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The Discriminative Stimulus Properties of the R_2 Isomer of Viminol[1]

JENNIFER E. SHOOK, MARY JEANNE KALLMAN AND WILLIAM L. DEWEY

Department of Pharmacology, Medical College of Virginia
Virginia Commonwealth University, Richmond, VA 23298

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SHOOK, J. E., M. J. KALLMAN AND W. L. DEWEY. *The discriminative stimulus properties of the R_2 isomer of viminol.* PHARMACOL BIOCHEM BEHAV **20**(1) 59–62, 1984.—Viminol is a pyrrylethanolamine derivative which exists naturally as a racemic mixture containing six different stereoisomers. Viminol has been reported to exert both potent analgesic activity and minimal dependence liability. The analgesic component of racemic viminol has been attributed to the R_2 isomer, while the antagonistic S_2 isomer appears to be responsible for minimizing the dependence liability of the racemate. We tested the R_2 isomer of viminol in rats trained to discriminate 3 mg/kg morphine sulfate from saline on a VI-15 sec schedule for sweetened milk reinforcement. The R_2 isomer resulted in dose dependent morphine-like responding, with complete generalization to the 2.5 mg/kg dose of R_2 viminol. The morphine-like discriminative stimulus properties of R_2 viminol were reversed by naloxone in a dose-dependent fashion, with total blockade by 0.1 mg/kg naloxone. R_2 viminol, like morphine, also had a biphasic effect on response rate with low doses increasing and high doses suppressing response rates. R_2 viminol had a overall shorter time course than that reported for morphine, and its different physiological and behavioral effects may not occur simultaneously. These data suggest that R_2 viminol exerts a subjective effect similar to that of morphine and supports the hypothesis that R_2 viminol has opiate activity despite its lack of structural relationship to the opiate series.

Drug discrimination Morphine Viminol R_2 isomer Operant disruption

VIMINOL is a pyrrylethanolamine derivative with potent analgesic activity [1]. The structures of morphine and viminol are presented in Fig. 1. Viminol lacks structural similarities with any of the known narcotic analgesics. Viminol has 3 assymetric carbons and exists naturally as a mixture of 6 stereoisomers [3,6]. The racemic mixture has a profile of effects similar to morphine except that viminol produces only mild physical dependence, while morphine produces severe physical dependence and has high abuse liability [1, 2, 3, 4]. The ability of racemic viminol to produce centrally mediated analgesia with low addiction liability appeared to be a separation of pharmacological properties which are usually considered to be interrelated.

The isolation and characterization of the separate stereoisomers revealed a collection of isomers, each with unique properties, some of which are opposing, which may result in the low addiction liability of the racemic mixture [3,4]. The R_2 isomer has been shown to be the isomer most similar to the opiate prototype morphine [3,4]. R_2 Viminol produced centrally mediated analgesia and was more potent than morphine in several classic analgesic tests [3]. R_2 Viminol, like morphine, has been shown to reduce the release of acetylcholine from the guinea pig ileum [4], cause catalepsy [4], show cross-tolerance to morphine [3], have antitussive activity [5,7] produce physical dependence [3], substitute for morphine in chronic morphinized monkeys (personal communication from Dr. M. Aceto, Medical College of Virginia,

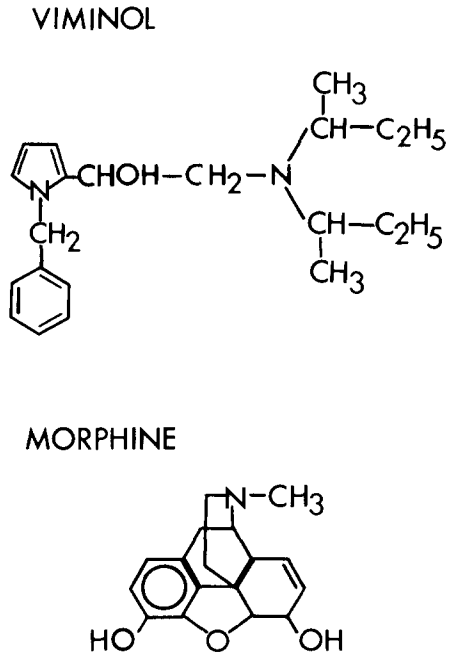

FIG. 1. Chemical structures of viminol and morphine.

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Richmond, VA) and be antagonized by naloxone [4]. R_2 Viminol has been shown to differ from morphine in its molecular structure, synthetic origin and its good oral activity [6]. R_2 Viminol has been shown to bind to opiate receptors but its relatively low affinity does not correlate well with its great *in vivo* potency [4].

We used the drug discrimination paradigm to compare the discriminative stimulus properties of R_2 viminol to those of morphine. Morphine produces a discriminative stimulus for which other narcotic analgesics can substitute [8]. And drugs which have been shown to produce morphine-like discriminative stimuli are highly correlated with those drugs which produce morphine-like subjective effects in humans [8]. Our primary interest was to determine if a similarity exists between the discriminative stimulus properties of R_2 viminol and morphine (which would imply a similarity of subjective effects in humans). In addition, the effects of R_2 viminol on response rate and the time course of both response rate and the discriminative properties were examined. Naloxone was also tested as a potential antagonist.

METHOD

Animals

Male Sprague-Dawley rats purchased from Dominion Animal Supplies Co., (Dublin, VA) were housed individually in temperature-controlled rooms with a 12-hour light/12-hour dark cycle. They received water ad lib and were fed Purina rat chow. All rats were maintained at approximately 85% of their free-feeding weights by daily adjustment of access to food.

Drugs

Morphine sulfate was obtained from Mallinkrodt Chemicals. The R_2 isomer of viminol was obtained from Zambon Research Laboratories, Milano, Italy. The vehicle for R_2 viminol was polyvinylpyrrolidine (PVP). Naloxone was obtained from Endo Laboratories.

Equipment

Standard operant chambers (Coulbourn Model E10-10), housed within light and sound attenuating outer chambers, were used. Two response levers, a central drinking trough, and a dipper which delivered sweetened milk reinforcement were located on one wall of each chamber. A recessed light was located above the trough, which was illuminated when reinforcement was presented. A 24 V houselight illuminated the chamber throughout each session. Solid state and electromechanical programming and recording equipment were located immediately adjacent to the chambers.

Discrimination Procedures

Rats were initially trained to lever press on a variable interval 15-sec schedule of sweetened milk presentation. Once trained to this operant task, rats were trained to press specifically the right or left lever depending on whether they were injected with drug or saline vehicle 30 min prior to the session. Each rat was assigned a particular drug-appropriate and saline-appropriate lever, and lever assignments were counterbalanced across the entire group (N=6 to 8). Fifteen min sessions were conducted daily and drug (D) and vehicle (V) injections were presented on a double alteration schedule (DDVVDDVV). On days of drug training (IP 3 mg/kg morphine sulfate, 30 min prior to testing), rats were reinforced for responses made only on the drug-appropriate lever. When given IP injections of saline (30 min prior to testing), only those responses made on the saline-appropriate lever were reinforced. The degree of discriminative control was determined by 2.5-min extinction tests at the beginning of every other session. During the extinction test period responding was not reinforced. While under training conditions, the 2.5-min extinction test was followed immediately by a 12.5-min training session during which responses made on the appropriate lever were reinforced. Criterion for testing of novel compounds was 80% or greater correct responding following consecutive saline and morphine injections. When testing novel compounds, animals were removed from the operant chambers immediately after the 2.5-min extinction tests and returned to their home cages. Extinction tests for R_2 viminol were performed as described above on the day after the animals reached testing criterion. Since animals did not always satisfy testing criterion simultaneously, R_2 viminol testing occurred independently in each animal. Morphine, R_2 viminol, and naloxone plus R_2 viminol were tested in that order in all rats and each drug was presented in order of low to high doses. For the time course determination, all rats were tested as described before except that preinjection times of R_2 viminol were varied and extinction tests were done at 5, 15, 30 and 60 min after injection. Preinjection times for all other tests were 30 min for R_2 viminol and 35 min for naloxone.

Data Analyses

For each test session, % drug bar responding, response rate and response rate expressed as % saline control were calculated. Percent drug bar responding was calculated by dividing the number of responses made on the drug appropriate lever by the total number of responses made during the 2.5-min test session. Response rate expressed as percent of saline control was calculated as the mean response rate for the test day divided by the mean response rate on the most recent saline test day.

RESULTS

After training had proceeded for approximately 12 weeks, animals were tested with various doses of morphine. The dose-response curve for morphine in rats trained to discriminate 3 mg/kg morphine from saline is presented in Fig. 2. Morphine resulted in dose-dependent increase in % drug-bar responding and only those doses equal to or greater than the training dose were generalized. From this, we concluded that the animals had learned the discrimination task and were ready for further testing. Morphine also caused a biphasic trend in response rates; at low doses, rates were increased and with higher doses, rates were decreased.

As seen in Fig. 3, the discriminative stimulus properties of the morphine training dose generalized to R_2 viminol in a dose-dependent fashion, with 2.5 mg/kg R_2 viminol producing a peak effect of 93% ($\pm$2.9) drug-bar responding. The PVP vehicle elicited saline-like responding. Comparison of the doses of morphine (3 mg/kg) and R_2 viminol (0.85 mg/kg) which resulted in 80% drug-bar responding shows that R_2 viminol is approximately 3.5 times more potent than morphine in producing this effect. R_2 Viminol also caused a biphasic effect on response rate similar to that of morphine, with low doses increasing and higher doses suppressing re-

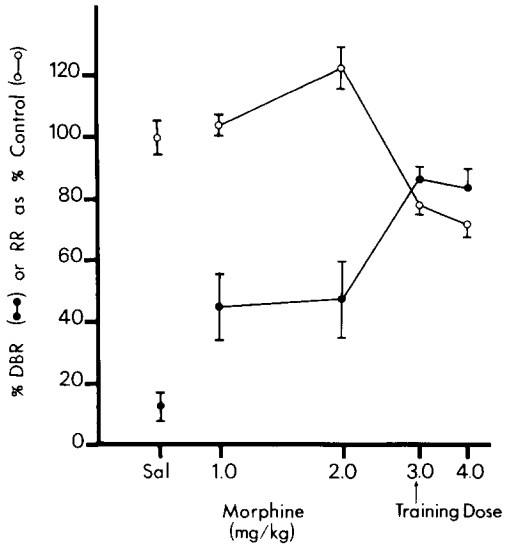

FIG. 2. Morphine dose effect curve. The % drug bar responding (%DBR) and the response rate expressed as % saline control values (RR as % control) following saline or various doses of morphine are represented by closed circles and open circles respectively. Each point represents the mean of 8 animals.

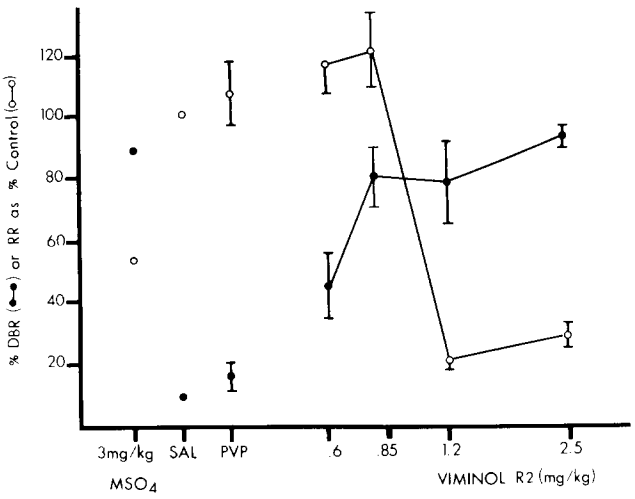

FIG. 3. R_2 Viminol dose-effect curve.The % drug bar responding (%DBR) and response rate expressed as a % of saline control values are represented by closed circles and open circles respectively. The %DBR and RR as % control for 3 mg/kg morphine sulfate (MSO_4), saline (Sal) and PVP are given for comparison. Each point represents the mean of 8 animals.

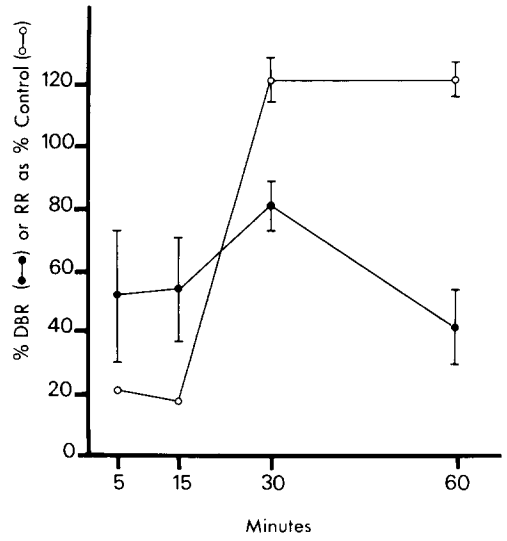

FIG. 4. Time course of actions of R_2 viminol. The % drug bar responding (%DBR) and response rate expressed as % saline control (RR as %) are represented by closed circles and open circles respectively. Extinction tests were performed at 5, 15, 30 and 60 min after injection of 0.85 mg/kg R_2 viminol. Each point represents the mean of 6 animals.

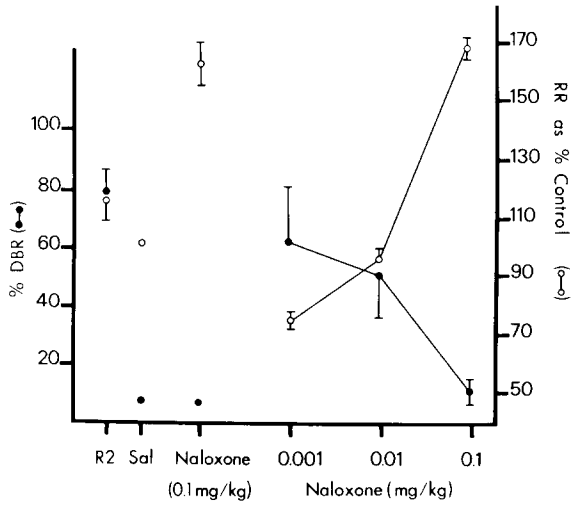

FIG. 5. Naloxone reversal of R_2 viminol. The % drug bar responding and response rate expressed as % saline control (RR as % control) are represented by closed circles and open circles respectively. The %DBR and RR as % control for 0.85 mg/kg R_2 viminol, saline (Sal) and 0.1 mg/kg naloxone are given for comparison. Naloxone and 0.85 mg/kg R_2 viminol were injected in that order at 35 and 30 min prior to extinction testing. Each point represents the mean of 6 animals except for the R_2 viminol and saline points which are the mean of 8 animals.

sponse rates. R_2 Viminol was also more potent than morphine in causing response rate suppression.

From the dose-effect curve for R_2 viminol, 0.85 mg/kg R_2 viminol was found to produce 80% drug-bar responding with facilitation of response rate. This dose was used at all time points (5, 15, 30, and 60 min) in the time course determinations. The results of this study are presented in Fig. 4. R_2 Viminol (0.85 mg/kg) elicited time-dependent generalization from the morphine training dose with peak effect (80% drug-bar responding) at 30 min (this was the preinjection time for all other experiments). Generalization fell to 40% at 60 min. At 30 min, response rates were increased and this effect persisted throughout the entire test session. Surprisingly, at 5 and 15 min this dose resulted in dramatic suppression of response rate with only 51 and 53% drug bar responding, respectively. From the dose-effect curve (Fig. 3), one

can see that a lower dose (0.6 mg/kg R_2 viminol) also resulted in an increase in response rates, and that all higher doses (1.2 and 2.5 mg/kg R_2 viminol) suppressed response rate at 30 min. R_2 Viminol apparently produces a predictable temporal pattern of effects on response rate with an initial phase of suppression followed by facilitation, and the duration of both phases is increased with increasing dosage.

The data in Fig. 5 demonstrate the dose-dependent reversal of the discriminative stimulus properties of R_2 viminol by naloxone. Naloxone (0.1 mg/kg) plus 0.85 mg/kg R_2 viminol resulted in only 10% drug bar responding (± 4.8), and thus completely blocked the morphine-like discriminative stimulus properties of R_2 viminol. We also expected rates to return toward control values with naloxone, but 0.1 mg/kg naloxone plus R_2 viminol instead caused a marked stimulation of response rate. Since 0.1 mg/kg naloxone alone also resulted in this marked increase in response rate, we have concluded that naloxone probably reverses the effects of R_2 viminol on response rate, but also exerts other effects which may influence response rate.

DISCUSSION

Rats trained to discriminate 3 mg/kg morphine from saline generalize to R_2 viminol in a dose-dependent fashion, with R_2 viminol being more potent than morphine in eliciting the morphine-like discriminative stimulus properties. These data imply that R_2 viminol causes subjective-effects similar to those of morphine. It has been proposed that the discriminative effects of a drug are predictive of its abuse liability as well as being predictive of its subjective effects in humans [8]. If this is true, our data suggest that R_2 viminol, like morphine, has a high abuse liability.

Like morphine, R_2 viminol also causes a biphasic effect on response rate, and again R_2 viminol is more potent than morphine in producing this effect. R_2 Viminol has a rapid onset of action and is relatively short acting in comparison to the reported time course for morphine [8]. R_2 Viminol (0.85 mg/kg) produced a strong initial suppression of response rate followed by an increase in rate, at which time the morphine-like discriminative stimulus properties became apparent. The response rate suppressing effects of R_2 viminol thus had a faster onset than its morphine-like discriminative properties, and these two effects also appeared to differ in duration (Fig. 4). The strong morphine-like discriminative stimulus properties of R_2 viminol were also blocked by naloxone.

The generalization of morphine stimulus control to R_2 viminol, the similarity of effects on response rate, and the reversal by naloxone suggest that R_2 viminol exerts morphine-like activity despite it lack of structural similarity to morphine. These data support the work of others who have shown the similarity of actions of R_2 viminol and morphine on isolated organ systems and various cellular phenomena. The evidence available to date suggests that R_2 viminol and morphine actions are mediated by a common receptor mechanism. R_2 Viminol has been shown to bind to opiate receptors but with only 1/10 [4] to 1/100 [6] the binding capacity of morphine. This interaction of R_2 viminol with opiate receptors probably contributes to its morphine-like activity, but this is not a perfect explanation because while morphine is more potent than R_2 viminol in *in vitro* binding, R_2 viminol is more potent than morphine *in vivo*. This discrepancy in potency may be explained by the fact that R_2 viminol crosses the blood-brain barrier more easily than morphine [3] and thus could conceivably yield proportionately greater brain levels than morphine.

The suggestion that R_2 viminol does interact with opiate receptors is in defiance of the strict structure-activity-relationship presently associated with the narcotic series. The ability of this novel structure to interact with opiate receptors may be attributed to the flexible nature of this molecule [6] which may enable it to interact in some way with the receptor. The S_2 isomer of viminol which may be responsible for minimizing the abuse liability of racemic viminol has been shown to antagonize the actions of R_2 viminol and morphine in several systems [6], and also worsens abstinence signs in opiate-withdrawn monkeys [6]. In contrast, S_2 viminol has been shown [4,6] to have very low affinity for opiate receptors ($ED_{50} > 2500$ nM). Thus the antagonistic activity of S_2 viminol cannot be attributed to displacement of R_2 viminol or morphine from an opiate receptor. This evidence suggests that these enantiomers are working through either different receptors or at different sites on the opiate receptor which results in increased specificity of effect.

Further studies on the pharmacology of and the interaction among all the isomers of viminol are needed, and hopefully from such studies, we can develop a greater understanding of the opiate receptors and develop new orally administered drugs and greater specificity of action.

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