ended the trial. Beginning 5.0 sec after the onset of the trial, 2.0-mA shock was delivered to the grid floor of the chamber every 3.0 sec in 1.0-sec pulse until the two-response chain was completed. The intertrial interval was 50 sec during which time the chamber was dimly illuminated by a red light. Experimental sessions ended after 21 trials or 30 min, whichever came first. The first trial of each session was considered a "warm-up" and was excluded from the data analysis. Training sessions were conducted 6 days/week. Either pentazocine (3.0 mg/kg) or vehicle was injected SC 30 min before each training session. Training continued until rats could complete reliably at least 18 of 20 trials (i.e., 90% exclusive of the first trial) on the appropriate choice lever under both conditions.

Discrimination Testing

Drug test sessions were conducted provided the rats satisfied the performance criterion in two consecutive training sessions. During test sessions, both choice levers were activated so that a response on either choice lever after the starting response terminated the trial. Test sessions and training sessions were identical in all other aspects. Except for the time-course experiment, all drugs and drug vehicles were administered SC 30 min before the start of the test session. When naltrexone and pentazocine were administered concomitantly in antagonism tests, injections were made a few seconds apart at different sites.

Data Analysis

The data were analysed in terms of the number of trials completed on the pentazocine-appropriate lever. Trials completed on the vehicle-appropriate lever were recorded but are not shown in the figures. All animals completed all trials of every session. A dose of test drug was considered to produce discriminative stimulus effects comparable to those produced by the training dose of pentazocine (3.0 mg/kg) if a rat completed at least 18 of 20 trials on the pentazocine-appropriate lever.

Drugs

The following drugs were used: (racemic) pentazocine base (Sankyo Co.), butorphanol (Bristol-Myers Co.), dextrorphan (Ro 01-6794, F. Hoffmann-La Roche & Co.), ethylketocyclazocine base (Sterling-Winthrop Research Institute), levorphanol tartrate (Takeda Chemical Industries Co. Ltd), methamphetamine hydrochloride (Dainippon Pharmaceutical Co. Ltd.), morphine hydrochloride (Shionogi & Co. Ltd.), nalorphine hydrochloride (Research Biochemicals Inc.), SKF 10,047 (Dr. W. R. Martin of University of Kentucky), and sodium pentobarbital (Pitman-Moore Inc.).

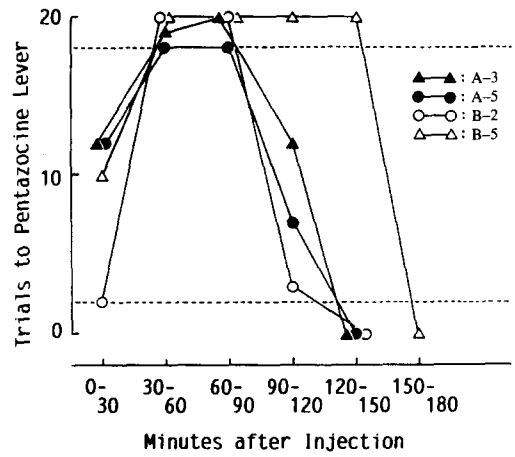


FIG. 1. Time courses of discriminative stimulus effects of pentazocine in the rat trained to discriminate 3.0 mg/kg of pentazocine from vehicle. Data are reported as the number of trials of a 20-trial session completed on the pentazocine-appropriate lever and are not shown. The dashed horizontal lines at 18 and 2 responses represent the criteria for pentazocine- and vehicle-appropriate responding, respectively, during training sessions. Each point represents a single observation in one rat. Different symbols denote different rats. Each point was determined on a separate day.

Pentazocine, ethylketocyclazocine and SKF 10,047 were dissolved in 8.5% lactic acid and 1.0 N sodium hydroxide in a 3:2 ratio, while all other drugs were dissolved in 0.9% saline. Drug solutions and vehicles were administered SC in a volume of 1.0 ml/kg. All drug doses are expressed in terms of the free base.

RESULTS

Pentazocine Cue

We at first trained 16 rats to discriminate 3.0 mg/kg of pentazocine from vehicle, but discarded 6 rats from the study within 20 sessions because of their poor performance. Only one rat (A-6) was trained until 62 sessions, but it did not reach the performance criterion. Although several sessions for "shaping" with vehicle injections were not included, the stimulus effects of pentazocine were established within 20 sessions after the start of discrimination training, and were well maintained thereafter. The time course of the stimulus control produced by the training dose (3.0 mg/kg) of pentazocine is presented in Fig. 1. The rat completed greater than 90% of the trials to the pentazocine-appropriate lever from 30 to 90 min after an injection except for one rat (B-5) from 30 to 150 min. A 0.3 mg/kg dose of pentazocine did not engender responding appropriate for pentazocine cue, while by increasing the dose to 1.0 mg/kg partial stimulus effects were evident (Fig. 2). A 3.0 mg/kg dose of pentazocine produced pentazocine-appropriate responding in every rat tested (Fig. 2).

Antagonism by Naltrexone

The results of administering the training dose of pentazocine (3.0 mg/kg) together with graded doses of naltrexone are shown in Fig. 3. The lowest dose of naltrexone (0.03 mg/kg) had no effect; the animals responded almost completely on the pentazocine-appropriate lever. Increasing the dose to 1.0 mg/kg abolished the pentazocine-appropriate responding in 2 out of 4 rats. However, a further 3-fold increase to 3.0 mg/kg was required before the

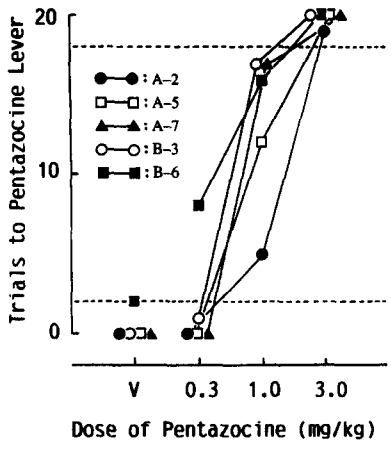


FIG. 2. Dose-response curves of discriminative stimulus effects of pentazocine in the rat trained to discriminate 3.0 mg/kg of pentazocine from vehicle (V). Other details are the same as in Fig. 1.

discriminative effects of pentazocine could completely be antagonized.

Other Opioids

In addition to pentazocine itself, five other opioid drugs were tested (Figs. 4 and 5). Four of these, butorphanol (0.3 mg/kg), nalorphine (3.0 mg/kg), morphine (3.0 mg/kg) and SKF 10,047 (1.0 mg/kg) substituted for pentazocine, respectively (Figs. 4 and 5). For ethylketocyclazocine (EKC), the highest dose (0.3 mg/kg) which could be tested produced levels of pentazocine-appropriate responding below 60% (Fig. 5). Rats treated with a 1.0 mg/kg dose failed to complete the session. Additionally, levorphanol (0.3 and 0.56 mg/kg), but not dextrorphan (10.0 and 17.5 mg/kg), produced responding appropriate for pentazocine cue in 4 rats tested (data not shown).

Nonopioid Psychoactive Drugs

Pentobarbital did not produce pentazocine-appropriate respond-

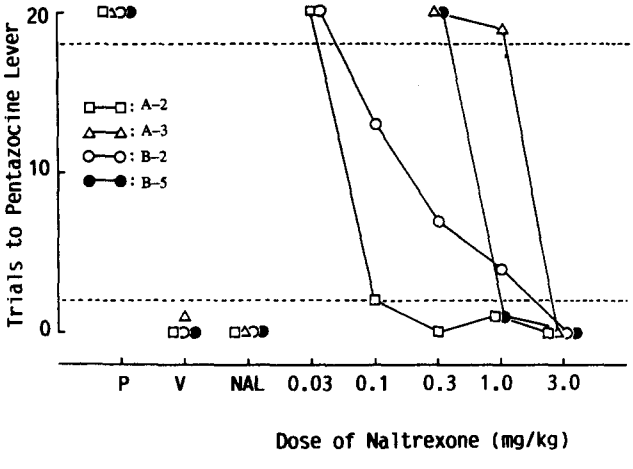
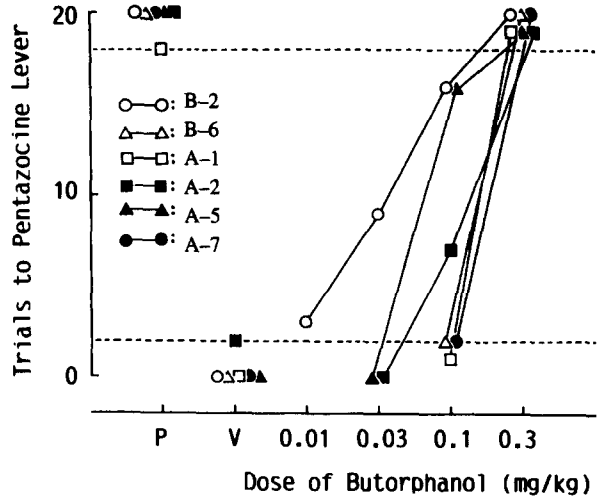


FIG. 3. Dose-response antagonism of the discriminative stimulus effects of 3.0 mg/kg of pentazocine by naltrexone (NAL) in the rat trained to discriminate 3.0 mg/kg of pentazocine (P) from vehicle (V). NAL: 3.0 mg/kg of naltrexone alone. Other details are the same as in Fig. 1.



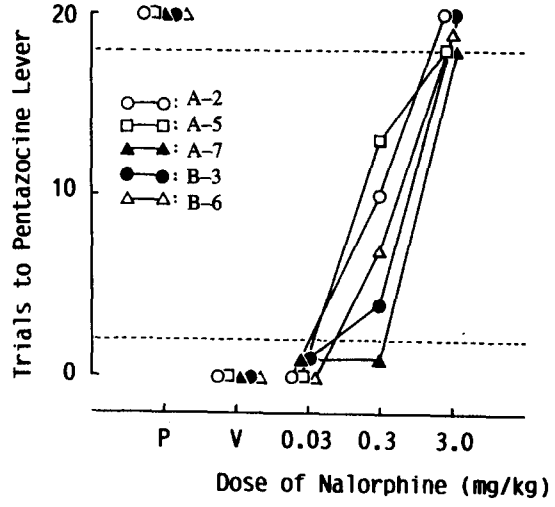


FIG. 4. Discriminative stimulus effects of graded doses of butorphanol and nalorphine in the rat trained to discriminate 3.0 mg/kg of pentazocine (P) from vehicle (V). Other details are the same as in Fig. 1.

ing at doses of 1.0 to 17.5 mg/kg. Rats failed to complete sessions when administered a 30.0 mg/kg dose of the drug. Methamphetamine, although a wide range of effective doses, engendered responding appropriate for pentazocine except for one rat (A-1) which could finish only 19 out of 20 trials.

DISCUSSION

Average training sessions to reach criterion for discrimination were within 20 and the discrimination was stable thereafter in the 9 rats used in the study. Although 16 rats were at first trained to discriminate 3.0 mg/kg of pentazocine from vehicle, 7 of them were discarded from the study because of their poor performance, suggesting that the discriminative stimulus effects of 3.0 mg/kg of pentazocine are rather weak to control the behaviors. Additionally, the duration of the stimulus effects was similar to that of pharmacological effects of the drug on nociceptive responses, conditioned avoidance responses, and brain monoamine levels (8,14).

The present results showing that the mu opioid agonist mor-

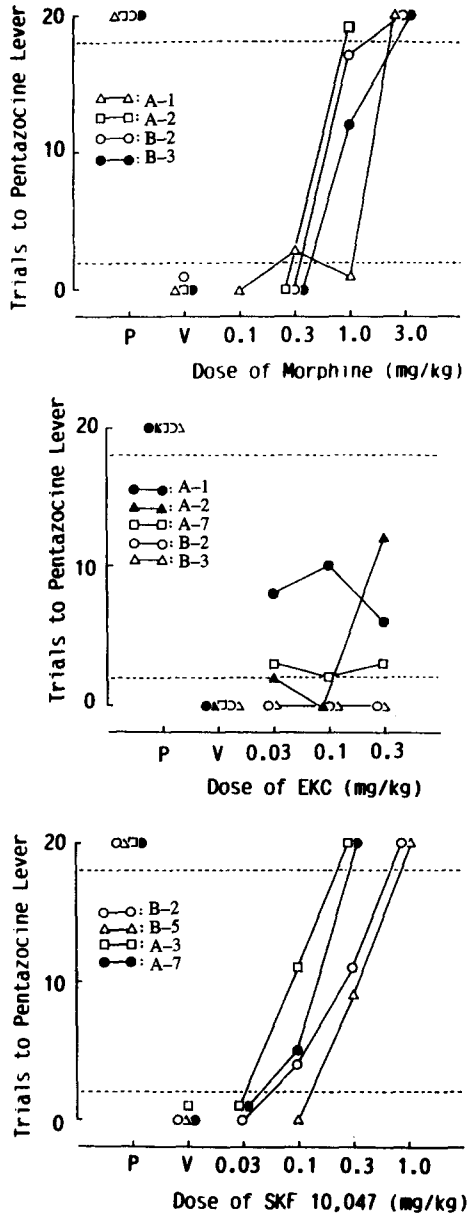


FIG. 5. Discriminative stimulus effects of graded doses of morphine, ethylketocyclazocine (EKC) and SKF 10,047 in the rat trained to discriminate 3.0 mg/kg of pentazocine (P) from vehicle (V). Other details are the same as in Fig. 1.

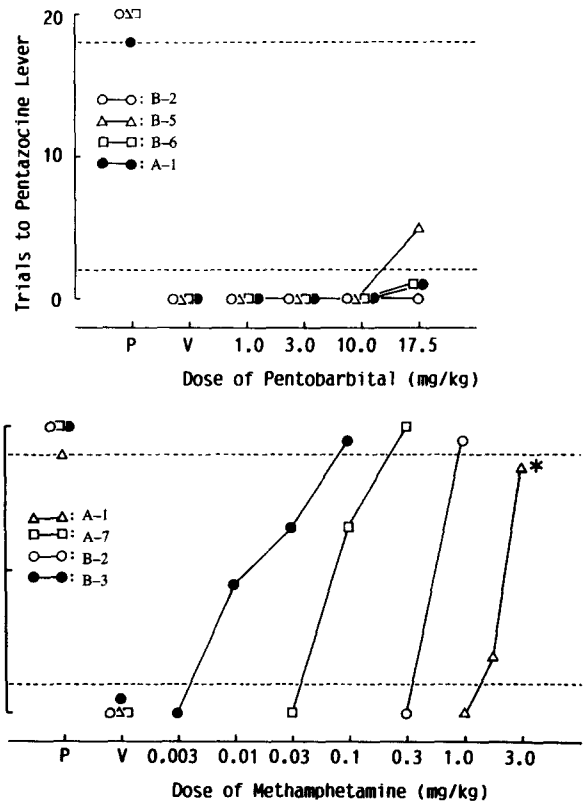


FIG. 6. Discriminative stimulus effects of graded doses of pentobarbital and methamphetamine in the rat trained to discriminate 3.0 mg/kg of pentazocine (P) from vehicle (V). *This rat completed only 19 of the 20 trials after an injection of 3.0 mg/kg of methamphetamine. Other details are the same as in Fig. 1.

phine generalized to pentazocine cue in the rat are consistent with those reported previously using food-reinforced responding (11). It has also been demonstrated that pentazocine produces stimulus effects in common with morphine in the rat (15). Similar results have been demonstrated in man (10). The cross-generalization between these drugs may be the outcome of their similar action at the mu opioid receptors. However, the doses of naltrexone to antagonize the stimulus effects of pentazocine were larger than those in the case of morphine (15). The different sensitivity of opioids to antagonism by naltrexone has previously been used as evidence that drugs are acting at different opioid receptors (1, 2, 4, 12). According to quantitative studies, the effects of pentazocine are different from those of morphine. The pA_2 value for pentazocine-naloxone is significantly lower than that for morphine-naloxone (19). Moreover, it is well known that the discriminative stimulus effects of morphine are antagonized by much lower doses (<0.1 mg/kg) of naloxone and naltrexone, while those of the kappa opioid agonist, ethylketocyclazocine are antagonized by more than 0.1 mg/kg doses of the antagonists (7,15). It is thus likely that the stimulus effects are associated with an opioid but non-mu mechanism.

The pentazocine cue did not generalize to the ethylketocyclazocine cue. Similar results have not been obtained in the rat, whilst the results agreed with those in the squirrel monkey (22). Additionally, a recent finding has indicated that monkeys trained to discriminate ethylketocyclazocine fail to generalize the effects of pentazocine (5). These suggest that the stimulus effects of pentazocine (3.0 mg/kg) are not mediated through a kappa component.

SKF 10,047, a prototypic agonist for sigma receptors, engendered responding appropriate for pentazocine. In the monkey trained to discriminate 3.0 mg/kg of pentazocine from vehicle, SKF 10,047 does not have any common discriminative effects with pentazocine (22). Although, in the rat trained to discriminate higher doses (3.0–5.6 mg/kg) of SKF 10,047 from vehicle, pentazocine has no common discriminative effects (17), the drug seems to have common stimulus effects with lower doses (0.3–1.0 mg/kg) of SKF 10,047 through sigma receptors.

The contribution of delta opioid receptors, on the other hand, would not be ruled out in the stimulus effects of pentazocine, even though pentazocine has much lower affinity for delta receptors in the rat brain (23). Unfortunately, the delta-selective compounds other than peptides which undergo rapid degradation by peripheral

enzymes when administered systemically are unavailable, thereby preventing the direct test of the hypothesis.

It is generally recognized that relatively low training doses produce relatively nonspecific discriminative effects (16,18). Although pentobarbital did not have any common discriminative effects with a 3.0 mg/kg dose of pentazocine, methamphetamine, an indirect dopamine agonist, produced responding appropriate for pentazocine, suggesting a nonspecific component in the discriminative stimulus effects of pentazocine. This would be compatible with the evidence that only 10 of 16 rats acquired the discrimination. The exact nature of the component, possibly dopamine system, remains to be determined.

Butorphanol (0.3 mg/kg), a mixed agonist-antagonist, generalized to pentazocine cue. This seems to be consistent with the fact that the drug has many pharmacological characteristics in common with pentazocine in animals and human subjects (3). The present results demonstrate that nalorphine generalized to pentazocine cue. However, nalorphine has been reported not to produce stimulus effects in common with pentazocine (10.0 mg/kg) in the rat with food reinforcement (VI-15 sec) (6). However, the evidence obtained by Hirshhorn (6) appears to be consistent with the idea that low doses of nalorphine produce a stimulus equivalent to that of a low dose of pentazocine. We may find, as did Hirshhorn (6), that with higher doses of nalorphine, the test stimulus no longer substitutes.

On the other hand, extensive studies have been conducted in both man and the dog to determine if indeed pentazocine has morphine-like activity. However, there is no evidence in man or the dog, unlike the present data, that pentazocine or nalorphine has any morphine-like agonistic activity (10) despite that doses of nalorphine, morphine and pentazocine cannot readily be equated among man, the dog and the rat. For example, pentazocine (<30.0 mg/kg) or nalorphine (<16.0 mg/kg) shows no activity in suppressing withdrawal abstinence in morphine-dependent subjects or dogs, while buprenorphine, a partial agonist, substitutes for morphine (9). Moreover, man should clearly distinguish low doses of pentazocine from low and high doses of morphine. In particular, although the experiments have not been done, a 3.0 mg/kg dose of pentazocine would never be mistaken for 0.25 or 0.5 mg/kg of morphine by human subjects. The same seems to be true for the dog. There are several potential explanations of these discrepancies between data: 1) the mechanisms of actions of opioids in the rat and monkey differ from their mechanisms in man and the dog (e.g., the specificities of the receptor differ from species to species), 2) the subjective effects that these drugs produce in the monkey and rat may differ from those produced in man and the dog, 3) subjects and animals may not be able to discriminate low doses of drugs with different mechanisms of action. However, the possibility of the statement 3 would be low, because it has been reported that the qualitative nature of the syndrome of pentazocine-elicited subjective effects associated with discriminative stimulus effects changes as a function of dose (10). Similar evidence has been obtained in the discriminative stimulus effects of morphine in the rat (16).

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