J Clin Pharmacol. 1981; 21:334S-340S.

The Antinociceptive Effects of Intrathecally Administered Levonantradol and Desacetyllevonantradol in the Rat

TONY L. YAKSH, Ph.D. Rochester, Minn.

Abstract: Levonantradol and its desacetylated metabolite (desacetyllevonantradol) produced a dose-dependent increase in the hot plate and tail flick response latencies following intrathecal administration in a dose range from 4 to 40 μg. No difference in potency between the two drugs was observed, as defined by the ED_{50} values obtained in either test. The duration of the effect of either drug was also dose dependent, ranging from 30 to 120 minutes. No effect on placing, stepping or righting reflexes or the ability of the rats to negotiate a 60° inclined plane was observed at these doses. At the highest doses, however, an exaggerated myoclonic response to abrupt muscle stretch was observed. None of those effects was antagonized by naloxone (2 mg/kg) administered intraperitoneally before or after levonantradol.

DELTA-9-tetrahydrocannabinol (THC) has been shown to possess antinociceptive properties in man and in animal pain models.[1–5] Levonantradol, a cannabinoid analog, also has been shown to exert this antinociceptive action. These effects have been reported to be independent of an opiate system by virtue of the inability to block their actions by naloxone[5–8] or to show displacement of opioid ligands in binding assays.[7] The clear effect of this family of drugs on perception has been associated with the change in the perceived pain threshold. Recent data have, however, lent credence to the possibility that these agents may, in addition, serve to modulate nociceptive spinal processing. A possible spinal site of action is suggested by the observation that the systemic administration of cannabinoids will antagonize spinal reflex activity such as the tail flick.[8] In addition, Gilbert[6] has reported that nantradol inhibits the skin twitch reflex in the spinal dog. Since these effects occur at doses which do not produce significant changes in motor behavior, the possibility is offered that the antinociception may be mediated by an action on some specific facet of nociceptive sensory transmission at the level of the spinal cord.

A direct test of this question is to examine the local effects in the spinal cord of these agents on the behavior of the intact and unanesthetized animal. The present experiments were to investigate the effects of intrathecally administered levonantradol and its deacetylated metabolite, desacetyllevonantradol, on the nociceptive threshold of the rat as measured by both a spinal reflex (tail flick) and a more complex behavioral task (hot plate).

Methods

Animal Preparation. Rats (male, Sprague-Dawley; 300 to 350 Gm) were implanted with a polyethylene catheter (PE-10; 0.5 mm o.d.). The catheter was inserted through the cisternal membrane, 7.5 to 8.0 cm to the

From the Department of Neurosurgical Research, Mayo Clinic, Rochester, Minn. 55901.

lumbar enlargement. Details of this procedure are given elsewhere.[10]

Intrathecal Injection Procedures and Drugs. Intrathecal injections of drugs or vehicle were made in a volume of 15 μl over a period of 10 to 15 seconds. Each injection was followed by an injection of 10 μl saline to flush the contents of the catheter. Levonantradol and desacetyllevonantradol hydrochloride were dissolved in spectral-grade dimethyl sulfoxide (DMSO, Sigma) and brought to volume with saline, such that the final concentration was 20% DMSO. This solution was used for all control or vehicle injections.

Behavioral Testing. The nociceptive thresholds were ascertained by the tail flick and hot plate (52.5°C) response. The measured response was a movement of the tail from the heat source (a focused projection bulb) for the tail flick, or by a licking of the hindpaws, jumping, or a rapid up-and-down motion of the hindpaw, for the hot plate. Tests were terminated if the animal failed to respond by 6 or 60 seconds on the tail flick and hot plate tests, respectively (cutoff times). Animals were tested twice in each procedure 24 hours before drug or vehicle injection. Motor capacity was assessed by examining hindpaw placing and stepping reflexes and the ability of the animal to negotiate a 60° inclined wire mesh surface.

Data Analysis. Parallel control (vehicle) and drug groups were always run on the same day. Animals were pretested (baseline) and assigned to each group so that the mean group response latencies were matched. Data are presented as either the response latency in seconds (mean ± S.E.) or as the maximum per cent effect (MPE) as follows:

$$MPE = \frac{\text{predrug} - \text{postdrug effect}}{\text{cutoff time} - \text{predrug effect}} \times 100$$

Statistical comparisons were carried out using a *t*-test for repeated measures. Differences reaching a $P < 0.05$ level of probability were considered as statistically significant. ED_{50} values with 95 per cent confidence intervals were determined as described by Goldstein[9] using doses which fell by inspection on or near the linear portion of the dose-response curve.

Results

Intrathecal injections of the DMSO/saline vehicle had no measurable effect on the hot plate and tail flick latencies over the time course of these experiments (Fig. 1). Levonantradol and desacetyllevonantradol, however, produced a significant, time-dependent elevation in the hot plate and tail flick response latencies. The duration of this effect was dose dependent, lasting from 30 to 120 minutes. There was no detectable difference between the drugs insofar as rate of onset or the overall duration was concerned (Fig. 1). The magnitude of the increase in the tail flick and hot plate response latencies was dose dependent for both levonantradol and desacetyllevonantradol over a dose range of 4 to 40 μg. The dose-response curves shown in Fig. 2 for the two drugs did not statistically deviate from parallelism on either the hot plate or tail flick tests. Comparison of the ED_{50} for the two drugs revealed no difference on either test (Table I).

To determine if the effects were altered by intraperitoneal administration of naloxone (2 mg/kg), animals were injected either 10 minutes before the intrathecal injection of levonantradol (40 μg) or vehicle, or 15 minutes after the intrathecal levonantradol (40 μg). As shown in Fig. 3, at no time was a detectable difference observed in the antinociceptive effects of intrathecal nantradol as measured by the hot plate response latency with either paradigm. Similar results were observed with respect to the tail flick test but are not presented here.

The intrathecal injection of low doses of either levonantradol or desacetyllevonantradol had no detectable effects on the behavior of the animal, aside from increasing its hot plate and tail flick response latency.

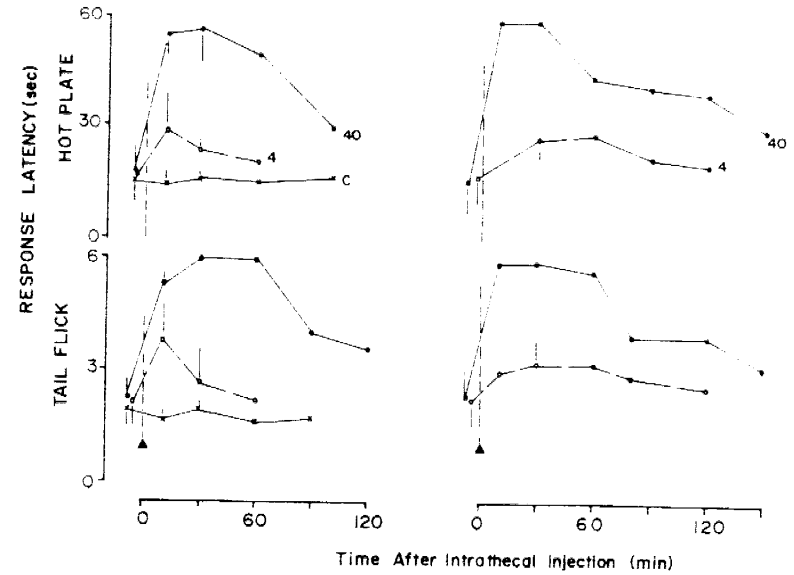

Fig. 1. Hot plate (top) and tail flick (bottom) response latencies (seconds) vs. time (minutes) after intrathecal injection of vehicle (C) (x) or 4 μg (o) or 40 μg (•) levonantradol (left) or desacetyllevonantradol (right). Each time-response curve represents the mean ± S.E. of four to eight animals.

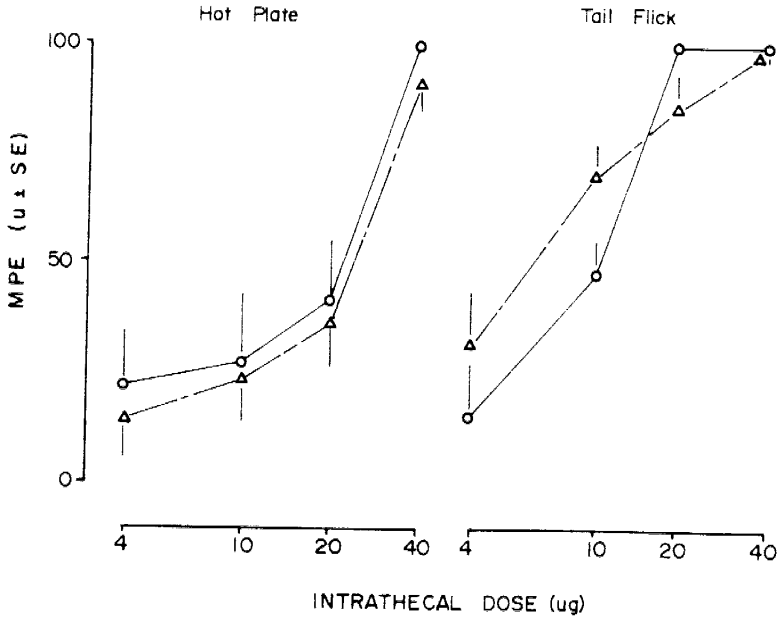

Fig. 2. Log dose-response curves for intrathecal levonantradol (o) and desacetyllevonantradol (Δ) on the hot plate (left) and tail flick (right) test. Each point presents the mean ± S.E. of a maximum per cent effect (MPE) for eight to 15 animals.

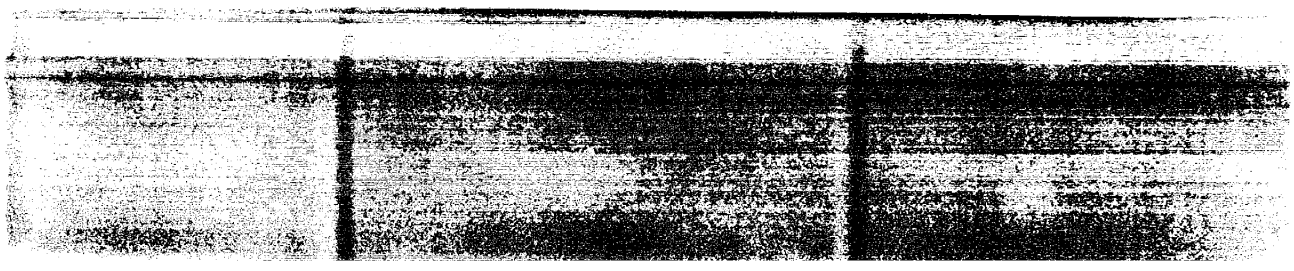

TABLE I

ED_{50} Values for Intrathecal Levonantradol and Desacetyllevonantradol on the Hot Plate and Tail Flick Tests

Drug	N	ED_{50} (μg ± S.E.)	
		Hot plate	Tail flick
Levonantradol	46	18 ± 4	8 ± 3
Desacetyllevonantradol	47	19 ± 5	6 ± 3

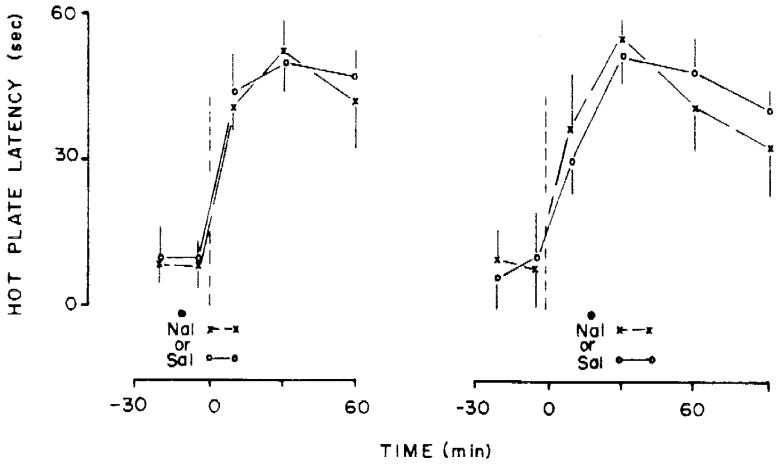

Fig. 3. Effects of naloxone (x) (2 mg kg i.p.) or saline (o) (1 ml/kg i.p.) on the antinociceptive effects of intrathecal levonantradol (40 μg) injected at the time indicated by the vertical dashed line. Each time-response curve presents the mean ± S.E. of five animals.

TABLE II

Per Cent of Rats as a Function of Dose of Intrathecal Levonantradol Showing Loss of Motor Function and/or Displaying the "Bouncing Rat" Syndrome

Levonantradol dose (μg)*	N	Per cent of group	
		Motor disability	Bouncing rat
0	22	0	0
4	12	0	0
10	15	0	5
20	12	0	58
40	18	0	100

* All injections in 15 μl/20% DMSO-saline.

As shown in Table II, however, at doses of 20 and 40 μg of either drug, there was an increasing incidence of an exaggerated myoclonus in response to abrupt muscle stretch. Thus, allowing the animal to fall on all four paws from a height of a few centimeters resulted in a springing behavior which we refer to as the "bouncing rat syndrome." At no dose was the ability of the animal to negotiate the 60° wire mesh impaired. Similarly, no effect on placing, stepping, or righting reflexes was observed. There appeared to be some evidence of an enhanced sensitivity to light stroking of the fur as indicated by the animals' squeaking behavior, but this was difficult to quantify.

At the 40-μg dose, catalepsy was observed after a 30- to 40-minute latency in all animals, suggesting the likelihood of a systemic or supraspinal effect resulting from diffusion.

None of the above effects was altered by the intraperitoneal administration of naloxone (2 mg/kg) either before or after the intrathecal injection of levonantradol.

Discussion

The present results indicate that the intrathecal injection of levonantradol or its desacetylated metabolite, desacetyllevonantradol, produced a dose-dependent elevation in the hot plate and tail flick response latencies. We believe the action of these drugs on spinal function reflect an antinociceptive effect, as the effects occurred at doses which did not significantly impair the animals' motor ability to make the response.

The similar magnitude and time course of the activity of the two agents does not permit us to exclude the possibility that all of the pharmacologic activity resides in the deacetylated metabolite. The parallelism of the dose–response curves on each test and the comparability of the slopes between tests is consistent with (but does not prove) the belief that the two drugs are working at similar sites and that the neural substrate affected by the two drugs in the spinal cord relevant to the two different tests is the same.

The pharmacology and physiology of the mechanisms whereby levonantradol produces its spinal effect are not known. The failure of extensive efforts to antagonize the behavioral effects of levonantradol with naloxone, argues against an opiate sensitive link, and is in agreement with results published by other laboratories (see introduction). Whether these cannabinoids act directly upon some links in the spinal nociceptive pathway or exert an influence via other modulatory systems such as those involving monoamines or GABA remain a subject for further investigation.

The "bouncing rat syndrome," reflecting an intensified monoclonic response to muscle stretch, has been observed following systemic administration. The present observations appear to suggest that the exaggerated reflex is due to an effect on spinal function. Whether this interaction is due to an action on the afferent portion of the gamma motor loop or on the repetitive discharge properties on the motor neuron is not presently known.

Acknowledgments

The author would like to thank Ms. Gail Harty for her technical assistance, Ms. Ann Rockafellow for preparing the manuscript, and Drs. George Milne and Ron Brown for supplies of levonantradol and desacetyllevonantradol. This work was supported in part by funds from the Mayo Foundation.

References

1. Bloom AS, Dewey WL. A comparison of some pharmacological actions of morphine and Δ^9-tetrahydrocannabinol in the mouse. *Psychopharmacology* (Berlin), 1978; **57**:243–248.
2. Brunk SF, Noyes R Jr, Avery DH, Canter A. The analgesic effect of Δ^9-tetrahydrocannabinol. *J Clin Pharmacol.* 1975; **15**:554.

3. Dewey WL, Harris LS, Kenney JS. Some pharmacological and toxicological effects of 1-*trans*-Δ^8-tetrahydrocannabinol and 1-*trans*-Δ^9-tetrahydrocannabinol in laboratory rodents. *Arch Int Pharmacodyn Therap.* 1972; **196**:133-145.

4. Noyes R Jr, Brunk SF, Avery DH, Canter A. The analgesic properties of Δ^9-tetrahydrocannabinol and codeine. *Clin Pharmacol Therap.* 1975; **18**: 84-89.

5. Sanders J, Jackson DM, Starmer GA. Interactions among the cannabinoids in the antagonism of the abdominal constriction response in the mouse. *Psychopharmacology* (Berlin). 1979; **61**:281-285.

6. Gilbert PE. A comparison of THC, nantradol, nabilone, and morphine in the chronic spinal dog. *J Clin Pharmacol.* 1981; **21**:311S-319S.

7. Milne GM, Koe BK, Johnson MR. Stereospecific and potent analgetic activity for nantradol. A structurally novel, cannabinoid-related analgetic. In: Harris LS, ed. *Problems of Drug Dependence, 1979.* NIDA Research Mongraph 27. Rockville, Md.; 1980: 84-92.

8. Milne GM, McIlhenny HM, Johnson MR, Koe BK, Weissman A. One way cross tolerance from morphine to nantradol—a potent, non-opioid analgetic. In: Way EL, ed. *Endogenous and Exogenous Opiate Agonists and Antagonists.* New York: Pergamon Press; 1980:367-370.

9. Goldstein A. *Biostatistics.* New York; Macmillan; 1968.

10. Yaksh TL, Rudy TA. Chronic catheterization of spinal subarachnoid space. *Physiol Behav.* 1976; **17**:1031-1036.

Address reprint requests to: Tony L. Yaksh, Ph.D., Mayo Clinic, Rochester, Minn. 55901.

Discussion of the Paper

Dr. Lassner: Is the effect segmental, that is limited to the caudal segments of the spinal cord?

Dr. Yaksh: Yes. We also test the animal's response to pinching of the paw. The animal will move the foot voluntarily, showing no motor disability, but as with morphine there is no vocalization.

Dr. Nahas: What you call the "bouncing rat" phenomenon has been observed in chronic intoxication of rodents given high doses of THC. It is called a "popcorn effect", and is associated with toxicity to the central nervous system.

Dr. Yaksh: Yes, I believe that toxicity is present, but also that the motor effect is in the spinal cord.

Dr. Nahas: Have you noticed development of tolerance to the analgesic effect?

Dr. Yaksh: We have not investigated tolerance development following direct intrathecal administration. Since we have seen analgesia using the intrathecal administration of morphine in cats and primates, our next plan was to proceed to study the intrathecal effects of levonantradol in the primate on shock titration. In preliminary experiments in the cat, intrathecal levonantradol blocks skin twitch, very much in line with what has been reported in the dog (Gilbert, this monograph).

Dr. Braude: Could you elaborate on your choice of DMSO as a solvent?

Dr. Yaksh: Histological studies in cats that have received acute DMSO injections show no gross evidence of toxicity. Behaviorally, we have not detected an effect.

Dr. Dewey: Have you administered levonantradol into the ventricle of the brain, and how does it compare with morphine potency when administered intrathecally?

Dr. Yaksh: We have not administered levonantradol intracerebrally. Intrathecally, levonantradol and morphine have about the same potency, in contrast to the difference observed systemically. The difference may reflect enhanced lipid permeability for levonantradol.

Dr. Aisner: DMSO in addition to being a solvent, will carry many solutes across cell membranes. Do you have any information that you are not measuring a systemic effect rather than a localized effect because of the solvent effect of the DMSO?

Dr. Yaksh: You can definitely smell DMSO on their breath. However, doses of 40 μg given in the femoral vein of an unanesthetized rat do not block the tail flick. In fact, if you did have a systemic effect, you wouldn't expect to see a segmental analgesia. I should mention, however, that catalepsy is seen after about sixty minutes. I am almost certain that that is a supraspinal effect, due to diffusion of the drug.