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Naloxonazine antagonism of levorphanol-induced antinociception and respiratory depression in Rhesus monkeys

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Abstract

The μ-opioid receptor antagonist effects of naloxonazine on levorphanol-induced thermal antinociception and respiratory depression were examined in rhesus monkeys. Levorphanol (0.032–3.2 mg/kg) produced dose-dependent increases in tail-withdrawal latencies from 50°C water in a warm-water tail-withdrawal assay and dose-dependent decreases in ventilation in both air and 5% CO_2 mixed in air. Naloxonazine (0.1–3.0 mg/kg) antagonized both the antinociceptive and ventilatory effects of levorphanol to a similar degree, and the antagonist effects of naloxonazine were greater after 1 h than after 24 h. Under all conditions, the antagonist effects of naloxonazine were fully surmountable. Schild analysis of the antagonist effects of naloxonazine after 1 h pretreatment in the antinociception assay yielded a pA_2 value of 7.6 and a slope of -0.50; by comparison, quadazocine yielded a pA_2 value of 7.5 and a slope of -1.05. These results suggest that naloxonazine acts as a potent and fully reversible μ-opioid receptor antagonist with a moderately long duration of action in rhesus monkeys. In addition, these results suggest that the antinociceptive and ventilatory effects of μ-opioid receptor agonists in rhesus monkeys are mediated by pharmacologically similar populations of μ opioid receptors.

Keywords: Antinociception; Respiratory depression; Opioid receptor; Naloxonazine; Levorphanol; Quadazocine; (*Macaca mulatta*)

1. Introduction

The opioid receptor ligand naloxonazine, which antagonizes morphine-induced antinociception in rats and mice for greater than 24 h, has been used to characterize two populations of μ-opioid receptor subtypes (μ_1 and μ_2) in radioligand binding experiments (Hahn et al., 1982; Ling et al., 1986). Shortly after administration, naloxonazine binds reversibly to a number of opioid receptors (including the μ_1 and μ_2 receptor subtypes) with affinities similar to the prototypic opioid receptor antagonist naloxone. After 24 h, only selective, wash-resistant binding to the μ_1-opioid receptor subtype remains. As the elimination half-life of naloxonazine in plasma has been reported to be less than 3 h, irreversible binding rather than slow elimination has been suggested to mediate the long-term antagonism of morphine-induced antinociception (Ling et al., 1986).

Although naloxonazine antagonizes the antinociceptive effects of morphine, it has been reported to be less effective in antagonizing other μ-opioid receptor agonist effects, and these differential antagonist effects may be related to naloxonazine's different binding profiles at μ-opioid receptor subtypes (e.g., Heyman et al., 1988; Paul and Pasternak, 1988; Pick et al., 1991). For example, naloxonazine has been shown to differentially antagonize the antinociceptive and respiratory depressant effects of morphine in rats (Ling et al., 1983, 1985). When studied 24 h after pretreatment with naloxonazine (10 mg/kg, i.v.), the morphine dose-effect curve for antinociception was shifted about 4-fold to the right, whereas the respiratory depressant effects of morphine were unchanged. These findings were taken to suggest that morphine-induced antinociception may be mediated by μ_1-opioid receptors (i.e. μ-opioid receptors irreversibly bound by naloxonazine and still blocked after 24 h), whereas morphine-induced respiratory depression may be mediated by μ_2-opioid receptors (i.e. μ-opioid receptors bound reversibly by nalox-

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onazine but not blocked after 24 h). Naloxonazine has also been reported to differentially antagonize other μ-opioid receptor-mediated effects in rodents. For example, naloxonazine antagonized μ-opioid receptor agonist-induced prolactin release but not gastrointestinal motility, antitussive effects or growth hormone release (Spiegel et al., 1982; Heyman et al., 1988; Kanei et al., 1993). Collectively, these studies encourage research to develop μ-opioid receptor analgesics free of the side-effects that complicate the use of existing opioids.

The purpose of the present study was to further evaluate the antagonist actions of naloxonazine by studying its effects on the antinociceptive and respiratory depressant effects of the μ-opioid receptor agonist levorphanol in rhesus monkeys. The antinociceptive effects of levorphanol were examined in a warm water tail-withdrawal procedure, in which opioids characteristically increase the latency of tail withdrawal from 50–55°C water (Dykstra and Woods, 1986; Walker et al., 1993). The ventilatory effects of levorphanol were determined in awake, seated rhesus monkeys breathing either air or 5% CO_2 mixed in air (Howell et al., 1988; Butelman et al., 1993). The antinociceptive and respiratory depressant effects of levorphanol were determined alone and at 1 and 24 h after pretreatment with naloxonazine. For purposes of comparison, the effects of levorphanol in the warm water tail-withdrawal assay also were determined after pretreatment with quadazocine, a reversible, μ-opioid receptor-selective antagonist (Dykstra et al., 1987).

2. Materials and methods

2.1. Subjects

Experiments were conducted in seven rhesus monkeys (*Macaca mulatta*) weighing between 4.5–12.0 kg. Four monkeys participated in tail-withdrawal procedures and three monkeys were used in ventilation experiments. Two subjects had not previously participated in drug studies, and all monkeys were drug free for at least one month prior to the beginning of the experiments. Subjects lived in individual home cages between sessions and had unrestricted access to water and food (PMI Feeds jumbo monkey diet, fresh fruits and vegetables). All housing and procedures were in accordance with the guidelines of the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (Department of Health and Human Services, NIH Publication No. 86-23, revised 1985).

2.2. Warm-water tail-withdrawal assay

During experimental sessions, monkeys were seated in acrylic primate chairs and the bottom 10 cm of their tails were shaved. An Apple IIe microcomputer was used to measure and record time intervals. Sessions consisted of five 20-min cycles. Tail-withdrawal latencies from 38 and 50°C water were measured for each subject immediately prior to the start of the experiment and 10 min after the start of each cycle. If the monkey did not withdraw its tail within 20 s, the warm water was removed, the timer was stopped, and a latency of 20 s was assigned to that measurement. Experimental sessions were conducted twice weekly.

2.3. Ventilation assay

During experimental sessions, each subject sat in a Plexiglas primate chair that was enclosed within a sound-attenuating chamber. Monkeys were trained to wear a plastic mask that covered the nose and mouth. The monkey's nose and mouth protruded into the mask through a hole in the center of a thin piece of flexible rubber that served as a dam; velcro straps were used to secure the mask to the monkey's head. Three tubes entered separate fitted holes in the opposite side of the mask and a port beneath the three tubes was covered with 150-mesh monel screen to serve as a resistance. A continuous flow (10 l/min) of gas entered the mask through one of the three tubes and was extracted through a second tube by a vacuum pump. Changes in the flow within the mask resulting from changes in ventilation were measured through the third tube by a pressure transducer connected to a polygraph; an integrator converted the flow signal to a volume measure. The apparatus was calibrated regularly by injecting known volumes of air into the mask. Data analysis was carried out by a computer (IBM/PC) that was connected to the polygraph and to the integrator. The number of inspirations per minute (f) was determined directly, and the minute volume (V_E) was determined by integration of the pressure-compensated plethysmograph signal after appropriate offset for bias flow.

Prior to drug studies, daily experimental sessions of 1–2 h duration were conducted with each monkey to establish stable patterns of ventilation in air and 5% CO_2 mixed in air. Sessions consisted of four 22-min cycles; in each cycle, subjects were exposed to 16 min of a continuous flow of air followed by 6 min of exposure to 5% CO_2 mixed in air. Values obtained for V_E and f during the last 4 min of each exposure to CO_2 in air and the last 4 min of each exposure to air were used for data analysis. When ventilation patterns stabilized within this procedure, drug sessions began. The effects of drugs generally were studied no more often than once per week in each monkey to minimize changes in sensitivity to levorphanol and to allow sufficient time for clearance of naloxonazine. Administration of drugs always followed sessions of stable ventilation.

2.4. Pharmacological procedures

The effects of levorphanol were evaluated using cumulative dosing procedures in which a complete levorphanol

dose-effect curve (0.1–10.0 mg/kg) was established during a single session by injecting sequentially higher doses i.m. in the thigh muscle at the beginning of each cycle. Each injection increased the total dose of levorphanol by 1/2 log increments. Experiments were designed to cover levorphanol dose ranges extending from doses that had no effect to doses that produced at least 50% of the maximum effect in all monkeys. In subsequent experiments, the effects of naloxonazine (0.1, 1.0 and 3.0 mg/kg) were examined by administering a single dose of naloxonazine i.m. in the thigh 1 or 24 h before cumulative dosing with levorphanol. The effects of quadazocine (0.01, 0.032, 0.1 mg/kg, i.m.) were tested by giving a single dose 30 min before a determination of the levorphanol cumulative dose-effect curve.

2.5. Data analysis

Tail-withdrawal latencies were transformed into percentages of maximum effect by the formula: [(test latency − baseline latency)/(20 s − baseline latency) × 100]. The effects of drugs on minute volumes in air and 5% CO_2 mixed in air are expressed as percent of control by dividing the minute volume obtained after each dose of levorphanol by the average minute volume obtained during the corresponding cycles in control sessions and multiplying by 100. When possible, ED_{50} values were determined by interpolation of the dose-effect function. Apparent pA_2 values were calculated from the ED_{50} values using Schild regression analysis (Tallarida and Murray, 1987). In all experiments, calculations were performed on the data for individual subjects, and the mean and standard error across subjects are reported.

2.6. Drugs

Levorphanol tartrate (Hoffman-La Roche, Nutley, NJ) and naloxonazine (Research Biochemicals, Natick, MA) were dissolved in small amounts of lactic acid or 0.1 N acetic acid and diluted to the desired concentration in sterile water. Quadazocine methanesulfonate (kindly provided by Sterling Winthrop, Collegeville, PA) was dissolved in sterile water. Doses are expressed as mg/kg of the salt form of the compound.

3. Results

3.1. Warm water tail-withdrawal assay

The monkeys always left their tails in 38°C water for the full 20 s, indicating that tail immersion alone did not elicit tail withdrawal. Baseline tail-withdrawal latencies from 50°C water averaged 1.0 (±0.04) s. Levorphanol produced a dose-dependent increase in tail-withdrawal latencies from 50°C water (Fig. 1). At the highest dose (3.2

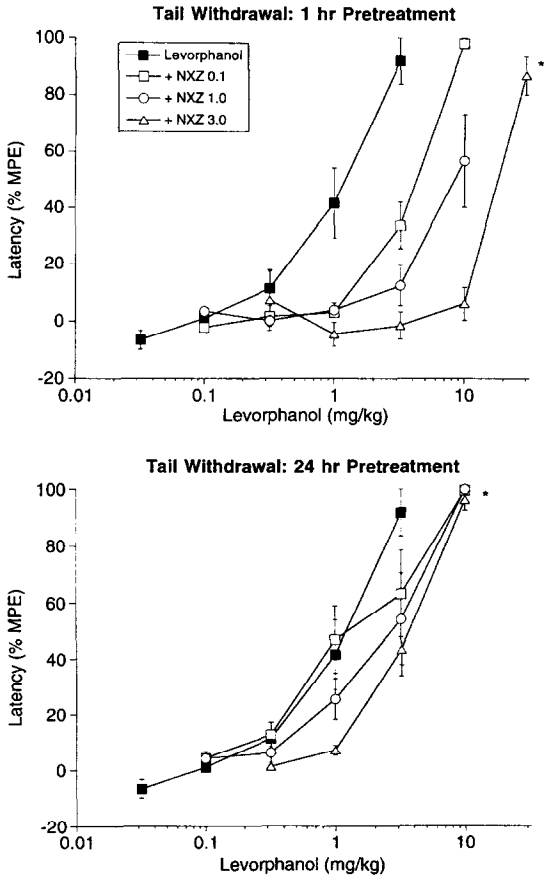

Fig. 1. Mean dose-effect curve for the antinociceptive effects of levorphanol alone and in combination with three naloxonazine (NXZ) doses after 1 h (top panel) and 24 h pretreatment (bottom panel). Abscissae represent the dose of levorphanol in mg/kg. Ordinates represent tail-withdrawal latency expressed as percentage maximum peak effect as described in the text ($n = 4$ except at points marked by asterisks in which $n = 3$, error bars represent 1 standard error).

Table 1
Mean ED_{50} values (mg/kg) and standard errors of the mean (in parentheses) for levorphanol alone and after 1 h and 24 h pretreatment with naloxonazine, or 0.5 h pretreatment with quadazocine ($n = 4$ for naloxonazine experiments and $n = 3$ for quadazocine experiments for tail-withdrawal assay, $n = 3$ with two determinations each for ventilation assay)

Treatment	ED_{50} (mg/kg)	
	Tail withdrawal	Ventilation
Levorphanol alone	0.68 (0.19)	
+ Quadazocine (0.5 h)		
0.01 mg/kg	1.21 (0.43)	
0.032	1.77 (0.40)	
0.1	6.72 (1.87)	
Levorphanol alone	1.21 (0.34)	0.26 (0.01)
+ Naloxonazine (1 h)		
0.1 mg/kg	4.22 (0.51)	
1.0	9.53 (2.29)	
3.0	18.89 (0.37)	3.44 (0.65)
+ Naloxonazine (24 h)		
0.1 mg/kg	1.68 (0.61)	
1.0	2.93 (0.85)	
3.0	3.66 (0.67)	1.71 (0.47)

mg/kg), latencies reached the 20 s maximum in three of four monkeys. ED_{50} values for the two determinations of levorphanol alone are shown in Table 1.

Quadazocine (0.01, 0.032 and 0.1 mg/kg) given 30 min before determination of the levorphanol cumulative dose-effect curve produced a dose-dependent increase in the levorphanol ED_{50} value (Table 1). The unconstrained apparent pA_2 value was 7.5 (±0.13), and the slope of the regression was −1.05 (±0.26).

Naloxonazine (0.1, 1.0 and 3.0 mg/kg) administered 1 or 24 h before the session shifted the levorphanol dose-effect curve rightward in a dose-dependent manner, resulting in graded increases in the levorphanol ED_{50} values (Fig. 1; Table 1). Each dose of naloxonazine produced a greater antagonist effect at 1 h than at 24 h. For example, the highest dose of naloxonazine (3.0 mg/kg) produced an approximately 15-fold increase in the levorphanol ED_{50} value after 1 h pretreatment, but only a 3-fold increase after 24 h pretreatment. Regardless of dose or pretreatment time, the antagonism produced by naloxonazine was fully surmountable by levorphanol. Schild analysis of the effects of 1 h pretreatment with naloxonazine gave an unconstrained apparent pA_2 of 7.6 (±0.29) with a slope of −0.50 (±0.09).

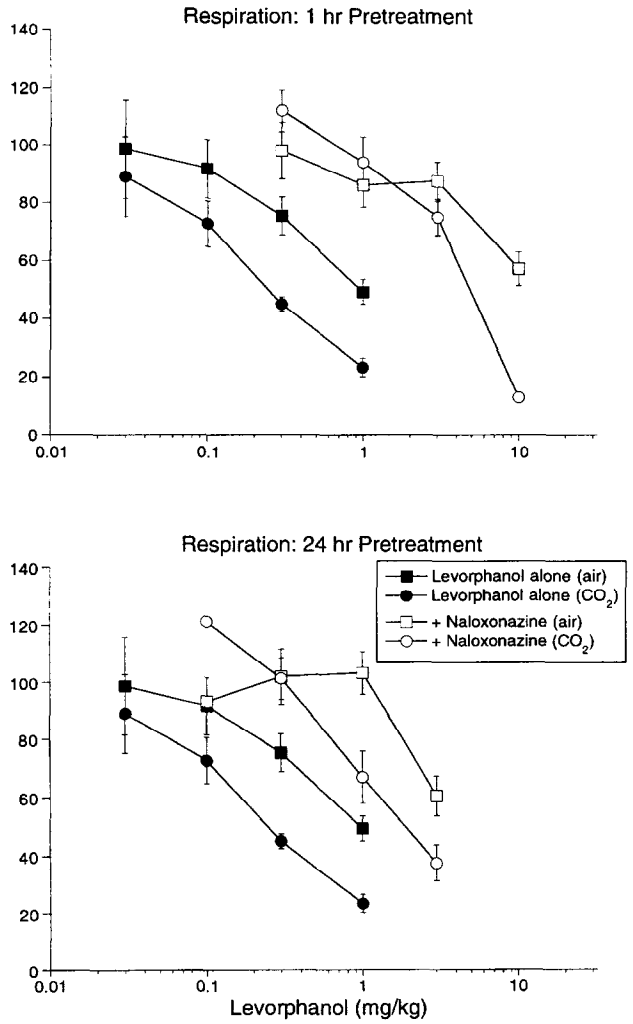

Fig. 2. Mean dose-effect curve for the respiratory effects of levorphanol alone and in combination with naloxonazine after 1 h (top panel) and 24 h pretreatment (bottom panel). Ordinates represent the minute volume in 5% CO_2 in air expressed as percentage of control. Each point represents the average of two determinations in each of three monkeys, other details as in Fig. 1.

3.2. Ventilation assay

Control values for ventilation were averaged across subjects and experiments. The average minute volume during exposure to air was 1236.74 (±16.16) ml/min. Exposure to 5% CO_2 mixed in air produced about a 4-fold increase in minute volume to 4466.75 (±77.20) ml/min. Levorphanol produced a dose-dependent decrease in ventilation during exposure to air and to 5% CO_2 mixed in air (Fig. 2). Maximal effects were observed following a dose of 1.0 mg/kg of levorphanol, which decreased the minute volume in air alone to 49% of control values and in 5% CO_2 in air to 23% of control values.

Naloxonazine (3.0 mg/kg) given either 1 or 24 h prior to determination of the levorphanol dose-effect curve antagonized the ventilatory effects of levorphanol in air and in 5% CO_2 mixed in air (Fig. 2). One hour pretreatment with naloxonazine produced an approximately 10-fold rightward shift in the levorphanol dose-effect curves. Twenty-four hour pretreatment with naloxonazine produced an approximately 4-fold shift in the dose-effect curves. At both pretreatment times, the antagonism produced by naloxonazine was fully surmountable by the highest doses of levorphanol (5.6 and 10.0 mg/kg).

4. Discussion

Levorphanol produced effects comparable to those of other μ-opioid receptor agonists in rhesus monkeys. In antinociception experiments, levorphanol produced dose-dependent increases in tail-withdrawal latencies that were antagonized by quadazocine, a μ-opioid receptor-selective antagonist. The in vivo apparent pA_2 value of 7.5 for quadazocine antagonism of levorphanol lies within the range of pA_2 values (7.3–8.2) reported for quadazocine antagonism of other μ-opioid receptor agonists in the tail-withdrawal assay in rhesus monkeys (Dykstra et al., 1987; Walker et al., 1993). In ventilation experiments, levorphanol dose dependently decreased the minute volume in air and in 5% CO_2 mixed in air in a manner similar to that previously reported for levorphanol and other μ-opioid receptor agonists in rhesus monkeys (Howell et al., 1988; Butelman et al., 1993). In addition, the reported pA_2 value of 7.33 for naltrexone antagonism of levorphanol in ventilation experiments is consistent with μ-opioid receptor mediation of levorphanol's effects (Howell et al., 1988).

These results suggest that levorphanol's antinociceptive and respiratory depressant actions in rhesus monkeys are similarly mediated by μ-opioid receptors.

In the present study, naloxonazine produced a dose-dependent and completely surmountable antagonism of levorphanol-induced antinociception in rhesus monkeys. Although these results agree with previous studies in rodents in finding that naloxonazine antagonizes the antinociceptive effects of levorphanol and other μ-opioid receptor agonists (Ling et al., 1983, 1985, 1986; Tive et al., 1992), these findings suggest that naloxonazine may have acted as a reversible rather than as an irreversible μ-opioid receptor antagonist. Specifically, naloxonazine differs from other irreversible or slowly dissociating μ-opioid receptor antagonists such as β-funaltrexamine (Ward et al., 1982) or clocinammox (Comer et al., 1992; Burke et al., 1994) both in the time course and in the surmountability of its antagonist effects. For example, clocinnamox has been shown to antagonize μ-opioid receptor agonist-induced antinociception in rhesus monkeys for several days, with peak antagonist effects observed for up to 72 h (Gerak et al., 1994; Zernig et al., 1994). In those experiments, clocinnamox also decreased the maximal antinociceptive effect of several μ-opioid receptor agonists in rhesus monkeys (Gerak et al., 1994; Zernig et al., 1994). In contrast, naloxonazine lost much of its effect within 24 h in the present experiments and never decreased the maximal effect of levorphanol. In rodent models of antinociception, the effects of naloxonazine antagonism have also been surmountable, as maximal effects of the agonists tested were reached even after large doses of naloxonazine (Ling et al., 1985, 1986; Pick et al., 1991). However, at least one study has documented that the maximum effects of levorphanol were depressed by naloxonazine (Tive et al., 1992).

These observations suggest that naloxonazine may have functioned as a reversible antagonist at the receptors mediating the antinociceptive effects of levorphanol. It is possible, however, that naloxonazine bound irreversibly to a subpopulation of μ-opioid receptors that were not necessary for the full expression of levorphanol's antinociceptive effects in the present experiments. In addition, it should be noted that higher doses of naloxonazine may have produced a longer duration of action and may have decreased the maximal antinociceptive effects of levorphanol. Doses higher than 3.0 mg/kg were not examined in this study because of the limited solubility of naloxonazine and because the acidity of the vehicle precluded administration of large injection volumes.

The in vivo apparent pA_2 values for naloxonazine (7.6) and quadazocine (7.5) in antagonizing levorphanol-induced antinociception were similar, suggesting that naloxonazine and quadazocine may possess similar affinities for the receptors mediating levorphanol-induced antinociception. However, comparison of pA_2 values may not be appropriate in this case. According to conventional receptor theory, the Schild regression plot for a fully reversible antagonist acting at a single, homogenous population of receptors should be -1, and a pA_2 value can be considered a reliable measure of antagonist affinity only when the Schild plot slope is -1 (Kenakin, 1987). Although the Schild plot slope for quadazocine (-1.05 ± 0.26) was close to the theoretical value of -1, the Schild plot for naloxonazine displayed a more shallow slope of -0.50 ± 0.09. The shallow slope of the naloxonazine Schild plot suggests that the derived pA_2 value may not be a reliable estimate of naloxonazine's true affinity for the receptors mediating levorphanol antinociception. There are at least two possible explanations for the shallow slope of the Schild plot for naloxonazine. First, Schild analysis is based upon the assumption that the effects of the antagonists are fully reversible (Kenakin, 1987), and as noted above, the present results do not preclude the possibility that naloxonazine binds irreversibly to a subset of the receptors mediating levorphanol antinociception. Second, a shallow slope may be produced when the agonist acts at multiple receptor types for which the antagonist has different affinities (Kenakin, 1987). Interestingly, either of these possibilities would be consistent with the differential activity of naloxonazine at μ-opioid receptor subtypes in vitro. However, additional experiments will be necessary to provide more conclusive evidence of functional μ-opioid receptor multiplicity in rhesus monkeys.

In the present study, both 1 h and 24 h pretreatment with naloxonazine antagonized levorphanol-induced respiratory depression and antinociception to the same degree, suggesting that both effects were mediated by pharmacologically similar types of opioid receptors. These results contrast with previous findings that 24 h pretreatment with naloxonazine antagonized morphine-induced antinociception but not respiratory depression in rats (Ling et al., 1983, 1985, 1986). Moreover, the present findings argue against the general conclusion that μ_1-opioid receptors mediate antinociception and μ_2-opioid receptors mediate respiratory depression. Although the reasons for these disparate findings are not clear, there are notable differences between the studies that may have contributed. These differences include the type of μ-opioid receptor agonist used (levorphanol vs. morphine and other μ-opioid receptor agonists) as well as the methods used to measure antinociception (warm water tail withdrawal vs. radiant heat tail flick) and respiratory depression (ventilation vs. arterial blood gas measurements). Another important difference between the present study and previous studies is the species used. Substantial species differences have been reported in the relative proportions and anatomical distributions of opioid receptor types (Buatti and Pasternak, 1981; Robson et al., 1985; Mansour et al., 1988). These findings suggest that, in different species, opioid receptor types may be differentially integrated into the biological substrates mediating various physiological and behavioral processes. As a result, the role of a given receptor type in mediating a given drug effect may vary across species.

Within the context of the issues raised by the present study, μ-opioid receptor agonist-induced antinociception and respiratory depression may be mediated by different μ-opioid receptor subtypes in mice and rats, but these effects appear to be mediated by similar μ-opioid receptor types in rhesus monkeys.

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