Trabajo científico

Naltrexone treatment decreases water intake without affecting alcohol consumption, and suppresses the binge eating after alimentary deprivation

El tratamiento con naltrexona decrementa el consumo de agua sin afectar el consumo de alcohol, y suprime el atracón de comida posterior a la privación alimentaria

Eliana Barrios De Tomasi, Jorge Juárez

Laboratorio de Farmacología y Conducta, Instituto de Neurociencias, CUCBA, Guadalajara, Jalisco, México

Resumen

Se estudió el efecto de la naltrexona en los patrones de consumo de alimento, alcohol y agua, en acceso libre y privación alimentaria. Ratas Wistar fueron tratadas con naltrexona (10mg/Kg/día, s.c.) o solución salina por 14 días. El consumo de agua se redujo entre 2 y 4 h después de la inyección de naltrexona en la condición alimentaria a libre demanda. Bajo privación periódica alimentaria, la naltrexona suprimió el atracón de alimento tras su re-exposición y redujo significativamente la ingesta de agua, sin afectar el consumo de alcohol. Los resultados sugieren la participación del sistema opioide en los mecanismos de regulación de ingesta de líquidos como un incentivo primario independientemente de la privación alimentaria, y apoyan que este sistema juega un papel importante en el componente apetitivo de la ingesta de alimentos, especialmente durante su restricción.

Abstract

Present study assessed the effects of naltrexone on the patterns of food, alcohol and water consumption under free-feeding and food deprivation in rats. Wistar rats were treated with either naltrexone 10mg/Kg/day, s.c. or saline solution at 0.2mL/day, for 14 days. Under free-feeding condition, water consumption decreased between 2 and 4 h after the naltrexone injection. Under periodic food deprivation, naltrexone suppressed the binge for food following the feeding re-exposure; decreased water intake more extensively than in free-feeding condition, and did not affect alcohol consumption. Results suggest the participation of opioid activity in the regulatory mechanisms of liquid intake as a primary incentive as much in free-feeding as in food deprivation condition, and findings support that opioid system plays an important role in the appetitive mechanisms of feeding behavior, particularly during food-deprivation.

Palabras clave: Naltrexona; Ingestión de alimento; Consumo de alcohol; Consumo de agua; Privación de alimento; Antagonista opioide; Sistema opioide.

Key words: Naltrexone; Food intake; Alcohol consumption; Water consumption; Food deprivation; Opioid receptor antagonist; Opioid system.

Correspondencia:
Dr. Jorge Juárez
Laboratorio de Farmacología y Conducta
Instituto de Neurociencias
Universidad de Guadalajara
Francisco de Quevedo #180, Col. Arcos Vallarta, CP 44130
Guadalajara, Jalisco, México
Tel: (0133) 37771150 ext. 33368 and 33369
Fax: (0133) 38180740 ext. 33351
E-mail: jjuarez@cencar.udg.mx

Fecha de recepción: 11 de diciembre de 2013
Fecha de recepción de modificaciones: 14 de abril de 2014
Fecha de aceptación: 9 de mayo de 2014

Introduction

There is evidence of a biochemical link between opiates and alcohol dependence; hence, many behaviors associated with the processes of reward and reinforcement may be controlled or modulated by distinct components of the endogenous opioid system. Opioid receptors and peptides are selectively involved in several components of the addictive processes induced by opioids, cannabinoids, psychostimulants, alcohol and nicotine.[1]

It is well known that alcohol consumption facilitates the release of beta-endorphins from the hypothalamus[2,3] hence, opioid receptor antagonists have been probed extensively in studies of the control of alcohol intake, most of which agree that opioid blockage by naltrexone (an non-selective opioid receptor antagonist) significantly reduces alcohol consumption in both human beings[4,5] and animals.[6-8]

Also, it has been shown that the restriction or deprivation of food intake affects the activity of the opioid system. Rats studied under a regimen of 50% of their normal daily chow consumption showed no changes in the β-endorphin levels in the arcuate nucleus when measures were taken on days 4, 8 and 12, but this peptide decreased after 16 days of food restriction.[9] Both food restriction for 2 weeks and food deprivation for 4 days reduce pro-opiomelanocortin (POMC) mRNA levels in the hypothalamic arcuate nucleus, but food restriction produces larger changes in peptide mRNA expression than does food deprivation.[10] In obese women, two months of a hypocaloric diet and an increase in physical exercise produced lower plasma levels of beta-endorphins.[11]

In contrast, other studies have described an increase in beta-endorphin (BE) immunoreactivity in response to food deprivation in the hypothalamus and anterior pituitary,[12] while basal in vitro BE release from hypothalamic explants increased after the first day of food deprivation and remained greater than that seen in a normally-fed control group with continuing food deprivation for 48, 72 and 96 h in rats.[13]

Naltrexone has been used clinically to treat alcoholism and opiate dependence and, less extensively, for the treatment of certain eating disorders, such as overeating, anorexia and bulimia. However, there are at least two problems involved in studies of the effects of naltrexone on alcohol consumption and eating behavior. The first is related to the time that elapses between administering the drug and assessing its effects, as most studies in the literature analyze the acute effects of the opioid receptor antagonist at a maximum of 6 h after administration and generally use food restriction, special alimentary diets or pharmacological induction of food intake, while few experiments analyze the patterns of food intake throughout the 24h/day under conditions of free access to food. The second problem concerns the nature of the conditions of food deprivation or restriction, because the incentive value of food varies according to motivational status, such that the underlying neurobiological substrate may be different in free-feeding vs. starved food conditions.[14, 15]

Given that the half-life of naltrexone is 2.7 + 1.0 h González and Brogden, (1988), it has been argued that its effect is more evident during the first hours after administration than later, when the active substance has undergone a metabolic process. With respect to this issue, we have found that while the effect of naltrexone treatment is indeed more evident during the first few hours after injection, it also shows an apparent rebound effect on food intake several hours later in the circadian cycle, and over the long term; i.e., during a course of chronic treatment. Moreover, these changes depend on the precise conditions of food deprivation. Thus, under free-feeding conditions food intake discretely decreases immediately after naltrexone administration. In contrast, under conditions of food deprivation, naltrexone has a clear short-term effect that inhibits deprivation-elicited overfeeding.[17] Hence, the motivational state produced by a pre-starved condition seems to play an important role in the effects of this opioid receptor antagonist.

A question that has required attention is whether alcohol consumption reduces food intake in direct proportion to the caloric content of the alcohol consumed. When alcohol consumption is the only source of fluids, at least three regulatory systems are involved: the fluid regulatory system, caloric regulation, and the ability to metabolize alcohol.[18] Body mass index (BMI) has been positively correlated with alcohol consumption; [19] i.e., as alcohol consumption increases BMI also rises, which supports the finding that alcohol can be a risk factor for obesity. However, other studies have found an inverse relationship between alcohol consumption and BMI; [20,21] that is, as alcohol consumption decreases, BMI increases. These discrepancies may be due to the scarce information concerning the relationship between patterns of alcohol and food consumption gathered on the basis of several days of simultaneous exposure.

Considering that ethanol exposure increases β-endorphin levels, it is possible that the endogenous opioid system might mediate some of the rewarding effects of alcohol. [22] On the other hand, opioids are also implicated in the orosensory reward components of eating. Whether alcohol-induced opioid release modifies subsequent food-induced opioid release is not well understood, but it has been shown that this is an additional, potential neural component of appetite stimulation due to alcohol.[23]

Many studies have focused on examining the effects of naltrexone on either alcohol consumption or food intake, but very few have been designed to analyze changes in both of these stimuli when they are available simultaneously. Moreover, there is no data available from studies of those effects under different conditions of alimentary deprivation. Considering that distinct motivational states for food-seeking may modulate the effects

of the opioid receptor antagonist, the scope of the present study was to assess the effects of naltrexone treatment on the consumption patterns of food, alcohol and water under conditions of free-feeding and food deprivation in rats.

Methods and material

Animals

Wistar male rats were obtained from a colony bred at the Institute of Neuroscience of the University of Guadalajara. Animals were maintained on a reversed 12-12 h light-dark cycle (lights on at 20:00).

General methods

At the age of 70 days, 40 rats were placed in individual cages, and at 75 days they were exposed to the different experimental conditions described in the following sections. Food, water and alcohol consumption were measured daily every 2 for 12 h during the dark phase (08:00 to 20:00) in both experiments 1 and 2, and once at the end of the light phase (20:00 to 08:00) in experiment 1. Body weight was recorded three times a week. All rats were assessed during three periods: a baseline of 7 days; 14 days of naltrexone treatment; and 7 days post-treatment.

The ethics committee of the institution approved the present study, and all procedures involving the animals, were performed in accordance with the Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council, 1996).

Period of Alcohol Induction

During all experimental periods, alcohol consumption was voluntary; that is, 2 bottles were available for 24 (experiment 1) or 12 h (experiment 2): one containing tap water and the other an alcohol solution.

For the purpose of familiarizing the rats with the taste of alcohol, all males were exposed to a solution prepared with tap water and different concentrations of ethanol. Concentrations began at 6% (99.8% Merck) v/v and were increased gradually by 2% every 2 days, until 10% was reached. This concentration was maintained until alcohol consumption became stable.

Experiment 1. Free-feeding condition

After assessment of the baseline period (BL), 20 rats were assigned alternately to two groups of 10 animals each on the basis of their respective amounts of alcohol intake during BL. This procedure was carried out in order to assure that the two groups had similar values for this variable before beginning the pharmacological treatment. During the treatment period, the rats in one group were treated with a single dose of naltrexone (Spectrum®) at 10mg/Kg/day, s.c. for 14 days. The rats in the other group were treated with a saline solution (Ss) at 0.2mL/day, s.c., also for 14 days. In order to analyze changes week-by-week, the 14-day period of naltrexone or saline treatment was divided into two periods of 7 days each (week 1 and week 2).

After the treatment period, the application of naltrexone and saline was interrupted and the different variables were assessed for 7 additional days; the time considered as the post-treatment period (PT). In this experiment, the rats in both groups were exposed continuously (24 h/day) to food, alcohol and water ad libitum during all periods.

The food, alcohol and water consumption corresponding to the 12 h of the light phase were measured daily at 08:00 h and then every 2 h until 20:00 h during the dark phase. At 08:00 h, the amount of food and alcohol consumption corresponding to the previous 12 h of the light phase was ascertained, but the distribution of this consumption during those 12 h could have differed among subjects; therefore, in order to determine food, alcohol and water consumption during the 2 h previous to the daily naltrexone administration, the drug and saline were injected daily at 10:00 h (i.e., 2 h after onset of the dark phase).

Experiment 2: Food-deprived condition

The procedure followed in this experiment was identical to that described for experiment 1, except that food and alcohol were available only for 12 h/day during the dark phase; i.e., from 08:00 to 20:00. To summarize: after a baseline period, 20 rats were assigned to two groups of 10 animals each using the same criteria described for experiment 1. During the treatment period, the animals were treated with either naltrexone at 10mg/Kg/day/rat, s.c., or with a saline solution at 0.2mL/day/rat, s.c., both for 14 days. Finally, a post-treatment period of 7 days without naltrexone or saline was assessed.

At 20:00 on each day, food and alcohol were withdrawn totally and replaced again ad libitum at 08:00 on the next day; i.e., the rats were food- and alcohol-deprived during the light phase of all periods of this experiment, though water was available ad libitum 24h/day throughout the procedure.

In this experiment, the rats were injected with either naltrexone or saline at 08:00, after the 12 h of daily food deprivation and immediately before the daily 12 h of exposure to food and alcohol.

Water consumption corresponding to the 12 h of the light phase was measured daily at 08:00; while food, alcohol and water consumption were all measured every 2 h from 08:00 to 20:00 during the dark phase.

Statistical analyses

Two-way ANOVAs (treatment (naltrexone, saline) X hours (8, 10, 12, 14, 16, 18, 20)) were used to assess the values for food, alcohol and water consumption obtained every 2 h during the 12 h of the dark phase. Two-way ANOVAs (treatment (naltrexone, saline)

X periods (LB, week 1, week 2 and PT)) were performed to assess the body weight and total daily consumption of food, alcohol and water. As mentioned above, the values for food, alcohol and water consumption obtained from 20:00 to 08:00 constituted the accumulated consumption during the 12 h of the light phase, and were analyzed separately using a one-way ANOVA to assess treatment differences among the different periods. A Tukey's test was used for post-hoc comparisons with the level of significance set at $p \leq 0.05$.

Results and discussion

When interaction among the factors proved to be significant, the factor of hours is described in terms of the number of hours that elapsed after the naltrexone or vehicle injection; all exceptions will be clearly indicated.

Experiment 1. Free-feeding Condition

The factor of hours was significant in all experimental periods and in all the variables analyzed (food, alcohol and water intake). In order to facilitate the description of this factor, the statistical data and multiple comparisons that resulted from the post-hoc test are shown in Table 1.

Food intake

The Naltrexone treatment had no effect in any period during free-feeding condition. Interaction among factors (treatment X hours) was significant in week 2 ($F(5,80)=6.27$, $p < 0.0001$), indicating that food intake was higher in the naltrexone group than in the saline group 8 h after injection in week 2 of treatment (Figure 1). Food intake showed a tendency to be lower in the naltrexone group than in the saline group at 2 h after injection in the week 1 of treatment, but the difference was not significant.

Considering that naltrexone has a half-life of 2.7 + 1.0 h,[16] we assumed that a decrease in food intake would be more evident immediately after injecting the opioid receptor antagonist. However, this effect occurred only as a tendency in the 2 h following injection, as food intake increased 8 h after naltrexone administration and significantly only in the second week. These results suggest that in free-feeding conditions, naltrexone seems to increase the rewarding value of food when the action of the drug has supposedly declined due to its metabolization, this supports a previous experiment conducted in the laboratory replicating the same methodology but without alcohol.[17] The explanation of the discrete increase in food intake in the second week of naltrexone treatment –which continued into the post-treatment period– could be structured as follows: it is well known that naltrexone treatment produces opioid receptor up-regulation after several days of continuous infusion, a phenomenon described even when discrete low doses of this drug (1mg/Kg, twice a day) are administered for just 1 week.[24] Therefore, the daily injection of 10 mg/Kg of naltrexone in this study may have produced opioid receptor up-regulation around the beginning of the second week of treatment. If this is the case, then the higher availability of opioid receptors in this period, together with the lower levels of naltrexone at 8 h after injection, may have increased the rewarding effects of food and, as a result, increased food intake.

Table 1. Hour factor results (experiment 1).

Food Intake	Statistics (main factor)	Significant differences among hours (Post-hoc test)
Baseline	($F(5,80)=23.23$, $p< 0.0001$)	8 > 2, 4, 10, 12 6, 12 > 2, 4, 10
Treatment week-1	($F(5,80)=8.73$, $p< 0.0001$)	6, 8 > 2, 4, 10 12 > 2, 4 10 > 4
Treatment week-2 Post-treatment	($F(5,80)=4.13$, $p= 0.0022$) ($F(5,80)=10.35$, $p< 0.0001$)	6, 8 > 4, 10, 12 6 > 2, 8, 10, 12 4 > 10, 12 2, 8 > 12
Alcohol intake		
Baseline	($F(5,80)=4.90$, $p= 0.0006$)	8, 12 > 2, 4 6, 10 > 2
Treatment week-1	($F(5,80)=19.91$, $p< 0.0001$)	8, 12 > 2, 4, 6, 10 6, 10 > 2, 4
Treatment week-2	($F(5,80)=12.90$, $p< 0.0001$)	12 > 2, 4, 6, 10 8 > 2, 4, 6 10 > 2, 4 6 >2
Post-treatment	($F(5,80)=35.88$, $p< 0.0001$)	8, 12 > 2, 4, 6, 10 6, 10 > 2, 4 4 > 2
Water intake		
Baseline	($F(5,80)=31.15$, $p< 0.0001$)	8 > 2, 4, 6, 12 12 > 2, 4, 6 6, 10 > 2, 4 4 > 2
Treatment week-1	($F(5,80)=24.70$, $p< 0.0001$)	8 > 2, 4, 6, 10, 12 12 > 2, 4, 10 6, 10 > 2, 4
Treatment week-2	($F(5,80)=11.02$, $p< 0.0001$)	8 > 2, 4, 6, 10, 12 6 > 2, 4 10, 12 > 4
Post-treatment	($F(5,80)=13$, $p< 0.0001$)	6, 8 > 2, 4, 10, 12 4 >2, 10, 12

Alcohol Consumption

A significant interaction between factors (treatment X hours) was observed in week 2 ($F(5,80)=4.03$, $p= 0.0026$). This result indicated that alcohol consumption was higher in the naltrexone group than in the saline group at 8 h after injection only in week 2 (Figure 2). The factor of treatment was not significant.

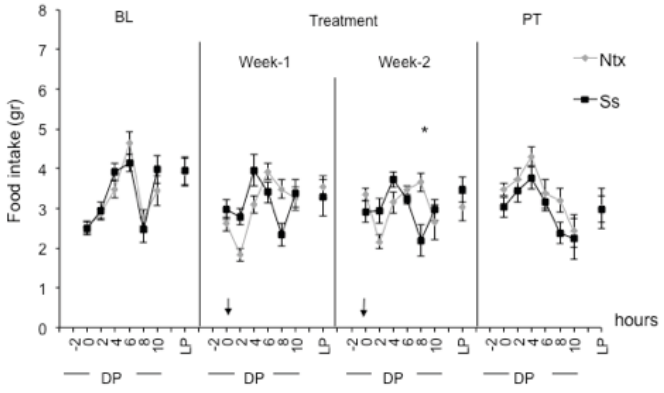

Figure 1. Effect of naltrexone (Ntx) at 10 mg/Kg, or saline (Ss), on food intake patterns (g) from 0 to 10 h following injection during the dark-phase (DP) and in the 12 h of the light-phase (LP) in free-feeding rats (Experiment 1). Arrows indicate the hour at which naltrexone or saline injection was applied. Data show the mean ± S.E.M for the 7 days of each period: baseline (BL), two weeks of naltrexone or saline treatment (week-1, week-2), and post-treatment (PT); significant differences between groups (*).

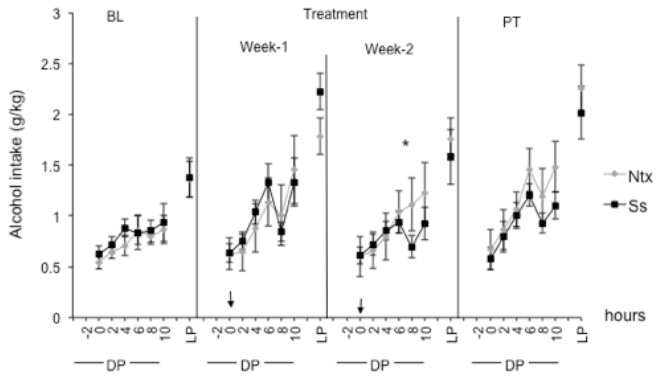

Figure 2. Effect of naltrexone (Ntx) at 10mg/Kg, or saline (Ss), on alcohol intake patterns (g/Kg) from 0 to 10 h following injection during the dark-phase (DP) and in the 12 h of the light-phase (LP) in free-feeding rats (Experiment 1). Arrows indicate the hour at which naltrexone or saline injection was applied. Data show the mean ± S.E.M for the 7 days of each period: baseline (BL), two weeks of naltrexone or saline treatment (week-1, week-2), and post-treatment (PT); significant differences between groups (*).

No significant differences appeared in relation to any of the factors when total daily alcohol intake was analyzed. Similarly, there were no significant differences in total daily alcohol consumption.

Naltrexone did not produce the expected decrease in alcohol consumption that has been documented in several other studies;[7,25-28] however, most such studies offered access to alcohol for only a few hours after naltrexone administration, in contrast to the free-access 24h/day permitted in this experiment.

In our study, the significant increase in alcohol intake 8 h after drug injection coincided with the observed increase in food intake at the same elapsed time. The explanation given for the changes in food intake in terms of possible opioid receptor up-regulation could be applied to changes in alcohol consumption, but we cannot discard the possibility that naltrexone affects only one variable, which in turn affects the other.

Water Consumption

The factor of treatment was not significant, but a significant interaction between factors (treatment X hours) was observed in week 1 ($F(5,80)=2.65$, $p= 0.0289$), and week 2 ($F(5,80)= 6.61$, $p< 0.0001$). This result indicates that water consumption was higher in the saline group than in the naltrexone group at 4 h after injection in week 1 and at 2 and 4 h after injection in week 2 (Figure 3).

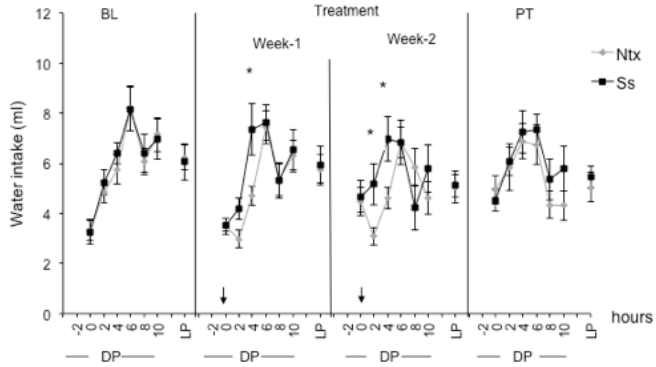

Figure 3. Effect of naltrexone (Ntx) at 10 mg/Kg, or saline (Ss), on water intake patterns (mL) from 0 to 10 h following injection during the dark-phase (DP) and in the 12 h of the light-phase (LP) in free-feeding rats (Experiment 1). Arrows indicate the hour at which naltrexone or saline injection was applied. Data show the mean ± S.E.M for the 7 days of each period: baseline (BL), two weeks of naltrexone or saline treatment (week-1, week-2), and post-treatment (PT); significant differences between groups (*).

There were no significant differences when total daily water intake was analyzed.

Naltrexone decreased water consumption during the 4 hours following injection, but intake then returned to values similar to those observed in the saline group in the remaining hours of the dark phase. These changes in water intake are more consistent with the hypothesis outlined with respect to food intake, suggesting an important participation of the opioid system in regulating the liquid intake mechanisms. Apparently, this effect cannot be generalized to include any liquid, since alcohol was not modified in the same way, and it is possible that this differential effect of naltrexone is related to the palatability characteristics and a preference for one liquid over another, as

described by Williams and Woods (1999).

Body weight

Body weight gain in each period was calculated by subtracting the final body weight obtained in the previous period from the body weight obtained at the end of each subsequent period for each rat. Analysis of this data showed significant interaction ($F(3,48)= 7.15$, $p= 0.0005$) between factors, and indicated a lower body weight gain in the naltrexone group than in the saline group in the first week of treatment. The main factor of treatment and period were not significant. This decrease in the body weight gain of Ntx group coincides with the tendency of the food consumption decrease in the same period for this group; this result agrees with other findings, which support that naltrexone produces a decrement in the body weight.[29,30] A discrete decrease in food intake, affecting body weight, could be due to a decrease in the appetite for, or the rewarding properties of, food intake, during the acute effects of naltrexone, particularly in free-feeding conditions when the caloric demand is not a challenge for animals.

Experiment 2. Food-deprived condition

As occurred in experiment 1, the factor of hours was significant in all experimental periods and for all the variables analyzed. The statistical data and multiple comparisons from the post-hoc test are shown in Table 2.

Food intake

Food intake after 12 h of food- and alcohol-deprivation and after the injection of naltrexone or saline was analyzed. The factor of treatment was significant in week 1 ($F(1,18)= 8.91$, $p= 0.0079$), indicating that food intake was lower in the naltrexone group than in the saline group, regardless of the hour.

Interaction between factors was significant in week 1 ($F(5,90)=7.69$, $p< 0.0001$) and week 2 ($F(5,90)=12.75$, $p< 0.0001$), a finding that indicated that the binge eating observed in both groups immediately after the 12 h of food deprivation in the baseline period was maintained in the saline group, but suppressed in the naltrexone-treated group; i.e., food intake was significantly lower in the naltrexone group than in the saline group at 2 h after injection in both weeks of treatment (Figure 4). This value observed in the saline group was significantly higher than any other value observed in the previous hours in either the naltrexone or saline group during both weeks of treatment. The binge eating that took place in the naltrexone group after deprivation was compensated in the post-treatment period.

Obviously, no analysis of food intake was conducted for the light phase because food was not available during that period. The analysis of total daily food intake showed that only the interaction between factors was significant ($F(3,54)= 4.77$, $p=0.0051$), and indicated higher food intake in the saline group than in the naltrexone group during week 1.

Table 2. Hour factor results (experiment 2).

Food Intake	Statistics (main factor)	Significant differences among hours (Post-hoc test)
Baseline	($F(5,90)=16.27$, $p< 0.0001$)	0 > 2, 4, 6, 8, 10
Treatment week-1	($F(5,90)=20.41$, $p< 0.0001$)	0 > 2, 4, 6, 8, 10 2, 6, 8 > 10
Treatment week-2	($F(5,90)=30.56$, $p< 0.0001$)	0 > 2, 4, 6, 10 8 > 6, 10 2, 4, 6 > 10
Post-treatment	($F(5,90)=30.60$, $p< 0.0001$)	0 > 2, 4, 6, 8, 10 4, 6, 8 > 10
Alcohol intake		
Baseline	($F(5,90)=4.87$, $p= 0.0005$)	8 > 0, 2 4, 6, > 0
Treatment week-1	($F(5,90)=11.97$, $p< 0.0001$)	4, 6, 8 > 0, 2 10 > 0
Treatment week-2	($F(5,90)=21.05$, $p< 0.0001$)	8 > 0, 2, 4, 6, 10 6, 10 > 0, 2 4 > 0
Post-treatment	($F(5,90)=21.06$, $p< 0.0001$)	8 > 0, 2, 4, 10 6 > 0, 2, 10 2, 4, 10 > 0
Water intake		
Baseline	($F(5,90)=2.78$, $p=0.0222$)	2 > 10
Treatment week-1	($F(5,90)=9.23$, $p< 0.0001$)	10 < 0, 2, 4, 6, 8
Treatment week-2	($F(5,90)=16.26$, $p< 0.0001$)	4, 8 > 0, 2, 10 6 > 0, 10 2 > 10
Post-treatment	($F(5,90)=6.24$, $p< 0.0001$)	8 > 0, 10 2, 4, 6 > 10

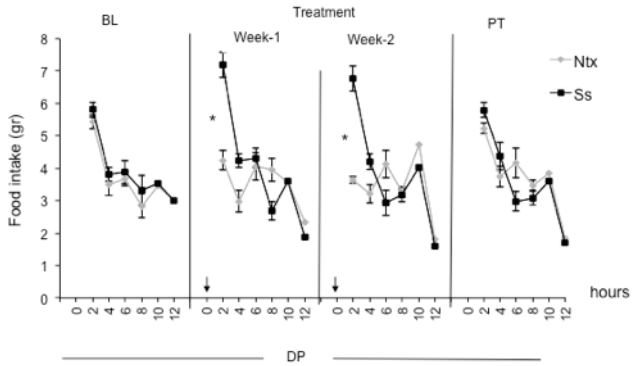

Figure 4. Effect of naltrexone (Ntx) at 10 mg/Kg, or saline (Ss), on food intake patterns (g) from 2 to 12 h following injection during the dark-phase (DP) in food-deprived rats (Experiment 2). Arrows indicate the hour at which naltrexone or saline injection was applied. Data show the mean ± S.E.M for the 7 days of each period: baseline (BL), two weeks of naltrexone or saline treatment (week-1, week-2), and post-treatment (PT); significant differences between groups (*).

Binge eating is a typical behavior that follows a period of food deprivation. Under this condition, naltrexone produced a significant decrease in food intake during the 2 h following injection, indicating a complete inhibition of the binge eating that was observed in the saline group. This effect is consistent with most other studies that have analyzed the effect of naltrexone on food intake in both humans[31,32] and rodents.[33-35] In most studies in rodents, the effects of naltrexone are studied in free-feeding animals,[33-35] and some have involved continuous infusions of the opioid receptor antagonist.[33] In the present experiment, in contrast, only a single dose of naltrexone was given; therefore, the decrease in food intake in only the two initial hours after naltrexone injection may be due to a decrease in the bioavailability of the drug over the course of the day.

In the food deprivation scheme used in this research, rats had the opportunity to compensate the amount of food consumed during the 12 h of the dark phase, the period in which food was available ad libitum. However, the initial decrease in food intake apparently did not produce a compensatory feeding behavior, since no differences were observed between groups in food intake in the subsequent hours. Therefore, the opioid blockage seems to decrease the appetite for, or the rewarding effects of, food intake, regardless of the previous period of deprivation.

Alcohol Consumption

There was no significant difference when alcohol consumption was analyzed, as both groups showed very similar patterns of consumption across all periods (Figure 5); nor was any significant difference found when total daily alcohol intake was analyzed.

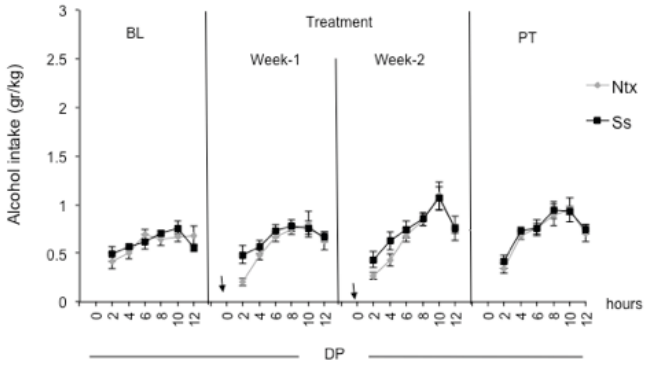

Figure 5. Effect of naltrexone (Ntx) at 10mg/Kg, or saline (Ss), on alcohol intake patterns (g/Kg) from 2 to 12 h following injection during the dark-phase (DP) in food-deprived rats (Experiment 2). Arrows indicate the hour at which naltrexone or saline injection was applied. Data show the mean ± S.E.M for the 7 days of each period: baseline (BL), two weeks of naltrexone or saline treatment (week-1, week-2), and post-treatment (PT); significant differences between groups (*).

Most studies agree that naltrexone decreases alcohol consumption,[7,14,24,27,28] a finding that differs from the results of this study, in which naltrexone did not affect alcohol consumption. We used standard animals in the present study i.e. the initial amount of alcohol consumption was not a criterion of inclusion in the study; therefore, it is possible that alcohol consumption was not a highly significant stimulus compared with food or water intake, at least in this condition of food-deprivation.

Water Consumption

The analysis of the factor of treatment in week 1 indicated that water consumption was significantly higher in saline than naltrexone regardless of the hour ($F(5,90)=7.02$, $p=0.0163$). The ANOVA showed significant interaction between factors in week 1 ($F(5,90)= 4.85$, $p>0.0001$) and week 2 ($F(5,90)=6.44$, $p< 0.0001$). This result indicates that water consumption was lower in naltrexone than saline at 2 and 4 h after injection in both weeks of treatment (Figure 6). Water consumption during the 12 h of the light phase was significantly lower in the naltrexone group than in the saline group during the two weeks of treatment: week 1 ($F(1,18)=7.63$, $p= 0.0128$) and week 2 ($F(1,18)=10.25$, $p=0.0049$).

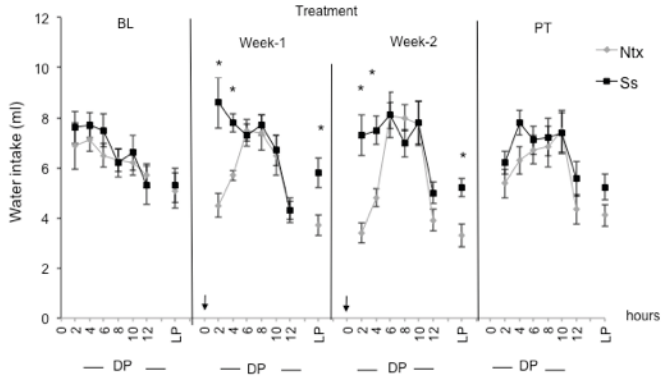

Figure 6. Effect of naltrexone (Ntx) at 10mg/Kg, or saline (Ss), on water intake patterns (mL) from 2 to 12 h following injection during the dark-phase (DP) and in the 12 h of the light-phase (LP) in in food-deprived rats (Experiment 2). Arrows indicate the hour at which naltrexone or saline injection was applied. Data show the mean ± S.E.M for the 7 days of each period: baseline (BL), two weeks of naltrexone or saline treatment (week-1, week-2), and post-treatment (PT); significant differences between groups (*).

Total daily water intake, showed significant differences with respect to the factor of treatment, regardless of period ($F(1,18)=4.51$, $p=0.0477$), and indicated higher water intake in the saline group than in the naltrexone group.

Barrios De Tomasi and Juárez (2011) found that water consumption was lower in the naltrexone group than in the saline group between 2-4 h after injection when a similar procedure

of two weeks of naltrexone treatment was used, but without alcohol exposure. In the present study, naltrexone decreased water consumption significantly during the 4 h following injection in both weeks, contrary to what was observed with respect to alcohol consumption. Therefore, the most relevant effects of Naltrexone in this experiment was observed on water intake, even without any deprivation of this liquid, and in the presence of alcohol consumption, which suggests the participation of opioid activity in the regulation mechanisms of liquid intake as a primary incentive, since consumption of alcohol diluted in water was not affected by naltrexone.

It has been shown that opioid activity modulates the reinforcing qualities of the preferred stimulus;[26] that is, when water is preferred over alcohol, naltrexone will affect water consumption but not alcohol intake, which could explain the present results.

It has been demonstrated that the amount of water intake is related to the amount of food ingested.[36] Although the effect of naltrexone on food consumption followed the same direction as that observed with respect to water consumption, the effect on the latter incentive was more evident, despite the fact that the animals were not water deprived in any way. Though these changes would seem to support an apparent interdependence between the consumption of these two incentives, a direct action of naltrexone on appetitive or consummatory water intake, per se, is suggested. It is noteworthy that the decrease in water consumption was maintained during the light phase, a behavior not observed in either experiment 1, under free-feeding conditions, or in the baseline and post-treatment periods of experiment 2. It seems clear that an interaction between food deprivation and naltrexone's action on the regulation of liquid intake accounts for the observed effects, but at present we have no explanatory rationale for such an effect.

Body weight

Body weight gain showed no any significant difference in experiment 2. Despite the inhibitory effect of naltrexone on food intake, body weight was not affected significantly, however in previous experiments[17] body weight gain showed biphasic changes throughout the study: it was lower in the naltrexone group than in the saline group in the first week of treatment, but was inverted in the post-treatment period; i.e., body weight was higher in the naltrexone group than in the saline group. The increase in body weight gain in the naltrexone group at the end of the study indicated an apparent compensatory effect that came as a response to the initial decrease in the first week of treatment, a result congruent with the absence of differences in total daily food intake between the groups in any period. This result may be due to the fact that body weight is regulated more efficiently than daily food intake, and it is possible that in food-deprived conditions this regulation becomes even more efficient. In this vein, Hadcock and Scott, (2005) found that naltrexone did not affect the body weight of lean rats, a result that would support this notion.

Conclusions

It is important to point out that the main objective of this study was to analyze the consumption patterns of alcohol and primary incentives such as food and water after a single daily dose of naltrexone. The rationale was that the kinetics described for this opioid receptor antagonist could generate a differential action throughout the day, and that food deprivation might be an additional factor in the possible differential effect of the drug.

It is clear that naltrexone decreased food consumption in food-deprived rats, which did not occur under the free-feeding condition. It might be expected that the effects of naltrexone would be less evident during food deprivation due to the nutritional requirements produced by this condition; however, contrary to that rationale, our results suggest that the opioid system could play a more important role in the appetitive mechanisms than in the consummatory mechanisms of feeding behavior.

Naltrexone had no effect on alcohol consumption in either the free-feeding or food deprivation condition; however, alcohol intake was lower during food deprivation. This is not surprising, as the rats showed no preference for alcohol over water, and food deprivation enhanced their seeking for the primary incentive, with the animals spending more time on feeding and water intake than alcohol consumption. It is possible that naltrexone has an evident effect when alcohol is a very relevant stimulus, as indeed occurs in conditions of alcohol dependency, but this problem was not examined in this research.

In general, reports in the literature have focused on the study of naltrexone's effects on food and alcohol consumption as they relate primarily to its underlying rewarding mechanisms. Results of this study give evidence that naltrexone also affects water consumption, apparently regardless of its effect on food intake. However, the effect of naltrexone was differential, as it depended on the different conditions of food deprivation.

References

1. Maldonado R. The endogenous opioid system and drug addiction. Ann Pharm Fr. 2010; 68:3-11.
2. Olive MF, Koenig HN, Nannini MA, Hodge CW. Stimulation of endorphin neurotransmission in the nucleus accumbens by ethanol, cocaine and amphetamine. J Neurosci. 2001; 21(RC184):1–5.
3. Marinelli PW, Quirion R, Gianoulakis CA. Microdialysis profile of β-endorphin and catecholamines in the rat nucleus accumbens following alcohol administration. Psychopharmacology. 2003; 169:60–67.

4. Setiawan E, Pihl RO, Cox SM, Gianoulakis C, Palmour RM, Benkelfat C, Leyton M. The effect of naltrexone on alcohol's stimulant properties and self-administration behavior in social drinkers: influence of gender and genotype. Alcohol Clin Exp Res. 2011; 35(6):1134-41.
5. Davidson D, Palfai T, Bird C, Swift R. Effects of naltrexone on alcohol self-administration in heavy drinkers. Alcohol Clin Exp Res. 1999;23: 195-203.
6. Bienkowski P, Kostowski W, Koros E. Ethanol-reinforced behaviour in the rat: effects of naltrexone. Eur J Pharmacol. 1999; 374:321–327.
7. Coonfield DL, Kiefer SW, Ferraro III FM, Sinclair JD. Ethanol palatability and consumption by high ethanol-drinking rats: manipulation of the opioid system with naltrexone. Behav Neurosci. 2004; 118:1089–1096.
8. Zalewska-Kaszubska J, Gorska D, Dyr W, Czarnecka E. Voluntary alcohol consumption and plasma beta-endorphin levels in alcohol-preferring rats chronically treated with naltrexone. Physiol Behav. 2008; 93:1005-1010.
9. Knuth UA, Friesen HG. Changes of beta-endorphin and somatostatin concentrations in different hypothalamic areas of female rats after chronic starvation. Life Sci. 1983; 33:827-833.
10. Brady LS, Smith MA, Gold PW, Herkenham M. Altered expression of hypothalamic neuropeptide mRNAs in food-restricted and food-deprived rats. Neuroendocrinology. 1990; 52:441-447.
11. Scavo D, Barletta C, Buzzetti R, Vagiri D. Effects of caloric restriction and exercise on b-endorphin, ACTH and cortisol circulating levels in obesity. Physiol Behav. 1988; 42:65-68.
12. Majeed NH, Lason W, Przewlocka B, Przewlocki R. Brain and peripheral opioid peptides after changes in ingestive behavior. Neuroendocrinology. 1986; 42:267-272.
13. Mitev Y, Almeida 0FX, Patchev V. Pituitary-adrenal function and hypothalamic beta-endorphin release in vitro following food deprivation. Brain Res. 1993; 30:7-10.
14. Rudski JM, Billington CJ, Levine AS. Naloxone's effects on operant responding depend upon level of deprivation. Pharmacol Biochem Behav. 1994; 49:377-383.
15. Nader K, van der Kooy D. Deprivation state switches the neurobiological substrates mediating opiate reward in the ventral tegmental area. J Neurosci. 1997; 17:383-390.
16. González JP, Brogden RN. Naltrexone: a review of its pharmacodynamic and pharmacokinetic properties and therapeutic efficacy in the management of opioid dependence. Drugs. 1988; 35:192-213.
17. Barrios De Tomasi E, Juárez J. Differential effects of chronic naltrexone treatment on food intake patterns and body weight in rats depend on their food deprivation status. Eur J Pharmacol. 2011; 650:261-267.
18. Larue-Achagiotis C, Poussard AM, Louis-Sylvestre J. Alcohol drinking, food and fluid intakes and body weight gain in rats. Physiol Behav. 1990;47:545-548.
19. Wannamethee SG, Sharper AG. Alcohol, body weight and body weight gain in middle-aged men. Am J Clin Nutr. 2003; 77:1312-1317.
20. Lahti-Koski M, Pietinen P, Heliovaara M, Vartiainen E. Associations of body mass index and obesity with physical activity, food choices, alcohol intake and smoking in the 1982-1997 FINRISK studies. Am J Clin Nutr. 2002; 75:809-817.
21. Rohrer JE, Rohland BM, Denison A, Way A. Frequency of alcohol use and obesity in community medicine patients. BMC Fam Pract. 2005; 6:17.
22. Gianoulakis C. Endogenous opioids and addiction to alcohol and other drugs of abuse. Curr Top Med Chem. 2004; 4:39–50.
23. Yeomans MR, Caton S, Hetherington MM. Alcohol and food intake. Curr Opi Clin Nut Metab Care. 2003; 6:639-644.
24. Parkes H, Sinclair JD. Reduction of alcohol drinking and upregulation of opioid receptors by oral naltrexone in AA rats. Alcohol. 2000; 21:215-221.
25. Gardell LR, Hubbell CL, Reid LD. Naltrexone persistently reduces rat's intake of a palatable alcoholic beverage. Alcohol Clin Exp Res. 1996; 20:584-588.
26. Williams KL, Woods JH. Naltrexone reduces ethanol and/or water-reinforced responding in rhesus monkeys: effect depends upon ethanol concentration. Alcohol Clin Exp Res. 1999; 23:1462-1467.
27. Stromberg MF, Volpicelli JR, O´Brien CP. Effects of naltrexone administered repeatedly across 30 or 60 days on ethanol consumption using a limited access procedure in the rat. Alcohol Clin Exp Res. 1998; 22:2186-2191.
28. Goodwin FL, Campisi M, Babinska I, Amit Z. Effects of naltrexone on the intake of ethanol and flavored solutions in rats, Alcohol. 2001; 25:9-19.
29. Kotz CM, Grace MK, Briggs JE, Billington CJ, Levine AS. Naltrexone induces arcuate nucleus neuropeptide Y gene expression in the rat. Am J Physiol Regulatory Integrative Comp Physiol. 1996; 271:R289–R29.
30. Marks-Kaufman R, Balmagiya T, Gross E. Modifications in food intake and energy metabolism in rats as a function of chronic naltrexone infusions. Pharmacol Biochem Behav. 1984; 20:911–916.
31. Spiegel TA, Stunkard AJ, Shrager EE, O´Brien CP, Morrison MF, Stellar E. Effect of naltrexone on food intake, hunger and satiety in obese men. Physiol Behav. 1987; 40:135-141.
32. Yeomans MR, Gray RW. Effects of naltrexone on food intake and changes in subjective appetite during eating: evidence for opioid involvement in the appetizer effect. Physiol Behav. 1997; 62:15-21.

33. Lang IM, Strahlendorf JC, Strahlendorf HK, Lutherer LO, Barnes CD. The effects of chronic administration of naltrexone on appetitive and water exchange in rats. Pharmacol Biochem Behav. 1982; 16:909-913.
34. Hobbs DJ, Koch JE, Bodnar RJ. Naltrexone, dopamine receptor agonists and antagonists and food intake in rats: 1. Food deprivation. Pharmacol Biochem Behav. 1994; 49:197-204.
35. MacDonald AF, Billington CJ, Levine AS. Effects of the opioid antagonist naltrexone on feeding induced DAMGO in the ventral tegmental area and in the nucleus accumbens shell region in the rat. Am J Physiol Regul Integrat Comparat Physiol. 2003; 285:R999-R1004.
36. Verplanck WS, Hayes JR. Eating and drinking as a function of maintenance schedule. J Comp Physiol Psychol.1953; 46:327-333.
37. Hadcock JR, Scott DO. Role of opiates and their receptors in the regulation of food intake and body weight. Drug Discove Today. 2005; 2:171-175.