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Antagonism by Nalmefene of Systemic and Intrathecal Morphine-Induced Analgesia in Mice[1] (42609)

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Abstract. Nalmefene is an orally active opiate antagonist structurally related to naloxone and naltrexone. In this study using two different strains of mice (Swiss Cox and ICR), the antagonist activity of nalmefene given subcutaneously (sc) was quantified by determination of the apparent pA_2 values against the antinociceptive activity (tail flick and hot plate tests) of morphine given sc or intrathecally (lumbar spinal cord). The apparent pA_2 values (constrained to a slope of -1) were 8.06 (7.79–8.33) in Swiss Cox mice and 7.81 (7.62–8.00) in ICR mice in the tail flick test with sc morphine. These values were larger than the corresponding value for naloxone in ICR mice, 7.35 (7.10–7.60). The hot plate test provided similar results: the apparent pA_2 values for nalmefene with sc morphine were 8.14 (7.89–8.39) in Swiss Cox mice and 7.81 (7.65–7.97) in ICR mice, values which were different from naloxone 7.33 (7.23–7.42) in ICR mice. Apparent pA_2 values for nalmefene with intrathecal morphine were not different from those for naloxone in the tail flick test. Thus, these sets of results suggest that it may be worthwhile to further determine whether systemic nalmefene might possibly possess an advantage over naloxone in antagonizing systemic side effects of morphine arising from local spinal morphine administration.

Nalmefene [17-(cyclomethyl)-4,5-epoxy-6-methylene-morphinan-3,4-diol] is a pure narcotic antagonist undergoing clinical trial (1–3). In comparison to naloxone, a pure narcotic antagonist marketed in the United States for acute parenteral use, nalmefene has the advantages of longer duration of action and better oral bioavailability (1–4). Nalmefene has been reported to have greater binding affinity than naloxone for rat brain μ-, κ-, and δ-opioid receptor subtypes and is 40 times more potent than naloxone in antagonizing morphine in the hot plate and tail clip analgesic tests (5).

When administered spinally (intrathecally (i.t.) or epidurally), morphine produces profound analgesia by a direct action on the spinal cord (6, 7). This analgesia can be antagonized by administration of sc naloxone, and an apparent pA_2 value of 7.0 has been reported in rats using the tail flick test (6). This apparent pA_2 value is similar to apparent pA_2 values reported for this antinociceptive test in mice when both morphine and naloxone are given sc (8, 9). Apparent pA_2 values for systemically administered nalmefene against sc or intrathecal morphine are not found in the literature. Therefore the purpose of the present study is to determine such values to see whether differences occur between naloxone and nalmefene. Different apparent pA_2 values would be expected between naloxone and nalmefene if the binding affinity and the potency are the important determinants. A difference may confer an advantage of one antagonist over another. For instance, if nalmefene were to be more potent than naloxone against systemically administered morphine and yet equally potent against intrathecally administered morphine, the possibility would exist that the systemic side effects induced by intrathecal or epidural morphine administration might be antagonized by nalmefene with minimal effect on spinal analgesia. As a first study, it was not feasible to measure such side effects as respiratory depression, urinary retention, and itching produced by spinally administered morphine. In lieu of measuring side effects produced by the morphine acting on other than a direct spinal site, we chose to determine apparent pA_2 values for sc nalmefene against sc morphine analgesia. For the latter, the rationale was that if nalmefene had a different apparent pA_2 than naloxone for sc morphine analgesia, this differential would carry over to

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antagonism of the side effects of morphine as well. This approach was taken since the same measurement (analgesia) could be made for the spinal and systemic effect of morphine without the use of a variety of techniques to quantify side effects such as respiratory depression, antidiuresis, or inhibition of intestinal transit in mice, complexities with which we have had previous experience (10–12). Also, a few naloxone apparent pA_2 values were determined to confirm literature values.

Materials and Methods. Male, Swiss Cox mice (Laboratory Supply, Indianapolis, IN) weighing 30–35 g were used in studies employing sc nalmefene with intrathecal or sc morphine. When these mice became unavailable, male ICR mice (30–35 g, Sasco, Inc., Omaha, NE) were used in studies which determined the effects of sc nalmefene or naloxone on sc morphine analgesia.

Analgesia. The radiant heat tail flick technique (13) was one test used to evaluate antinociception. Response times before drug administration were 2–4 sec and a maximum cut-off time of 10 sec was allowed in drug-treated animals. The procedure of Dewey *et al.* (14) was used to convert tail flick latency times to percentage maximum possible effect (% MPE):

$$\%\text{MPE} = (\text{drug time} - \text{control time})/(\text{cut-off time} - \text{control time}) \times 100.$$

The response latency on a 55°C hot plate was recorded as another index of antinociception. Time required for the naive animal to lick its paws was 8–10 sec. A cut-off time of 30 sec was used as the maximum response time. Percentage MPE was calculated as in the tail flick procedure.

For each ED_{50} determination, four or more different doses of morphine sulfate were used with 8–10 mice given each dose. Each animal was given only one morphine dose and usually was used for both antinociceptive tests. In the procedure of Dewey *et al.* (14), the graded analgesic response expressed as the %MPE is used as if the data represented quantal responses of the sample population and ED_{50} values were derived by the procedure of Litchfield and Wilcoxon (15). It appears that in both tests with the use of the cut-off times, a combination of quantal and graded responses is measured (16). Analgesic ED_{50} values in the present report were calculated using a computerized form of the Litchfield and Wilcoxon procedure (17).

Drugs. Nalmefene HCl was obtained from Key Pharmaceuticals (Miami, FL). Morphine sulfate was purchased from Mallinckrodt Chemical Works (St. Louis, MO). Naloxone HCl was obtained from National Institute on Drug Abuse. Dose designations used are for the salt forms of the drugs.

Drugs were dissolved in 0.9% NaCl (w/v) and administered in a volume of 0.1 ml/10 g body weight sc or 5 μl intrathecally. The method of Hylden and Wilcox (18) was used for i.t. injection into the lumbar spinal intrathecal space.

Time relationship study. To determine the time of maximal antagonistic effect, nalmefene (10 μg/kg) was administered sc at various times before or after morphine (25 mg/kg, sc, or 10 μg, i.t.). Tail flick response was measured 20 min after sc or 5 min after i.t. morphine, at the respective peak times of morphine-induced analgesia (19). When concurrent sc morphine and nalmefene injections were made, different sites of injection were used. Separate groups of animals were used for each time interval. For the hot plate test, time to maximal response for morphine was determined to be 20 min after sc and 15 min after i.t. administration. Time for maximal antagonistic activity of nalmefene (10 μg/kg) was determined as in the tail flick paradigm above. Time for maximal antagonistic activity of naloxone (20 min) was determined previously (20).

pA_2 determinations. At fixed doses of nalmefene or naloxone given 20 min before the analgesia test, the morphine ED_{50} values were determined at the peak time of morphine action (see above). The dose ratio of the ED_{50} values in the presence and absence of antagonist was calculated and the procedure of Arunlakshana and Schild (21) was used to plot log (dose ratio − 1) versus −log (dose antagonist in mole/kg). Apparent pA_2 values with 95% confidence intervals and slopes (±SEM) were obtained using the computerized Schild method (17). In addition, apparent pA_2 values with 95% confidence intervals were obtained using the constrained Schild plot (constraining the slopes to unity) (17). The apparent pA_2 value is expressed as moles of antagonist per

kilogram of body weight and represents the amount of antagonist which will double the ED_{50} dose of morphine.

Results. To establish the peak time for antagonism, nalmefene was given at various times in relation to the peak time of action of morphine. In Figs. 1A and 1B, the dose of morphine was 25 mg/kg, sc, given 20 min before the analgesic tests. The peak effect of sc nalmefene occurred at 20 min for both tail flick and hot plate tests, with substantial antagonism present at 10 and 30 min. Similarly, against i.t. morphine, Figs. 1C and 1D, the peak times for sc nalmefene were 20 min in the tests. Thus, in subsequent experiments, the nalmefene was given sc at 20 min with peak times for morphine as given above (see Materials and Methods).

Table I presents the ED_{50} values determined in Swiss Cox and ICR mice when fixed doses of sc nalmefene were given along with various doses of sc morphine. The ED_{50} values for i.t. morphine in Swiss Cox mice in the presence of fixed doses of sc nalmefene are shown in Table II. The data in Table III were obtained from ICR mice using sc naloxone as the antagonist with sc morphine.

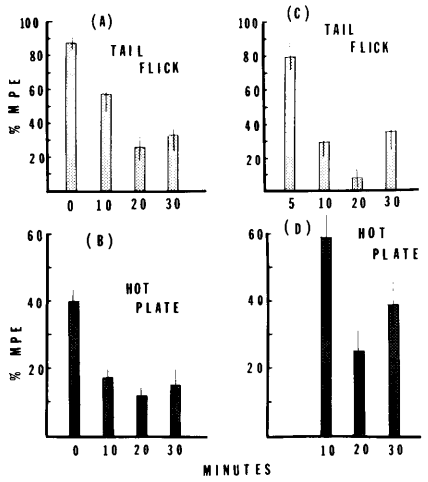

FIG. 1. Time course of the antagonistic effect of nalmefene, 10 μg/kg, sc, against morphine given sc or i.t. in the tail flick and hot plate test in the Swiss Cox mice. (A, B) Morphine, 25 mg/kg, sc, given 20 min before the tail flick and hot plate test. (C, D) Morphine, 10 μg/mouse, i.t., given 5 and 15 min before the tail flick and hot plate test, respectively. The abscissa indicates the time at which the nalmefene was given before the test.

TABLE I. EFFECT OF NALMEFENE HCl (sc) ON TAIL FLICK AND HOT PLATE ED_{50} VALUES OF MORPHINE SULFATE (sc) IN SWISS COX AND ICR MICE

Dose nalmefene (μg/kg)	ED_{50} morphine (95% confidence interval, mg/kg)	
	Tail flick	Hot plate
Swiss Cox mice		
0	12.1 (8.9–16.4)	20.0 (13.5–29.6)
5.0	22.2 (12.8–38.6)	36.8 (22.1–61.3)
7.5	59.4 (39.2–90.0)	83.5 (57.1–122)
10.0	46.1 (34.5–61.5)	100 (60.7–165)
12.5	44.5 (27.4–72.3)	150 (96.7–235)
15.0	101 (74.8–136)	170 (116–250)
ICR mice		
0	4.8 (3.2–7.1)	13.3 (9.3–19.2)
10	16.7 (7.1–39.4)	43.4 (25.9–72.5)
15	16.1 (7.7–33.3)	56.4 (32.5–97.8)
30	19.2 (12.9–28.6)	59.2 (33.8–103)
60	61.8 (33.8–113)	161 (116–224)
100	98.3 (53.5–181)	194 (110–341)

Note. Morphine and nalmefene were given sc 20 min before the analgesia test.

From each morphine ED_{50} value obtained in the presence of antagonist, the dose ratio for the ED_{50} values was calculated and apparent pA_2 values were computed. Table IV lists the apparent pA_2 values and slopes obtained from Schild plots, as well as apparent pA_2 values computed by constraining the Schild slopes to unity. Apparent pA_2 values for sc nalmefene with sc morphine calculated from

TABLE II. EFFECT OF NALMEFENE HCl (sc) ON TAIL FLICK AND HOT PLATE ED_{50} VALUES OF MORPHINE SULFATE (i.t.) IN SWISS COX MICE

Dose nalmefene (μg/kg)	ED_{50} morphine (95% confidence interval, μg, i.t.)	Dose nalmefene (μg/kg)	ED_{50} morphine (95% confidence interval, μg, i.t.)
Tail flick		Hot plate	
0	2.1 (0.7–5.9)	0	2.4 (1.2–4.5)
1.5	4.3 (1.7–10.4)	3.0	6.3 (4.3–9.1)
2.5	6.4 (2.4–16.9)	4.0	10.2 (4.6–22.7)
3.0	16.8 (10.1–27.9)	5.0	6.7 (3.9–11.1)
5.0	14.0 (4.3–46.0)	7.0	10.9 (4.6–23.5)
		10.0	11.7 (6.7–20.6)

Note. Nalmefene was given 20 min before the analgesia tests. Morphine was given 5 min before the tail flick and 15 min before the hot plate tests.

TABLE III. EFFECT OF NALOXONE HCl (sc) ON TAIL FLICK AND HOT PLATE ED_{50} VALUES OF MORPHINE SULFATE (sc) IN ICR MICE

Dose naloxone (μg/kg)	ED_{50} morphine (95% confidence interval, mg/kg)	
	Tail flick	Hot plate
0	4.8 (3.2–7.1)	13.3 (9.3–19.2)
50	14.4 (10.3–20.3)	47.9 (31.3–73.2)
100	29.6 (13.5–64.8)	86.8 (58.2–130)
200	82.4 (57.5–118)	203 (148–279)
400	167 (115–242)	315 (241–409)

Note. Morphine and naloxone were given sc 20 min before the analgesia tests.

both Schild plots and constrained slope plots in both antinociceptive tests were similar in the two strains of mice. Thus, method of analgesia measurement and mouse strain showed similar sensitivity to the antagonism of morphine effect by nalmefene. When sc nalmefene was given with i.t. morphine, again similar pA_2 values were obtained in the tail flick and hot plate tests in Swiss Cox mice, an indication that both tests showed similar sensitivity to nalmefene action. However these values were different from those for sc naloxone/sc morphine (Table IV).

Discussion. Comparison of apparent pA_2 values obtained by constraining the slopes to unity represents a method of comparing values based on competative theory which relates the pA_2 value to the K_B (9). Since the slopes shown in Table IV varied from 1, for purposes of comparison the results will be discussed using the apparent pA_2 values obtained by constraining the slopes to one. Apparent pA_2 values for sc nalmefene with sc morphine were not different between the tail flick and the hot plate tests in the two strains of mice: 8.06 (Swiss Cox) and 7.81 (ICR) in the tail flick test and 8.14 (Swiss Cox) and 7.81 (ICR) in the hot plate test. These values were significantly different from the apparent pA_2 values ob-

TABLE IV. APPARENT pA_2 VALUES FOR NALMEFENE AND NALOXONE WITH MORPHINE ON THE TAIL FLICK AND HOT PLATE TESTS

Injection, mouse, test	pA_2 (95% confidence interval)	Slope (±SEM)	Constrained pA_2 (95% confidence interval)
Nalmefene sc/morphine sc			
Swiss Cox mice			
Tail flick	7.91 (7.47–8.35)	−1.50 (0.60)	8.06 (7.79–8.33)
Hot plate	7.88 (7.74–8.04)	−1.95 (0.28)	8.14 (7.89–8.39)
ICR mice			
Tail flick	7.83 (7.26–8.40)	−0.97 (0.21)	7.81 (7.62–8.00)
Hot plate	7.98 (7.44–8.52)	−0.81 (0.14)	7.81 (7.65–7.97)
Naloxone sc/morphine sc			
ICR mice			
Tail flick	7.09 (6.91–7.27)	−1.39 (0.08)	7.35 (7.10–7.60)
Hot plate	7.26 (6.91–7.61)	−1.08 (0.10)	7.33 (7.23–7.42)
Nalmefene sc/morphine i.t.			
Swiss Cox mice			
Tail flick	8.46 (7.76–9.14)	−1.52 (0.66)	8.63 (8.26–9.00)
Hot plate	8.53 (7.39–9.67)	−0.64 (0.32)	8.28 (8.11–8.45)

tained for sc naloxone/sc morphine in ICR mice: 7.35 (tail flick) and 7.33 (hot plate). These latter naloxone values are similar to those previously reported (ca. 7) by others (8, 9) in various outbred strains of mice. Thus, the present study shows that a smaller dose of sc nalmefene than naloxone was needed to double the analgesic ED_{50} value of sc morphine. This result is consistent with the report of the greater binding affinity and potency of nalmefene to antagonize morphine analgesia (5).

For sc nalmefene with i.t. morphine, the apparent pA_2 values were the same for the tail flick (8.63) and hot plate (8.28) tests. Furthermore, these values for nalmefene were not different from the value for naloxone administered sc with i.t. morphine which in the tail flick test was 8.53 (8.00–9.06) (22). Thus, sc nalmefene and naloxone were equally potent against i.t. morphine.

The set of findings that the relative potency of nalmefene is greater (pA_2 = 8.06, 7.81, 8.14, 7.81) than that of naloxone (pA_2 = 7.35, 7.33) against systemic (sc) morphine and that the two antagonists show no such potency difference against spinally induced morphine analgesia can be interpreted in two ways. First, if the same difference between nalmefene and naloxone were to exist for antagonizing the side effects of morphine as for antagonizing the analgesic effect of systemically administered morphine, it is possible that systemically administered nalmefene might be better than naloxone at antagonizing the side effects evoked systemically by spinally applied morphine. That is, this antagonism might occur without as much effect on spinal analgesia as that produced by naloxone. Second, the argument can be made that even if the greater potency of nalmefene over naloxone were applicable to the systemic side effects produced by spinally applied morphine, nalmefene would not have an advantage over naloxone since the pA_2 of nalmefene for sc morphine is the same as for i.t. morphine, that is, about 8. Along this line, we find that the apparent pA_2 value for sc naloxone in Swiss Cox mice with sc morphine is 7.05 (6.62–7.47) for respiratory depression (10) compared to its value of 8.53 for i.t. morphine analgesia (22). These values would suggest that naloxone would antagonize the side effect of respiratory depression at the expense of spinally induced analgesia. In spite of this kind of argument, the difference between nalmefene and naloxone is sufficiently large to warrant further work especially since the nalmefene apparent pA_2 value for morphine-induced side effects is not known. Also, the difference between nalmefene and naloxone might be greater than seen in these experiments with mice since oral administration of nalmefene might provide a better correspondence to the time of occurrence of the side effects.

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