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European Journal of Pharmacology 495 (2004) 63–66

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# Chronic administration of nalmefene leads to increased food intake and body weight gain in mice

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Received 10 May 2004; accepted 14 May 2004

**Abstract**

Nalmefene is an orally available opioid receptor antagonist that has been shown to suppress appetite in humans, but its effects on chronic food intake and body weight remain unclear. Here, we report that chronic (21-day) oral administration of nalmefene at 2 or 10 mg/kg/day in diet-induced obese (DIO) mice led to significant increases (9–11%) in cumulative food intake. Mice in the nalmefene-treated groups also gained body weight at a rate faster than the control. Body composition analysis showed that the extra body weight gains in the treated animals were mostly due to increased fat accumulation. Since acute nalmefene treatment showed a trend toward a decrease rather than an increase in food intake, it is possible that the orexigenic effect of chronic oral administration of nalmefene was caused by pharmacologically active metabolites rather than the drug itself. Our results argue against the potential use of nalmefene for treating human obesity.


*Keywords:* Opioid receptor antagonist; Nalmefene; Food intake; Body weight

## 1. Introduction

There is a considerable amount of evidence implicating the central opioid systems in the regulation of energy homeostasis. Opioid receptor agonists, including morphine and endogenous opioid peptides β-endorphin and dynorphin, are known to increase food intake (Glass et al., 1999; Reid, 1985; Sanger and McCarthy, 1981). In addition, elevated levels of endogenous opioid peptides were observed in obese or fasting animals (Ferguson-Segall et al., 1982; Herve and Fellmann, 1997; Margules et al., 1978; Welch et al., 1996). Opioid receptor antagonists, on the other hand, have been shown to suppress feeding in a variety of species (Billington et al., 1985; de Zwaan and Mitchell, 1992; Deviche and Wohland, 1984; Glass et al., 1999; Yeomans and Gray, 2002). Significantly, it has been reported that chronic administration of naltrexone (Glass et al., 2002; Marks-Kaufman et al., 1984; Shaw, 1993) or LY255582 ((3*R*,4*R*)-1-(*S*)-3-hydroxy-3-cyclohexylpropyl-4-(3-hydroxyphenyl)-3,4- dimethyl-1-piperidine) (Shaw, 1993; Statnick et al., 2003), two non-selective and structurally distinct opioid receptor antagonists, inhibited long-term body weight gain in rats. Based on these observations, it was proposed that blockade of the central opioid pathway by opioid receptor antagonists may represent a potential means to treat human obesity (Statnick et al., 2003; Yeomans and Gray, 2002).

Naloxone, naltrexone and nalmefene are members of a class of high-affinity, non-selective opioid receptor antagonists that have been approved for clinical use to treat opioid-induced constipation, opioid overdose and other illnesses. In humans, all three drugs have demonstrated an efficacy in suppressing short-term appetite (de Zwaan and Mitchell, 1992). Several human trials have also been conducted to assess the effects of chronic oral administration of naltrexone or naloxone on body weight, but the results were inconclusive (de Zwaan and Mitchell, 1992). Compared to naloxone or naltrexone, nalmefene has the highest oral bioavailability and a relatively long half-life in humans (Dixon et al., 1986, 1987). The drug has also shown good safety and tolerability in humans (Dixon et al., 1986, 1987; Mason et al., 1999). However, the potential of nalmefene as an oral medicine for treating human obesity has not been evaluated. In the present study, we attempted to determine the effects of chronic oral nalmefene on feeding in a preclinical animal model. We

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doi:10.1016/j.ejphar.2004.05.025

dosed diet-induced obese (DIO) mice orally with nalmefene once a day for 21 days at 2 or 10 mg/kg/day, and measured food consumption and body weight changes.

## 2. Materials and methods

### 2.1. Animals, drug administration and body composition analysis

Male DIO C57BL/6 mice of 8 months old weighing 44–50 g were purchased from Taconic Farms (Germantown, NY, USA), which had been maintained on high fat diets for 6 months (5 months at Taconic on TD97070, 60% kcal from fat, Harlan Teklad, Madison, WI, USA; 1 month at Merck on D12451, 45% kcal from fat, Research Diets, New Brunswick, NJ, USA). The mice were individually caged under a 12-h light/dark cycle (lights off at 17:00 h) in a temperature- and humility-controlled environment with free access to food and water. Nalmefene (Akzo Nobel, The Netherlands) at 2 or 10 mg/kg/day was administered by oral gavage using 5% Tween 80 and 0.5% methylcellulose as the vehicle. Before drug administration, mice were acclimated to once daily oral dosing with vehicle alone for approximately 1 week and then assigned randomly to individual experimental groups ($n = 10$ per group) to ensure equivalent mean body weights at the start of the treatment (Fig. 2A, day 0). Dosing was carried out daily for 21 days approximately 45 min before the onset of dark cycle (17:00 h). On the first day, food intake at 4 h into the dark cycle was measured. Subsequently, measurements were taken each day just prior to drug administration to determine daily body weight change and food consumption. Body composition was determined before and after the treatment period using an NMR analyzer (Minispec, Bruker Optics, Billerica, MA, USA). All animal protocols used in the study were in accordance with the NIH Guide for Care and Use of Laboratory Animals and approved by the Merck Research Laboratories Institutional Animal Care and Use Committee (IACUC) at Rahway, NJ.

### 2.2. Statistical analysis

All data shown are the mean ± standard error of the mean (S.E.M.). The differences in daily food intake were determined using one-way analysis of variance (ANOVA) with repeated measures. The effects of time and nalmefene treatment on body weight change were determined by two-way ANOVA, and any differences at a given time point were analyzed by Dunnett's test. The differences in cumulative food intake and body composition changes between vehicle- or nalmefene-treated groups were analyzed by one-way ANOVA, followed by Dunnett's test for multiple comparisons. In all cases, significance was at $P < 0.05$.

## 3. Results and discussion

The effect of nalmefene on food intake in male DIO mice is shown in Fig. 1. Food intake at 4 h after the initial dosing was slightly decreased in nalmefene-treated groups (Fig. 1A, 4 h), although the effect was not statistically significant. Over the period of 24 h after each dosing, food intake in nalmefene-treated groups was generally increased as compared to the vehicle group (Fig. 1A, days 1–21; $F = 6.50$, $P < 0.001$; repeated-measures one-way ANOVA). Overall, nalmefene at 2 or 10 mg/kg/day increased the 21-day cumulative food intake by 9% ($P < 0.01$) and 11% ($P < 0.01$), respectively, over the vehicle group (Fig. 1B).

Fig. 2 shows the effect of nalmefene on body weight in male DIO mice. The two nalmefene-treated groups showed a dose-dependent trend toward gaining body weight at a rate faster than the vehicle group, and from day 13 on (except for

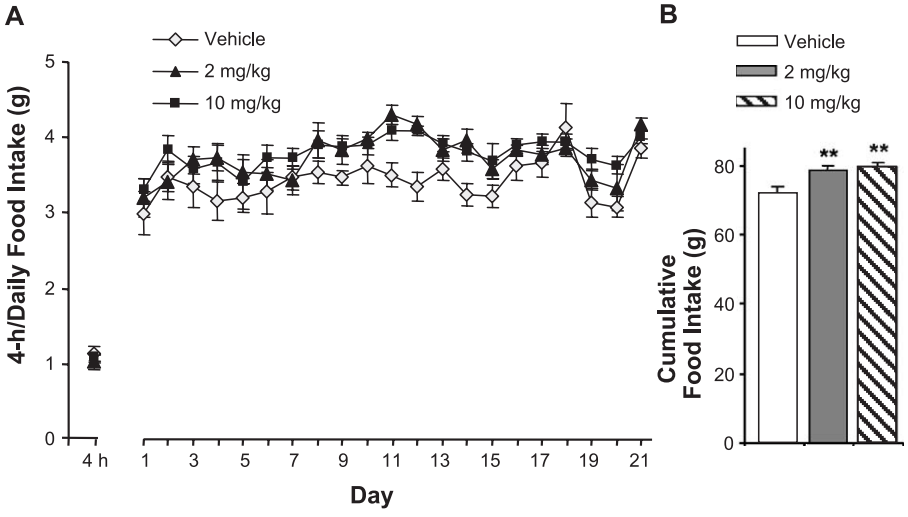


Fig. 1. Effect of nalmefene treatment (2 or 10 mg/kg/day) on food intake in male DIO mice. (A) Food intake at 4 h following the first dosing and 24 h (daily) after each dosing; (B) 21-day cumulative food intake. $^{**}P < 0.01$ vs. vehicle. Values shown are the mean ± S.E.M. ($n = 10$ per group).

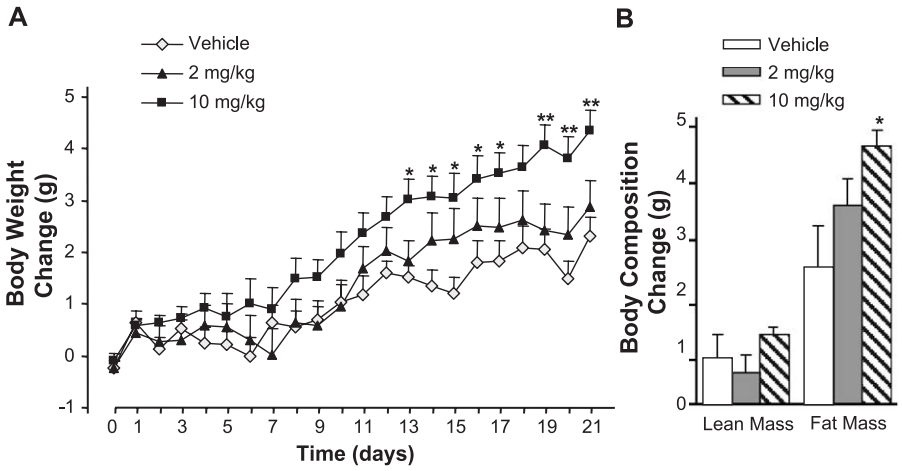


Fig. 2. Effect of nalmefene treatment (2 or 10 mg/kg/day) in male DIO mice on (A) cumulative body weight changes and (B) body composition changes after 21-day nalmefene treatment. *$P<0.05$, **$P<0.01$ vs. vehicle. Values shown are the mean ± S.E.M. ($n=10$ per group).

day 18) the mean body weight of the 10 mg/kg/day nalmefene-treated group became significantly higher than the control (Fig. 2A). In vivo body composition analysis by NMR showed that all three treatment groups gained similar amounts of lean mass over the 21-day period, but the 10 mg/kg/day nalmefene-treated group gained significantly more fat (Fig. 2B).

To our knowledge, this is the first report describing the chronic effect of nalmefene on feeding and body weight in mice. Previously, it was reported that a single oral dose (2.5 mg) of nalmefene led to a significant suppression of short-term appetite in humans (Yeomans et al., 1990), and that nalmefene by subcutaneous injection also reduced acute food intake in rats (McLaughlin and Baile, 1983; Shaw et al., 1991). Recently, we also reported that oral administration of nalmefene in mice demonstrated a dose-dependent trend toward a reduction in acute food intake although the effect was not statistically significant even at the highest dose (20 mg/kg) (Chen et al., 2004). The chronic effect of nalmefene on food intake and body weight in humans has not been reported. In rats, subcutaneous injection of nalmefene twice a day for up to 21 days led to a modest suppression of food intake and body weight gain in obese or diabetic lean rats (Levine et al., 1988; McLaughlin et al., 1986). Continuous oral dosing by including nalmefene in the feed also decreased food intake and body weight in both lean and obese rats, although the initial strong effects were partially offset by subsequent increases (McLaughlin et al., 1986). Our current study sought to determine if nalmefene demonstrated anti-obesity efficacy in a chronic, daily oral dosing paradigm. We dosed male diet-induced obese mice by oral gavage once a day for 21 days and observed, surprisingly, a slight but generally an increase in food intake that was evident during the initial days of dosing and persisted over most of the treatment period (Fig. 1A). As a result, the nalmefene-treated mice also showed a consistent trend of gaining body weight faster than the vehicle group. Thus, our results are in contrast to the weight-suppressing effects observed in rats chronically exposed to nalmefene, although different dosing routes and regimens were used in the rat studies.

The actual cause of the orexigenic effect of chronic oral nalmefene in mice is unclear. It is unlikely that nalmefene itself was the cause because the drug acutely showed a trend in decreasing rather than increasing food intake (Fig. 1A and Chen et al., 2004). The apparent delay in the onset of the orexigenic effect following nalmefene dosing may indicate the action of an accumulating metabolite(s). Nalmefene (as well as naloxone and naltrexone) is structurally similar to morphine, which is an agonist for the μ-subtype opioid receptor and has long been known to stimulate appetite in rodents (Levine et al., 1988; Sanger and McCarthy, 1981). It is possible that following oral administration, in vivo metabolism of nalmefene generated derivatives that might possess morphine-like agonist activities, leading to stimulation of the opioid pathway and increased appetite. The disposition of nalmefene in mice has not been reported. However, it was reported that in rats nalmefene is converted predominantly to an *N*-dealkylated intermediate, nornalmefene, before undergoing glucuronidation and being secreted (Murthy et al., 1996). Interestingly, nornalmefene was reported to have affinity for opioid receptors and in vivo efficacy as an agonist (Murthy et al., 1996). Alternatively, the increased food intake and weight gain in mice following chronic nalmefene administration could be a result of neural and/or physiological changes associated with chronic opioid blockade. For example, it is known that chronic exposure to opioid receptor antagonists leads to supersensitivity to opioid receptor agonists, which could be due to up-regulation of opioid receptors, particularly of the μ- but also the δ-subtype as demonstrated in two recent studies in mice treated chronically with naltrexone (Bailey et al., 2003; Lesscher et al., 2003). Evidence arguing against this hypothesis, however, includes the observation that chronic parenteral administration of naltrexone suppressed food intake and body weight gain in rats (Glass et al., 2002; Shaw, 1993). In addition, another opioid receptor antagonist, LY255582,

which belongs to the *trans*-3,4-dimethyl-4-phenylpiperidine series that are structurally distinct from naloxone, naltrexone or nalmefene (Zimmerman and Leander, 1990), has also been shown to consistently suppress both food intake and body weight gain in rats after chronic administration by either injection or oral dosing (Shaw, 1993; Shaw et al., 1991; Statnick et al., 2003). Those observations suggest that chronic opioid blockade does not necessarily induce in vivo compensatory changes that may lead to hyperphagia. In conclusion, our results highlight the potential agonist activities associated with oral administration of nalmefene and thus argue against the potential use of nalmefene for treating human obesity.

## Acknowledgements

We thank Lauren Shearman and Alison Strack for critical reading of the manuscript and Jennifer Adams for the help with the NMR analyzer.

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