MDAN-21: A Bivalent Opioid Ligand Containing mu-Agonist and Delta-Antagonist Pharmacophores and Its Effects in Rhesus Monkeys

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MDAN-21, 7\textsuperscript{′}-[2-[7-\{(5α,6α)-4,5-Epoxy-3,14-dihydroxy-17-methylmorphin-6-yl\}-aminocarbonyl]metoxy\}-acetylamino\}-heptylaminocarbonyl]-methoxy\}-acetylamino\}-naltrindole, a bivalent opioid ligand containing a mu-opioid receptor agonist (derived from oxymorphone) linked to the delta-opioid receptor antagonist (related to naltrindole) by a spacer of 21 atoms, was reported to have potent analgesic properties in mice. Tolerance, physical dependence, and conditioned place preference were not evident in that species. The finding that bivalent ligands in this series, with spacers 19 atoms or greater, were devoid of tolerance and dependence led to the proposal that MDAN-21 targets heteromeric mu-delta-opioid receptors. The present study focused on its effects in nonhuman primates (Macaca mulatta), a species with a physiology and behavioral repertoire not unlike humans. With regard to opioids, this species usually better predicts clinical outcomes. MDAN-21 substituted for morphine in morphine-dependent monkeys in the remarkably low dose range 0.006–0.032 mg/kg, subcutaneously. Although MDAN-21 failed to produce reliable thermal analgesia in the dose range 0.0032–0.032 mg/kg, intramuscularly, it was active in the same dose range and by the same route of administration, in the capsaicin-induced thermal allodynia assay. The results suggest that MDAN-21 may be useful in the treatment of opioid dependence and allodynia. The data provide additional evidence that opioid withdrawal is associated with sensitized pain.

1. Introduction

The alkaloid morphine has been used in the treatment of pain, cough, and diarrhea. Unfortunately, unpleasant and/or potentially dangerous side effects such as respiratory depression, nausea, vomiting, and constipation can accompany its use. Psychological and physiological processes such as abuse, tolerance, and physical dependence have been associated with chronic use and limit the utility of morphine and other mu-opioid agonists in the treatment of chronic pain.

In a continuing search for potent analgesics free of these undesirable side effects, many analogues of morphine and numerous semisynthetic and synthetic derivatives have been introduced. The evolution of this search was summarized in a succinct review of the exciting but vain quest for the Holy Grail of opioid research [1]. Results of an investigation of the effects of leucine and methionine enkephalin on morphine-induced analgesia suggested an interaction between mu- and delta-opioid receptors [2]. Over a decade later, investigators found that naltrindole, a selective delta-opioid receptor antagonist, blocked the morphine tolerance without diminishing its antinociceptive potency [3]. These reports and the finding that G-protein-coupled opioid receptors existed as heterodimers: mu-kappa [4], mu-delta [5, 6], and kappa-delta [7] led to a hypothesis-driven synthesis [8] of a series of conjugates containing the mu-opioid receptor agonist (derived from oxymorphone) linked with delta-opioid receptor antagonist (related to naltrindole) with spacers ranging from 16 to 21 atoms [9]. Studies in...
mice indicated that 7’-[2-[(7-α6α)]-4,5-Epoxy-
3,14-dihydroxy-17-methylmorphin-6-yl]-aminocarbonyl]-
metoxy]-acetylamino]-heptylaminocarbonyl]-methoxy]-acety-
lymino]-naltrindole or MDAN-21, (see Figure 1), with a
25.4 Å spacer, (21-atoms), was the most potent analgesic
of the series. Significantly, naloxone, an opioid receptor
antagonist, failed to precipitate withdrawal signs after
chronic administration. It was subsequently shown that
MDAN-21-treated mice failed to develop conditioned place
preference [10]. These properties suggested that MDAN-21
may, to a significant extent, fulfill the long quest for a
strong opioid analgesic with greatly reduced side e

ccts. The rhesus monkey study
models were selected because of their excellent relationship
profile in nonhuman primates. The rhesus monkey study
on nociception and capsaicin-induced thermal allodynia assays
were maximally dependent on morphine. At least 4 mon-
keys/treatment were used and a minimal two-week wash-out
period was allowed between testing. The assay was modified
as indicated below [13, 14]. It was initiated by the injection
(s.c.) of the test drug or control substances (morphine and
vehicle) into animals in a group that had not received mor-
phine for 14-15 hr and showed definite signs of withdrawal.
Each animal was randomly chosen to receive one of the
following treatments: (a) a dose MDAN-21; (b) morphine
sulfate control (4.0 mg/kg); (c) vehicle control (1.0 mL/kg).
Withdrawal signs were scored, absent, or present, once
during each of five consecutive 30-min observation periods.
Withdrawal signs included slowing of movement, drowsiness
(sitting with eyes closed and lethargic or being indifferent
to surroundings), fighting, vocalizing, rigidity of abdom-
inal muscles, vocalization during palpation of abdominal
muscles, restlessness (pacing), tremors, coughing, retching,
vomiting, wet-dog shakes, and masturbation. The observer
was “blind” regarding the assignment of treatments. The
cumulative number of withdrawal signs was analyzed using
the Kruskal-Wallis Analysis of Variance (ANOVA) and post
hoc Mann-Whitney U Tests. The Stat View statistical package
(Brainpower, Agoura Hills, CA) was utilized for these
analyses. In all cases, significance (P) was set at 0.05.

2.2. Substitution for Morphine Assay. Twenty-four monkeys
in the weight range 3.5–7.5 kg comprised the group tested.
The assay was based on that originally described by Deneau
[12]. Modifications follow. Morphine was given subcuta-
nously (s.c.) daily at 6 AM, 12 noon, and 6 PM. All the
animals had received morphine for at least 3 months and
were maximally dependent on morphine. At least 4 mon-
keys/treatment were used and a minimal two-week wash-out
period was allowed between testing. The assay was modified
as indicated below [13, 14]. It was initiated by the injection
(s.c.) of the test drug or control substances (morphine and
vehicle) into animals in a group that had not received mor-
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hoc Mann-Whitney U Tests. The Stat View statistical package
(Brainpower, Agoura Hills, CA) was utilized for these
analyses. In all cases, significance (P) was set at 0.05.

2.3. Assay of Thermal Nociception. Three adult rhesus mon-
keys were studied in this assay as described previously [15].
They were seated in acrylic restraint chairs so that their tails
hung down freely. During tail-withdrawal measurements, the
bottom 15 cm of each monkey’s shaved tail was immersed
in a thermal container of warm water. If the subject did
not withdraw its tail within 20 s, the tail was removed from
the water by the experimenter, and a latency of 20 s was
assigned to that measurement. Experiments were conducted
no more than once a week. A stopwatch was used to measure
and record time intervals. Each test session consisted of
multiple cycles. Before MDAN-21 administration, baseline
tail-withdrawal latencies from 38 and 50°C water were
determined. Testing continued only if tail withdrawal from
38°C water did not occur before the 20 s cutoff, and if tail
withdrawal occurred in ≤2 s from 50°C water. All monkeys

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\text{Figure 1: Chemical structure of MDAN-21.}
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met this criterion before every test session. The effects of MDAN-21 intramuscularly (i.m.) were evaluated using a
time-course procedure, in which a single dose of MDAN-
21 (0.0032–0.32 mg/kg) was administered at the start of the
session, and tail withdrawal latencies from 50°C water were
re-determined 3, 6, 10, 18, 32, 56, and 100 min after the
injection.

MDAN-21 was completely ineffective up to the highest
doses tested in two monkeys. Accordingly, a follow-up exper-
iment was conducted in these two monkeys to determine
whether MDAN-21 might function as an antagonist of
the high-efficacy mu-agonist methadone. For these studies,
monkeys were pretreated with vehicle or 0.32 mg/kg MDAN-
21 15 min before treatment with 0.32 mg/kg methadone.
Tail-withdrawal latencies were then determined 10, 20, and
30 min after methadone administration. Drug effects were
expressed as Percent Maximum Possible Effect (%MPE)
using the following equation: “%MPE = [(Test Latency –
Baseline Latency)/(20 – Baseline Latency) *100],” where “Test
Latency” was the tail-withdrawal latency from 50°C water
obtained during each cycle after drug administration, “Base-
line Latency” was the latency from 50°C water during the
baseline determinations before drug injection, and “20” was
the cutoff latency in s.

2.4. Assay of Capsaicin-Induced Thermal Alldynia. Three
different male rhesus monkeys 5–12 kg were studied. Mon-
keys were seated in acrylic restraint chairs as described
above. To determine tail-withdrawal latencies, the lower
15 cm of each monkeys shaved tail was immersed into a
thermal container of warm water heated to the designated
temperature (see below for temperatures). The latency in
seconds for the monkey to remove its tail from the water
was measured using a hand-held stopwatch. If the subject
did not withdraw its tail within 20 s, the tail was removed
from the water by the experimenter, and a latency of 20 s was
assigned to that measurement. Experimental sessions were
conducted once per week. At the beginning of each session,
tail withdrawal latencies were determined for each monkey
from water heated to 38, 42, 46, and 50°C, and the order of
temperature presentations was randomized across sessions.
By this procedure, baseline temperature-effect curves were
determined in each monkey at the beginning of each
session, and the highest temperature to produce a tail
withdrawal latency >15 s was identified. Water heated to this
“threshold” temperature then served as the thermal stimulus
for subsequent studies of allodynia during that session. The
threshold stimulus intensity was 42°C for two monkeys and
46°C for the third monkey throughout the study.

Allodynia was elicited by topical application of capsaicin
as described previously [15, 16]. Following baseline tail
withdrawal latency determinations, a topical patch treated
with capsaicin solution or vehicle was prepared as described
below (see Drugs), and the patch was applied to a region
approximately 7 cm from the bottom of the tail for 5 min.
After 5 min, the patch was removed. Tail withdrawal latencies
were then re-determined 15, 30, 45, and 60 min after patch
removal using the thermal stimulus identified from the
baseline temperature-effect curve in each monkey (i.e., 42°C
in two monkeys, 46°C in the third monkey). MDAN-21
(0.0032–0.32 mg/kg, i.m.) was administered 15 min before
application of the capsaicin patch. Morphine (1.0 mg/kg,
i.m., 15 min pretreatment) was tested for comparison.

Raw tail withdrawal latencies obtained 15, 30, 45, and
60 min after removal of the capsaicin patch were con-
verted to Percent Maximum Possible Effect (%MPE) using
the equation %MPE = [(Test Latency – Capsaicin Alone
Latency)/(20 – Capsaicin Alone Latency) *100], where “test
latency” was the tail withdrawal latency obtained at each time
point after drug pretreatment + capsaicin patch treatment,
and “Capsaicin Alone Latency” was the latency obtained
at the corresponding time point after treatment with the
capsaicin patch alone.

2.5. Drugs. MDAN-21 was synthesized by Dr. Eyup Akgun
(Medical Chemistry Laboratory, Dr. Philip Portoghese,
Director). Morphine sulfate was purchased from Mallinck-
rodt, Inc., Hazelwood, MO. Methadone HCl was provided
by the NIDA Drug Supply Program. All drugs were dissolved
in sterile water for injection (Hospira, Inc., Forest Hills,
IL). Capsaicin (Sigma Chemical Co., St. Louis, MO) was
dissolved in vehicle composed of 70% alcohol and 30% sterile
water and was delivered transdermally (topical patch) as
described previously [15, 16]. The concentration of capsaicin
in the solution was individually determined for each monkey
as the lowest concentration to produce sustained decreases
in tail-withdrawal latencies from the threshold temperature
to ≤5 s throughout the 1 hr testing period (0.61 mg/mL
(2 mM)) for all three monkeys in the study. Within 30 s
of preparing the capsaicin patch, it was secured onto the
monkey’s tail with elastic tape and left on for 5 min.

3. Results

3.1. Substitution for Morphine in Morphine-Dependent Rhesus
Monkeys Assay. Results of this assay are illustrated in
Figure 2. It is evident that MDAN-21 had a short onset and
long duration of action, that is, it effectively suppressed with-
drawal as soon as 30 min and was still effective at 150 min.
MDAN-21 and morphine suppressed the full spectrum of
withdrawal signs exhibited by the vehicle controls. The effective
dose range was 0.006–0.03 mg/kg. The Kruskal-Wallis
ANOVA P values are as follows: 30 min-0.01; 60 min-0.0002;
90 min-0.0002; 120 min-0.0001; 150 min-0.0001. Post hoc
Mann-Whitney comparisons with probability values of 0.05
or less are indicated in figure legends and with appropriate
superscript letters in Figure 2.

3.2. Assay of Thermal Nociception. The mean ± S.E.M
baseline tail withdrawal latency from 50°C was 0.75 ±
0.18 s. Figure 3 shows the time course of antinociceptive
effects produced by doses of 0.0032–0.32 mg/kg MDAN-21
in three individual monkeys. In monkey M1470 (one of
the two males), MDAN-21 doses of 0.0032 and 0.032 mg/kg
produced a dose-dependent increase in tail withdrawal
latencies that peaked after 18 and 32 min and then dissipated
after 56 min. However, in the other two monkeys, MDAN-
21 doses of 0.032 and 0.32 mg/kg were largely ineffective,
producing only small increases in tail-withdrawal latencies after 6 min in monkey M1472. Higher doses were also tested in M1472 (2.6 mg/kg) and M1475 (1.0 mg/kg) and these doses were also ineffective (maximum effect of 15% MPE at any time in either monkey; data not shown).

Figure 4 shows the effects of pretreatment with vehicle or MDAN-21 on the antinociceptive effects of 0.32 mg/kg methadone in monkeys M1472 and M1475. After vehicle pretreatment, methadone produced a time-dependent antinociception that peaked after 20–30 min. Pretreatment with 0.32 mg/kg MDAN-21 had no effect on methadone antinociception in either monkey.

3.3. Capsaicin-Induced Thermal Alldynia Assay. The mean ± S.E.M baseline tail withdrawal latency at the threshold temperature to mean ± S.E.M. values of 1.71 ± 0.42, 1.66 ± 0.23, 1.65 ± 0.41, and 1.49 ± 0.10 s at times 15, 30, 45, and 60 min after capsaicin patch removal, respectively. Figure 5 shows that pretreatment with MDAN-21 (0.0032–0.32 mg/kg) produced a dose- and time-dependent prevention of capsaicin-induced allodynia. The highest dose of 0.32 mg/kg MDAN-21 produced peak antiallodynic effects after 15 min, and these effects dissipated after 45 to 60 min. For comparison, the antiallodynic effect of 1.0 mg/kg morphine peaked after 30 min and remained at levels greater than 50% MPE in all three monkeys at 45 min and in two of three monkeys at 60 min.

4. Discussion
MDAN-21 was very effective in suppressing withdrawal signs in morphine-dependent monkeys. Its action was prompt.
It had a remarkably long duration of action compared to that in the capsaicin-thermal assay in nondependent monkeys. Perhaps, MDAN-21 is much more effective in situations involving physical dependence. Increased pain sensitivity in chronic pain patients and opioid abusers has been reported [17–19]. MDAN-21 failed to produce a reliable antinociceptive effect in the assay of thermal antinociception in nondependent monkeys. In one monkey, a dose of 0.032, MDAN-21 was fully effective, but neither this dose nor a 10-fold higher dose of 0.32 mg/kg was active in the other two monkeys. Conversely, MDAN-21 was as effective as morphine in all monkeys tested in the assay of capsaicin-induced thermal allodynia.

This profile of results is consistent with the possibility that MDAN-21 does not readily cross the blood-brain barrier in rhesus monkeys [15]. On the other hand, suppression of withdrawal signs in morphine-dependent monkeys by MDAN-21 suggests that it crosses the blood-brain barrier. It is known that quaternary compounds, do not readily cross the blood-brain barrier. Indeed, quaternary morphine compounds were reported virtually inactive in morphine-dependent rhesus monkeys and practically devoid of antinociceptive activity in mice [20]. The variability in effects produced by MDAN-21 in the assay of thermal nociception did not appear to result from low efficacy of MDAN-21 at mu-opioid receptors. If MDAN-21 had failed to produce antinociception in 2 of 3 monkeys due to low efficacy, then it would be expected to antagonize the effects of a higher efficacy mu-agonist in these two monkeys. However, MDAN-21 failed to antagonize the antinociceptive effects of methadone in either monkey. Nevertheless, it is possible that higher doses might have achieved a robust antinociceptive effect. Finally, species differences in mu-delta interactions on neural substrates that mediate thermal antinociception may be involved [21–23].

Nevertheless, MDAN-21 was potently active in the capsaicin-induced thermal allodynia assay in the dose range of 0.032–0.32 mg/kg. It also attenuated withdrawal signs in morphine-dependent monkeys in spontaneous withdrawal for a longer time period. The results are in accord with the findings of other investigators that pain sensitivity is increased in opioid-treated animals, chronic pain patients
and opioid abusers (see references above). The results also suggest that MDAN-21 may be useful in the pharmacotherapy of chronic pain and chronic opioid use.

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References


