

D₂, but Not D₁ Dopamine Receptor Agonists Potentiate Cannabinoid-Induced Sedation in Nonhuman Primates^{1,2}

JUSTIN P. MESCHLER,³ FRANCIS A. CLARKSON, PATRICIA J. MATHEWS, ALLYN C. HOWLETT,³ and BERTHA K. MADRAS

Department of Psychiatry, Harvard Medical School, New England Regional Primate Research Center, Southborough, Massachusetts

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ABSTRACT

In primates, CB₁ cannabinoid receptor agonists produce sedation and psychomotor slowing, in contrast to behavioral stimulation produced by high doses of dopamine receptor agonists. To investigate whether dopamine agonists attenuate the sedative effects of a cannabinoid agonist in monkeys, we compared the effects of D₁ or D₂ dopamine receptor agonists on spontaneous behavior in three to six cynomolgus monkeys (*Macaca fascicularis*) alone and after administration of a low dose of the CB₁ agonist levonantradol. Alone, the CB₁ cannabinoid receptor agonist levonantradol (0.01–0.3 mg/kg) induced sedation, ptosis, and decreased locomotor and general activity. Alone, D₂-type dopamine agonists quinolorane (0.001–1.0 mg/kg; *n* = 4) or pergolide (0.01–1.0 mg/kg) or a D₁ dopamine agonist 6-chloro-7,8-dihydroxy-1-phenyl-2,3,4,5-tetrahydro-3-allyl-[1H]-3-benzazepine (0.3–3.0 mg/kg) produced either no effect or promoted hyperactivity. Thirty minutes after

administration of a threshold dose of levonantradol (0.03 mg/kg), D₂-type agonists, but not the D₁ agonist, precipitated marked sedation, ptosis, and decreased general activity and locomotor activity. These data indicate the following: 1) D₂, but not D₁ dopamine agonists, potentiate sedation in monkeys treated with a CB₁ cannabinoid agonist, at doses of agonists that alone do not produce sedation; 2) the threshold dose for cannabinoid-induced sedation is reduced by D₂ agonists, but not by a D₁ dopamine agonist, differentiating D₁ and D₂ dopamine receptor linkage to cannabinoid receptors; and 3) modulation of D₂ dopamine receptor activity by a nonsedating dose of a cannabinoid agonist has implications for the pathophysiology and treatment of dopamine-related neuropsychiatric disorders and drug addiction. Cannabinoid agonists and D₂ dopamine agonists should be combined with caution.

Cannabinoids induce a broad spectrum of pharmacological effects on movement, thermoregulation, seizures, emesis, nociception, appetite, endocrine regulation, memory, reward, sensory perception, and cognition. In the past decade, pivotal discoveries have transformed our understanding of the neurobiological basis for cannabinoid pharmacology. The concept of an endogenous cannabinoid system in brain was shaped by the discovery of the G protein-coupled CB₁ cannabinoid re-

ceptor (Howlett et al., 1990). Receptor-mediated effects of cannabinoids were confirmed with the demonstration of a high and positive correlation between the relative potencies of drugs for producing cannabinoid-like effects in vivo and binding potencies in vitro (Compton et al., 1993). The unique brain distribution of the CB₁ receptor (Herkenham et al., 1990) facilitated identification of a cloned gene encoding the CB₁ cannabinoid receptor (Matsuda et al., 1990) and galvanized efforts to isolate endogenous cannabinoids from brain (Di Marzo et al., 1998).

The CB₁ cannabinoid receptor displays unusual properties, including the dual capacity to inhibit or stimulate adenylate cyclase via Gi/o or Gs proteins (Glass and Felder, 1997; Bonhaus et al., 1998) and brain density considerably higher than the majority of G protein-coupled receptors (Herkenham et al., 1990). Implicit in these properties is the potential for the CB₁ cannabinoid receptor to modulate the function of other receptor systems in brain, and evidence to support this premise is mounting (Pacheco et al., 1993; Ameri, 1999; Lédent et al., 1999; Vasquez and Lewis, 1999).

The focus of the present research is dopamine-cannabinoid interaction in primates. Coadministration of cannabinoid

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³ Current address: Department of Pharmacology and Physiological Science, Saint Louis University, 1402 S. Grand Blvd., St. Louis, MO 63104.

ABBREVIATIONS: SKF 81297, 6-chloro-7,8-dihydroxy-1-phenyl-2,3,4,5-tetrahydro-3-allyl-[1H]-3-benzazepine; EtOH, ethanol.

and dopamine agonists leads to reciprocal facilitative or antagonistic behavioral and biochemical effects in rodents (Bidaut-Russell and Howlett, 1991; Castellano et al., 1997; Glass and Felder, 1997; Tanda et al., 1997; Ameri, 1999; Giufridda et al., 1999). The relevance of these findings to normal brain function in primates and to dopamine-related neuropsychiatric diseases is not known. The ascendancy of dopamine agonists as potential treatments for cocaine addiction (Haney et al., 1998; Levin et al., 1999), merging with the frequent use of cannabinoids in the cocaine abusing population (Gfroerer and Brodsky, 1993), creates a compelling need to investigate dopamine-cannabinoid interaction in primates. Equally significant, cannabinoid-dopamine receptor interaction may be relevant to the pathophysiology of schizophrenia because endogenous cannabinoids, which are released by D₂ dopamine receptors in rodents, are elevated in cerebrospinal fluid of schizophrenics (Leweke et al., 1999).

In nonhuman primates, CB₁ cannabinoid receptor agonists alone produce ptosis, sedation, and ataxia (Young et al., 1981; Beardsley et al., 1987) that is replicable in humans with the cannabinoid agonist levonantradol (Jain et al., 1981). Select dopamine agonists targeting the D₂ dopamine receptor produce modest increases in general activity and environmental exploration in Old World rhesus monkeys (Ferguson et al., 1996). In contrast, D₁ dopamine receptor agonists do not stimulate locomotor activity in normal monkeys but promote grooming behavior and head movements (Rosenzweig-Lipson et al., 1994; Gnanalingham et al., 1995). Overall, CB₁ cannabinoid receptor agonists produce sedation and a generalized slowing of motor function as a function of dose, whereas D₁- or D₂-type dopamine receptor agonists do not elicit sedation but may promote heightened activity in monkeys.

Given the apparently opposing behavioral effects produced by activation of these two receptor systems in monkeys, we hypothesized that dopamine agonists would counteract the decreased general and locomotor activity and increased sedation engendered by the CB₁ cannabinoid receptor agonist. We also hypothesized that the combined effects of a cannabinoid agonist with either a D₂ or D₁ dopamine agonist would differ. To test these hypotheses, the CB₁ cannabinoid agonist levonantradol and D₂/D₃ dopamine receptors agonists pergolide and quinolorane, or the D₁ agonist SKF 81297 (6-chloro-7,8-dihydroxy-1-phenyl-2,3,4,5-tetrahydro-3-allyl-[1H]-3-benzazepine) were administered alone and in combination to Old World cynomolgus monkeys (*Macaca fascicularis*). Drug interaction studies were performed with doses of levonantradol and dopamine agonists that alone had no observable sedative effects in monkeys. Coadministration of D₂ dopamine and cannabinoid receptor agonists in primates potentiated cannabinoid-induced sedation at doses of each drug that alone did not produce sedation. This first report of reciprocal modulation of the behavioral effects of cannabinoid and dopamine agonists has therapeutic implications.

Materials and Methods

Subjects. Six male cynomolgus monkeys with average age 4.7 ± 2.1 years (range 3–9 years) and a weight range of 3.5 to 6.0 kg were used for the study. The monkeys were housed in individual cages in a controlled temperature and humidity environment, and fed monkey chow ad libitum and fresh fruit. The monkeys were on a 12-h

light/dark cycle and all of the studies were performed during the light cycle. Each monkey served as its own control. At least 72 h elapsed between sessions, involving drug testing or recording of baseline behavior.

Drugs. Drugs were chosen on the basis of dopamine receptor selectivity or clinical relevance. We recently completed a systematic study in nonhuman primate brain of the affinities and D₁/D₂ receptor selectivities of a wide range of D₁ dopamine receptor agonists and found SKF 81297 to be 343-fold selective for the D₁ over the D₂ receptor. We also recently observed that SKF 81297 alleviates advanced parkinsonian symptoms in a cynomolgus monkey model of Parkinson's disease and thus appears to have high efficacy in this primate species. Pergolide is a clinically approved drug recently tested as a treatment for cocaine addiction. Quinelorane is a highly selective D₂/D₃ dopamine agonist. Levonantradol (free base) was a generous gift from Pfizer Inc. (Groton, CT). Pergolide (methanesulfonate) and quinolorane (dihydrochloride) were purchased from Research Biochemicals (Natick, MA) and quinolorane also was generously donated by E. Lilly (Indianapolis, IN). SKF 81297 was generously donated by SmithKline Beecham (King of Prussia, PA). The drugs were dissolved in the following vehicles: levonantradol [5% ethanol (EtOH), 5% Emulphor, and 90% saline], quinolorane (5% EtOH and 95% sterile water), pergolide (5% 1 N HCl and 95% sterile water), and SKF 81297 (10% EtOH, 0.2% ascorbic acid in water). Each drug dose was normalized to the molecular weight of the free base form with a conversion factor. All drugs were administered in volumes no greater than 0.7 ml i.m. just before the session taping.

Session Protocols. The effects of drugs on unconditioned behaviors in monkeys were monitored in a large cage (91 × 99 × 83 cm) especially adapted for videotaping. Sessions were video taped in the presence of other monkeys to maintain a familiar environment. Before drug treatment, baseline behavior was filmed for each subject during at least three different periods of the day (morning, midday, and afternoon). No significant differences in unconditioned behavior were detected between these times of day and drug studies were conducted at various times during the day.

Vehicle was administered for the first session in every drug trial to determine the effect of the vehicle and to measure whether the monkey exhibited typical behavior on that particular session day. For quinolorane and pergolide, the drugs were administered on a cumulative dosing schedule every 0.5 h after the first injection of vehicle. After each dose, the monkey was video taped for 0.5 h. Due to the slow onset of i.m.-administered levonantradol (McIlhenny et al., 1981), the drug was administered on a cumulative dosing schedule every hour with scoring performed on the last 0.5 h of every session.

A 3-fold higher dose of levonantradol (0.1 mg/kg), if followed by a single dose of quinolorane (0.01 mg/kg) 30 min later, produced a total loss of locomotor and general activity and extreme sedation (data not shown). The subject was unarousable and aside from respiratory movements, no other movement was detected for >2 h. Based on this observation, replication of this experiment and evaluation of a full dose range of levonantradol in combination with dopamine agonists were not conducted. In subsequent experiments, a dose-response curve was generated and a dose was identified (0.03 mg/kg) that produced no measurable behavioral changes (Fig. 1). We also determined that the effects of levonantradol after a single dose of 0.3 mg/kg lasted for at least 2 h. Therefore, in the drug combination studies, dopamine agonists were administered within 2 h after levonantradol administration.

When levonantradol was combined with the dopamine agonist quinolorane or pergolide, the following protocol was used. First, a levonantradol vehicle was administered i.m. and a 0.5-h session was taped. Subsequently, a dose of 0.03 mg/kg was administered i.m. and a 0.5-h session was recorded. One-half hour after the levonantradol was administered, quinolorane or pergolide was administered every 0.5 h with a cumulative dosing schedule. A maximum of three doses of quinolorane or pergolide was administered after levonantradol

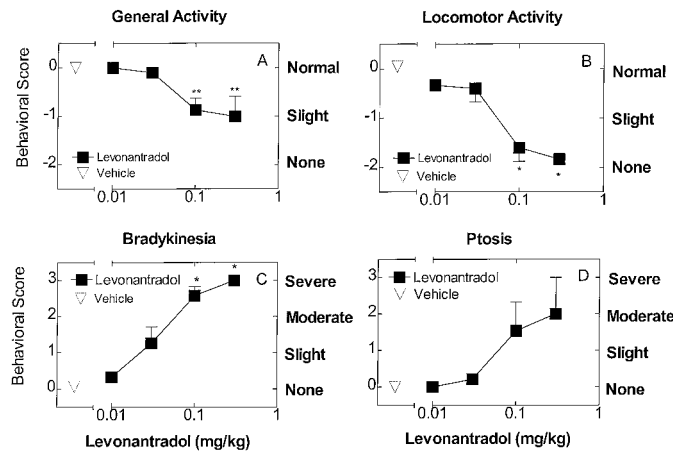


Fig. 1. Effects of a CB₁ cannabinoid agonist levonantradol (i.m.) on spontaneous behavior in monkeys. Various doses of levonantradol were administered to three cynomolgus monkeys: Top left (A) general activity, top right (B) locomotor activity, bottom left (C) bradykinesia, and bottom right (D) sedation. Statistical significance from vehicle is denoted by **P* < .05 or ***P* < .01.

administration to ensure that observations were made during the duration of action of levonantradol. The direct effects of levonantradol for the period of time elapsed during the drug interaction studies were measured in each subject as follows: levonantradol (0.03 mg/kg) was administered followed by three sessions in which vehicle was administered instead of dopamine agonists.

Rating Scale. The Rating Scale (Table 1) was designed to assess the effects of CB₁ cannabinoid and dopamine receptor agonists on behavior. It was developed specifically for this set of experiments, was designed to minimize qualitative measures, and is recorded in numerical units that can be analyzed statistically. The rating scale was used by an observer blind to the procedures and drug treatments, and enabled development of a quantitative measure of activity with the videotaped animal behaviors. Inter-rater reliability was assessed by two observers, blind to the procedures, both of whom rated the same six tapes and applied a numerical value to each observation with both drug-treated and nondrug-treated animals. Comparison of the scores of the two raters, working independently, revealed >90% agreement in the quantitative analysis of four parameters of behavior with this rating scale. Each measure was scored based on the number and type of movements detected per unit time. Each 0.5-h session was rated six times. Each rating period lasted 2 min from 0 to 2, 5 to 7, 10 to 12, 15 to 17, 20 to 22, and 25 to 27 min. All ratings were averaged over the span of the entire session and the score was recorded as the behavioral rating for that monkey with the given drug regimen.

Data Analysis. Statistics were performed by comparing the score of a group of monkeys given a drug dose regimen in a session, to the same group administered vehicle. Statistical significance is denoted throughout as **P* < .05 or ***P* < .01. All values were compared to vehicle with Dunnett's test using the statistics program GB Stat (Dynamic Microsystems, Silver Spring, MD). Error bars represent standard error of the mean.

Results

To determine the behavioral effects of a CB₁ cannabinoid receptor agonist in monkeys, the CB₁ cannabinoid receptor agonist levonantradol was administered to three monkeys at various doses (from 0.01 to 0.3 mg/kg; Fig. 1). Levonantradol produced a statistically significant and dose-dependent decrease in locomotor (Fig. 1A) and general activity (Fig. 1B) and an increase in bradykinesia (Fig. 1C). Ptosis or eyelid closure increased but did not reach statistical significance

TABLE 1

Rating scale and description of unconditioned behaviors of male cynomolgus monkeys

Monkeys were videotaped and the tapes were rated by an independent observer, blind to the experimental procedures.

Rating → Parameter ↓	-2	-1	0	+1	+2	+3
General activity	None: no movement of any kind observable	Slight: only monoaxial movements detected and/or one to three triaxial movements	Normal: three or more triaxial movements	Mild hyperactivity: two to four bursts of frenzied, rapid, triaxial movement with sudden onset	Excessive hyperactivity: four or more bursts of frenzied, rapid, triaxial movement with sudden onset	Severe: all movements slower than normal
Locomotor activity	None: no locomotion present	Slight: one to six steps detected	Normal: seven or more steps detected	Mild hyperactivity: two to four bursts of frenzied locomotion	Excessive hyperactivity: four or more bursts of frenzied locomotion	Severe: eyelids closed intermittently and are partially open at other times
Bradykinesia			None: all movement speed normal	Slight: at least two movements detected and slower than normal	Moderate: at least half of all movements slower than normal	Severe: eyelids closed all the time
Ptosis (eyelid closure)			None: eyes fully open, appears alert/awake	Slight: eyelids partially open but never closed	Moderate: eyelids close intermittently and are partially open at other times	

(Fig. 1D). The failure of ptosis to reach statistical significance can be attributed to one monkey that did not experience sedation when administered levonantradol in the dose range tested.

The effects of the three dopamine receptor agonists quinolorane, pergolide (D₂-type dopamine receptor agonists), and SKF 81297 (a D₁ dopamine receptor agonist) were tested and behavior was rated with the same scale. In contrast to levonantradol, quinolorane (0.001 to 1.0 mg/kg; Fig. 2), pergolide (0.01 to 1.0 mg/kg; Fig. 3), and SKF 81297 (0.3 to 3.0 mg/kg; Fig. 4) did not diminish locomotor or general activity and did not produce sedation in any of the subjects. Quinolorane produced hyperactivity in three of six subjects. Collectively, CB₁ cannabinoid receptor agonists produced sedation and a generalized slowing of motor function, as a function of dose, whereas D₂-type dopamine receptor agonists produced no sedation or promoted activity in monkeys.

Given the apparently opposite effects of agonists at D₂ and CB₁ receptor systems in monkeys, we hypothesized that dopamine receptor agonists would counteract the decreased general and locomotor activity and increased sedation engendered by the CB₁ cannabinoid receptor agonist. To test this hypothesis, levonantradol was administered followed by quinolorane. At all doses tested, quinolorane alone generally did not decrease activity, nor did it produce sedation (Fig. 2). Instead, quinolorane increased general activity and locomotor activity in three of six subjects.

Four subjects were treated with a dose of levonantradol that alone produced no sedation, and various doses of the D₂ dopamine receptor agonist quinolorane 30 min later. The subthreshold dose of levonantradol (0.03 mg/kg) was chosen based on the levonantradol dose-response curve (Fig. 1). At this dose, levonantradol failed to produce significant changes in general and locomotor activity or sedation in any of the subjects tested. In subjects pretreated with this dose of levonantradol, quinolorane produced a dose-dependent and statistically significant decrease in locomotor activity and a dose-dependent and statistically significant increase in ptosis and overall sedation. These effects were observed at doses of either drug, which, when given alone, did not reduce activity or produce sedation (Fig. 2, B and C). Quinolorane in combination with levonantradol also produced a decrease in general activity that did not achieve statistical significance at any of the doses tested (Fig. 2A).

To determine whether this effect generalizes to other D₂ agonists, the anti-Parkinsonian D₂ dopamine receptor agonist pergolide was tested in combination with levonantradol. Pergolide given alone did not produce significant sedation or reduce general or locomotor activity. In one subject, pergolide produced general hyperactivity but did not cause sedation in any of the animals at any of the doses tested. In four subjects pretreated with a subthreshold dose of levonantradol (0.03 mg/kg), increasing doses of pergolide produced a significant and dose-dependent decrease in general and locomotor activity (Fig. 3, A and B) and a significant and dose-dependent increase in sedation (Fig. 3C).

To determine whether potentiation of levonantradol was restricted to D₂-type dopamine receptor agonists, the highly selective D₁ dopamine receptor agonist SKF 81297 was tested in monkeys. In all three subjects, SKF 81297 (0.3–3.0 mg/kg) administered alone failed to produce sedation or marked changes in locomotor or general activity (Fig. 4).

After pretreatment with a subthreshold dose of levonantradol (0.03 mg/kg), increasing doses of SKF 81297 (0.3–3.0 mg/kg) failed to produce sedation, or a decrease in general or locomotor activity in the subjects (Fig. 4). In one of the subjects, the combination of drugs produced hyperactivity and anomalous behavior (aggressive and escape behavior, including leaps into the cage corners, and flailing at nonexisting objects). These behaviors were recorded by the observer but are not listed in the rating scale. Collectively, these observations suggest that D₂-type dopamine receptor agonists but not a D₁ dopamine receptor agonist exacerbate the sedation and motor depression engendered by a subthreshold dose of the CB₁ cannabinoid receptor agonist levonantradol.

Discussion

This is the first reported demonstration of reciprocal modulation of the behavioral effects of cannabinoid and dopamine agonists in primates. The behavioral stimulation produced by D₂ dopamine agonists at high doses was attenuated, and the sedative effects of a CB₁ cannabinoid agonist were magnified, when the two drug classes were coadministered to primates. Equally unanticipated, profound sedation occurred at doses of each drug that alone did not produce sedation. Potentiation of cannabinoid-induced sedation was limited to D₂ agonists, which distinguishes the functional sequelae of D₁ and D₂ dopamine receptors and their linkage to cannabinoid receptors in primates. The lowering of the threshold dose for a cannabinoid agonist to induce sedation has implications for the treatment of cocaine addiction and pathophysiology of dopamine-related neuropsychiatric disorders.

The objectives of this study were to test the hypothesis that D₁ and D₂ agonists would attenuate cannabinoid-induced sedation in primates. The CB₁ agonist levonantradol was chosen because of its selective pharmacological profile and relatively high affinity (Little et al., 1988). Levonantradol is also a prodrug that is converted to the active metabolite desacyllevonantradol and both levonantradol and the metabolite are agonists at CB₁ cannabinoid receptors in brain (McIlhenny et al., 1981). Levonantradol produced sedation, ptosis, and reduced motor activity, effects similar to those reported for the active ingredient of marijuana Δ^9 tetrahydrocannabinol in rhesus monkeys (Beardsley et al., 1987).

The D₂ dopamine agonist quinolorane improves motor activity in a primate model of Parkinson's disease (Goulet and Madras, 2000). Pergolide (van den Buuse, 1995), which also increases motor activity in Parkinson's disease, is under evaluation as a treatment for cocaine addiction (Haney et al., 1998, Levin et al., 1999). In contrast to levonantradol, high doses of D₂ agonists did not induce sedation, and quinolorane increased activity in some subjects. Given the apparently opposing actions of a CB₁ cannabinoid receptor agonist and D₂-type dopamine receptor agonists in monkeys, we postulated that the D₂-type dopamine receptor agonists would antagonize the sedative effects of a CB₁ cannabinoid receptor agonist.

To test this hypothesis, a single subthreshold dose of levonantradol (0.03 mg/kg) was used, after a pilot study with a 3-fold higher dose combined with quinolorane precipitated profound, prolonged sedation. If used alone, the same dose of levonantradol that was tested in the combination studies did

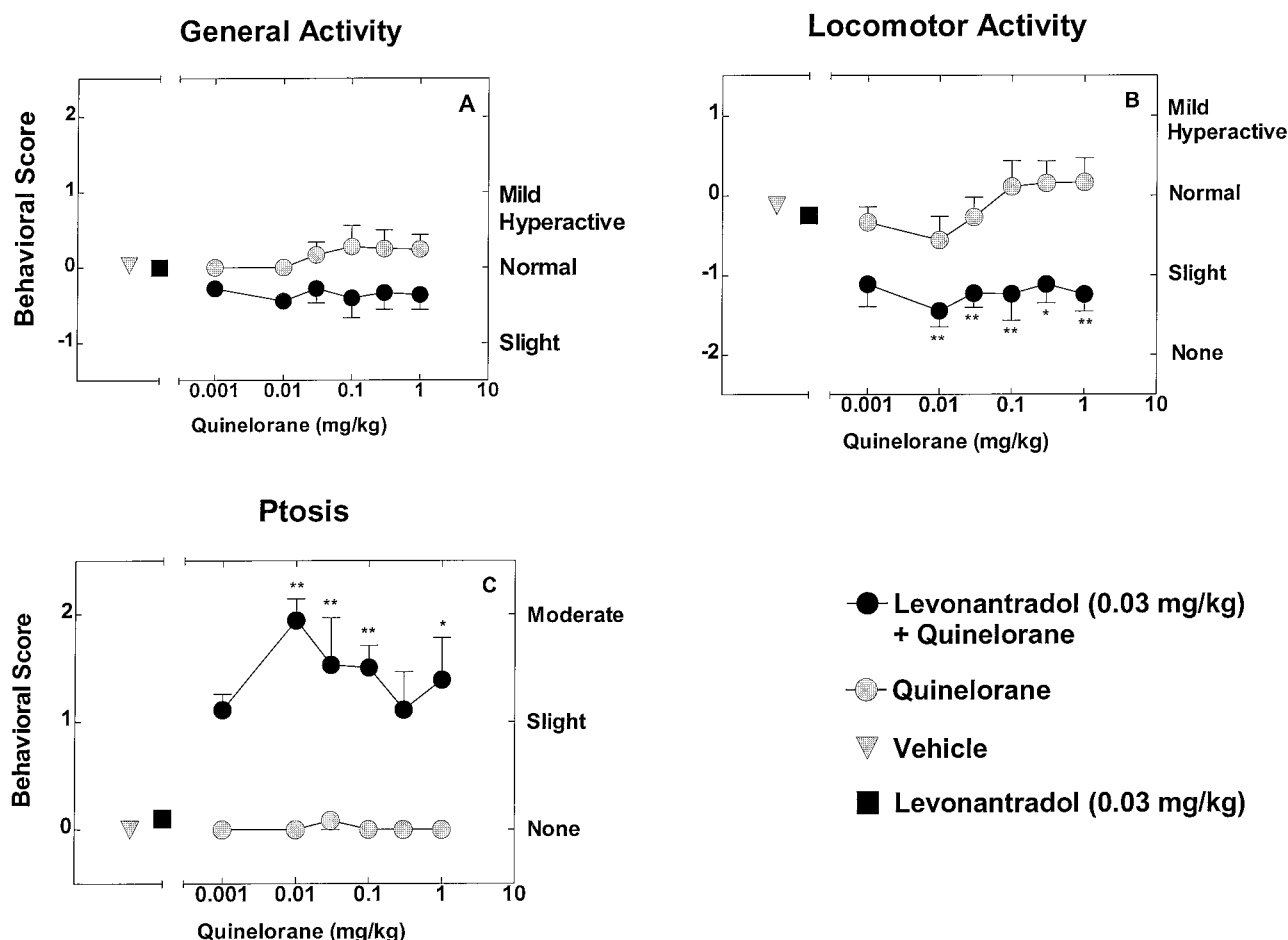


Fig. 2. Effects of combining the D_2 dopamine receptor agonist quinelorane with the cannabinoid agonist levonantradol on spontaneous behavior in monkeys. In separate sessions, various doses of quinelorane were administered alone i.m. (○) to determine its effects on activity. In a different session, vehicle (▽) or a subthreshold dose of levonantradol [0.03 mg/kg (■)] were administered alone i.m. followed 30 min. later by various doses of quinelorane to four cynomolgus monkeys. The effects of various doses of quinelorane with (●) or without levonantradol (○) were quantified on a rating scale designed to measure general activity (A), locomotor activity (B), and ptosis (C). Statistical significance from vehicle is denoted by * $P < .05$ or ** $P < .01$.

not produce statistically significant behavioral effects and was termed ineffective or subthreshold. Bradykinesia was not reported for the combination studies because in the majority of sessions, sedation was so profound that movement of any kind was not observable. Accordingly, it was impossible to quantify bradykinesia.

The results of combining D_2 agonists with a subthreshold dose of levonantradol agonist were unanticipated. Both D_2 agonists precipitated a profound loss of general and motor activity and induced ptosis, although quinelorane was effective at lower doses. Given the structural dissimilarities of pergolide and quinelorane and their similar effects in combination with levonantradol, it is unlikely that they interacted with levonantradol at a pharmacokinetic level but rather, centrally. Although low doses of quinelorane alone did not produce sedation, the drug combination resulted in a peak effect within a narrow dose range. Maximum potentiation of sedation was elicited with a low dose of quinelorane, which did not increase with higher doses. These findings differed from the results with pergolide, which yielded a clear dose-response effect. These drugs have different affinities and selectivities for D_1/D_2 and D_3 receptors and these differences may account for the more robust dose-response effects observed with pergolide.

D_2 dopamine receptor activation lowered the threshold for cannabinoid receptor induction of sedation, an effect that did not generalize to D_1 - CB_1 receptor interaction. In rodents, a precedent for functional linkage of CB_1 - D_2 , but not CB_1 - D_1 receptors has been established for regulation of anandamide release, neuroendocrine effects, and motor activity (Rodriguez de Fonseca et al., 1995; Maneuf et al., 1997; Giuffrida et al., 1999). This pattern does not generalize to memory consolidation because both D_1 and D_2 receptor agonists antagonized anandamide reduction of memory consolidation in mice (Castellano et al., 1997). In rat striatal neurons, both receptors are colocalized (Herkenham et al., 1990), CB_1 and D_2 receptors share signal transduction pathways (Howlett et al., 1990) and these pathways converge (Bidaut-Russell and Howlett, 1991; Glass and Felder, 1997). Reciprocal modulation of behavior by a cannabinoid receptor agonist combined with D_2 but not D_1 dopamine agonists may reflect differences in signal transduction of the two dopamine receptor subtypes. In this regard, D_1 dopamine receptors stimulate adenylate cyclase, D_2 dopamine receptors inhibit adenylate cyclase (Bidaut-Russell and Howlett, 1991), and CB_1 cannabinoid receptors either stimulate or attenuate cyclase activity in various regions of rodent brain (Bonhaus et al., 1998). Paradoxically, incubation of a D_2 dopamine receptor ago-

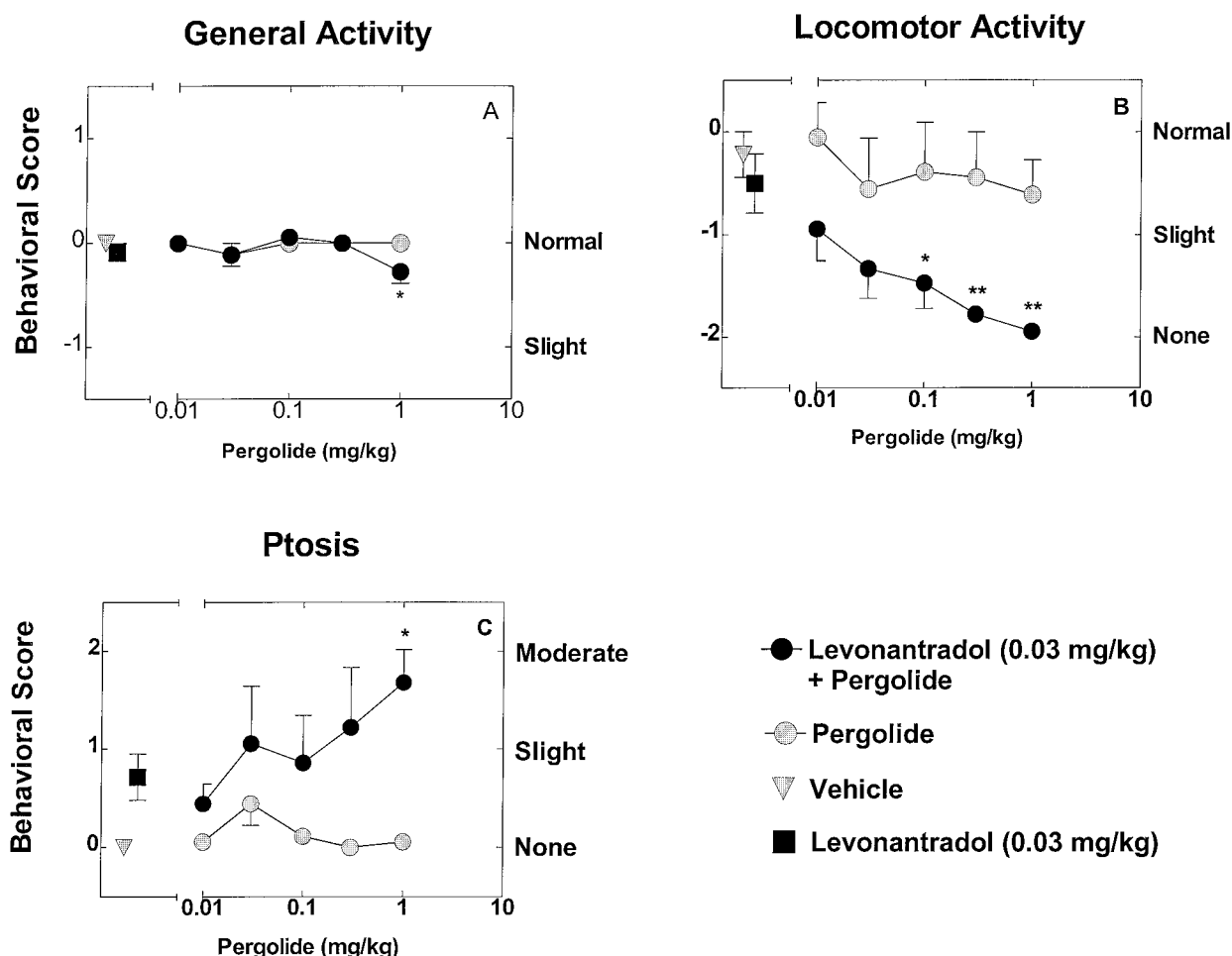


Fig. 3. Effects of combining the D₂ dopamine receptor agonist pergolide with the cannabinoid agonist levonantradol on spontaneous behavior in monkeys. In separate sessions, various doses of pergolide were administered alone i.m. (○) to determine its effects on activity. In a different session, vehicle (▽) or a subthreshold dose of levonantradol [0.03 mg/kg (■)] was administered alone (i.m.) followed 30 min. later by various doses of pergolide to cynomolgus monkeys. The effects of various doses of pergolide with (●) or without levonantradol (○) were quantified on a rating scale designed to measure general activity (A), locomotor activity (B), and ptosis (C). Statistical significance from vehicle is denoted by **P* < .05 or ***P* < .01.

nist with the CB₁ cannabinoid receptor agonist in cultured rat striatal neurons stimulated cAMP accumulation, even though each drug separately inhibited forskolin-stimulated cAMP accumulation (Glass and Felder, 1997). Nevertheless, extrapolations made from rodent to primate brain are speculative because regional differences in cannabinoid receptor/G-protein coupling and cannabinoid agonist-specific trafficking of intracellular responses (Breivogel et al., 1997; Bonhaus et al., 1998) may differ in the two species.

In primates, it is likely that the functional consequences of cannabinoid linkage to D₁ or D₂ dopamine receptors are region- and function-specific. Our observations were restricted to motor activity and sedation, but other psychoactive or cognitive functions may have been modulated by this drug combination. Possible mechanisms may include reciprocal modulation of receptor binding, receptor trafficking, sequestration of G proteins by the CB₁ receptor (Vasquez and Lewis, 1999) within neurons expressing both receptors, modulation of autoreceptor-induced release of endogenous dopamine or anandamide, or neural networks involving both receptor systems and involving a feed back inhibition loop (Diana et al., 1998; Giuffrida et al., 1999). Several of these possibilities require the coexistence of cannabinoid and do-

pamine receptors within the same neurons. Preliminary data from this laboratory indicate that CB₁ receptors are colocalized with D₂ dopamine receptors (>20% of neurons) in primate striatum, whereas CB₁-D₁ receptor colocalization is <5% (Tsaion et al., 1999). In this regard, D₂ agonists stimulate anandamide release in rodent brain at concentrations sufficient to produce behavioral effects, thereby limiting the behavioral response to D₂ agonists (Giuffrida et al., 1999).

If generalizable to the active ingredient of marijuana, Δ⁹-tetrahydrocannabinol (THC), and to other clinically relevant D₁, D₂, or D₃ dopamine receptor agonists (Eden et al., 1991; Mierau et al., 1995; Caine et al., 1997), the findings have significant clinical implications. D₂-type dopamine receptor agonists (e.g., pergolide) are important medications for Parkinson's disease and the therapeutic potential for D₁ agonists is mounting (Goulet and Madras, 1998; Rascol et al., 1999). The present study highlights the possible consequences of combining cannabinoid agonists with D₂-type Parkinson's disease medications, thereby reducing the effectiveness of the dopamine agonists and enhancing the sedative and possibly other psychoactive properties of cannabinoids. Conversely, D₂ but not D₁ dopamine agonists may augment the therapeutic effects of cannabinoids while reducing the dose of

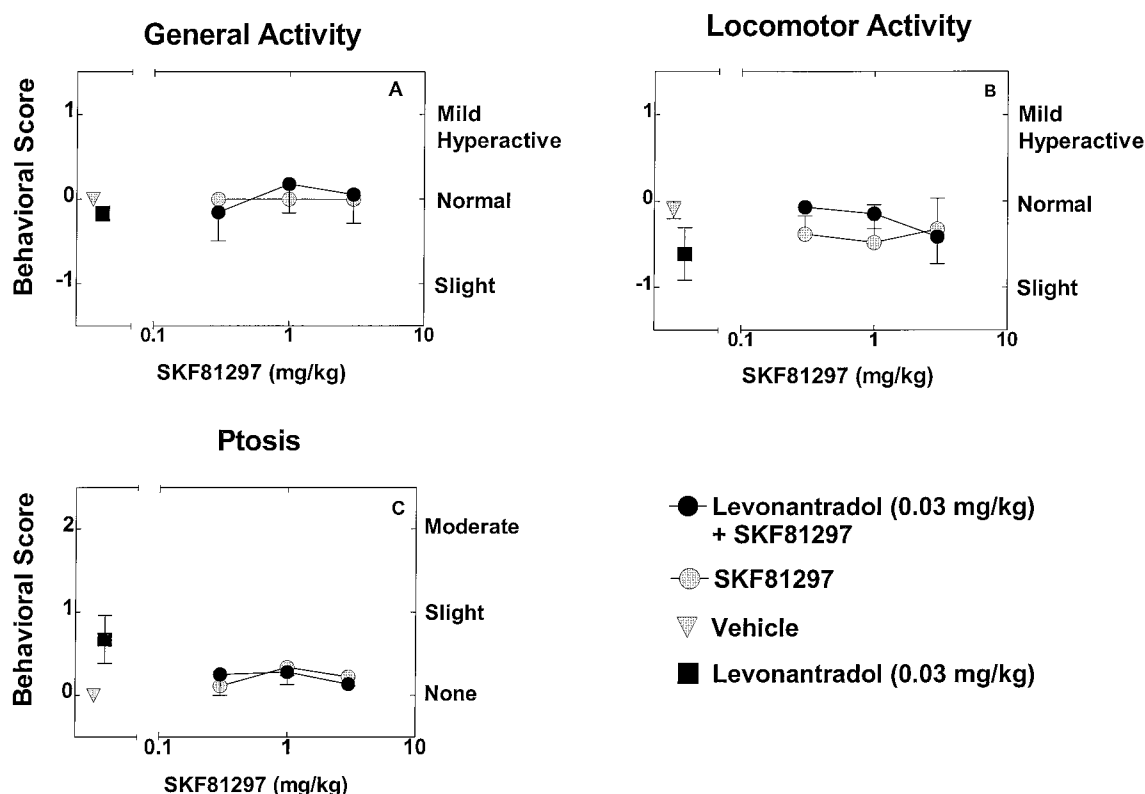


Fig. 4. Effects of combining the D_1 dopamine receptor agonist SKF 81297 with the cannabinoid agonist levonantradol on spontaneous behavior in three cynomolgus monkeys. In separate sessions, various doses of SKF 81297 were administered alone i.m. (○) to measure its effects on activity. In a different session, vehicle (▽) or a subthreshold dose of levonantradol [0.03 mg/kg (■)] was administered alone (i.m.) followed 30 min. later by various doses of SKF 81297. The effects of various doses of SKF 81297 with (●) or without levonantradol (○) were quantified on a rating scale designed to measure general activity (A), locomotor activity (B), and ptosis (C). Statistical significance from vehicle is denoted by * $P < .05$ or ** $P < .01$.

cannabinoids needed to obtain an effect. More research is needed to determine whether D_2 (or D_1) agonists potentiate the full spectrum of cannabinoid-induced effects, or whether, as in the present study, the interaction is confined to sedative properties.

The potential for combined use of these drug classes may increase in the drug abusing population because D_2 -type dopamine receptor agonists (e.g., pergolide, ropinerole, and pramipexole) are under investigation as potential drug therapies for cocaine addiction (Witkin, 1994; Caine et al., 1997). Cocaine addicts often are polydrug users (Gfroerer and Brodsky, 1993), and marijuana use is cited frequently by this cohort. If the findings extend to Δ^9 -tetrahydrocannabinol (THC) (the active CB_1 cannabinoid receptor agonist in marijuana), D_2 -type dopamine receptor agonists may produce unexpected enhancement of the distinct effects of marijuana. The clinical implications warrant further studies with additional drugs, other primate species, and chronic administration (Diana et al., 1998; Jentsch et al., 1998). Illicit drug users commonly self-administer drugs (cocaine, amphetamine, and methylphenidate) that elevate extracellular dopamine levels, alone or together with marijuana (Gfroerer and Brodsky, 1993). Because these drugs indirectly stimulate both dopamine receptor subtypes (D_1 and D_2), combined activation of both receptor subtypes may minimize CB_1 - D_2 potentiation.

In conclusion, nonsedating doses of a D_2 agonist combined with a nonsedating dose of a cannabinoid agonist lower the threshold dose for cannabinoids and precipitate profound

sedation in primates. The results may reflect an important link between dopamine and cannabinoid systems in primate brain, of relevance to the pathophysiology of dopamine-related neuropsychiatric diseases. Schizophrenia (Leweke et al., 1999), Gilles de la Tourette syndrome (Muller-Vahl et al., 1998), and narcolepsy (Smith and Cohen, 1988) are treated largely with dopamine receptor drugs. The evidence implicating cannabinoid-dopamine linkage in schizophrenia (Leweke et al., 1999) and Gilles de la Tourette syndrome (Muller-Vahl et al., 1998) warrants further investigation of this interaction in primate brain. Although the observations made in the present study were restricted to locomotor activity and sedation, dopamine agonists also may potentiate the psychoactive effects of cannabinoids. Notwithstanding this speculation, cannabinoids and D_2 dopamine agonists should be combined with caution.

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Send reprint requests to: Bertha K. Madras, Ph.D., Division of Neurochemistry, New England Regional Primate Research Center, 1 Pine Hill Dr., Box 9102, Southborough, MA 01772-9102. E-mail: bertha_madras@hms.harvard.edu
