ELSEVIER

Pharmacology, Biochemistry and Behavior 72 (2002) 699–706

PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR

www.elsevier.com/locate/pharmbiochembeh

Naloxone nonselective suppression of drinking of ethanol, sucrose, saccharin, and water by rats

Martha J. Cichelli*, Michael J. Lewis

Temple University, Philadelphia, PA 19122, USA

Received 12 June 2001; received in revised form 7 January 2002; accepted 28 January 2002

Abstract

Naloxone, a nonselective opioid antagonist, has been demonstrated to reduce oral self-administration of ethanol (EtOH) in rats. Conflicting conclusions have been drawn about the effects of naloxone on consumption of non-EtOH control liquids. A preliminary meta-analysis found large and homogeneous effects of naloxone on EtOH consumption and heterogeneous effects on the consumption of control liquids. Although many of the authors concluded that their control liquid results were ''not significant,'' when they were combined using meta-analytic techniques, it was apparent that there were some strong, but widely divergent, effect sizes. In the first experiment in the current study, 60 male Sprague–Dawley rats were trained to drink 10% EtOH in tap water over 3 weeks of limited-access sessions. Then, their limited-access consumption was measured in single-bottle tests of four liquids (water, 10% EtOH in water, an isocaloric sucrose solution, and an ''equally sweet'' saccharin solution) 15 min following an intraperitoneal injection of either saline or 1.0 mg/kg naloxone. Every animal was tested 36 times in a counterbalanced order: three times for each liquid following an injection of naloxone and six times for each liquid following an injection of saline. There were distinct differences in the quantity of each liquid consumed in the saline trials. However, the suppression percentages for each liquid in the naloxone trials were identical ($\sim$50%). There were significant correlations, in the range of .23–.42, between the mean amount of each liquid consumed during saline trials for each animal and the suppression percentage during naloxone trials for the same animal and liquid. When the animals were divided into high, low, and medium drinkers for each liquid, the low drinkers demonstrated a much lower suppression after naloxone treatment than did the other two groups. The data confirm that blockade of opioid receptors suppresses consumption of both EtOH and non-EtOH liquids to a degree that is related to the amount of voluntary, untreated consumption of the liquids.

Keywords: Ethanol; Naloxone; Self-administration; Suppression of drinking

1. Introduction

Many experiments have shown that the nonspecific opioid antagonist, naloxone, markedly reduces self-administration of ethanol (EtOH) in a variety of paradigms using rats. Some of these studies claim that this reduction in consumption is specific to EtOH solutions in that the drug treatment does not significantly reduce consumption of a control liquid (Froehlich et al., 1991; Mehiel, 1996; Myers and Critcher, 1982; Samson and Doyle, 1985). Others claim that the drug treatment reduces consumption of both the EtOH and control solutions; in some studies, the reduction of consumption of the control liquid is statistically significant (Weiss et al., 1990), while in some others it is not (Sandi et al., 1988).

Because the existing animal studies have come to conflicting conclusions on this question, we conducted a meta-analysis of a collection of the rat studies looking at the effects of naloxone on the consumption of both EtOH and control solutions (either water or water sweetened with sucrose or saccharin) to suggest reasons for the different conclusions. We found homogeneous effect sizes for suppression of EtOH consumption but extreme heterogeneity among the control solution effect sizes.

One hypothesis about the variability is suggested in one of the included studies (Pulvirenti and Kastin, 1988). This is that naloxone has a greater inhibiting effect on the water consumption of ''low-alcohol-preferring'' animals than on ''high-alcohol-preferring'' animals. In addition, several authors (Myers and Critcher, 1982; Pulvirenti and Kastin,

* Corresponding author. 404 Fox Road, Nazareth, PA 18064, USA. Tel.: +1-610-837-5240; fax: +1-610-746-7900.

E-mail address: mcichelli@rcn.com (M.J. Cichelli).

0091-3057/02/$ – see front matter
PII: S0091-3057(02)00736-0

1988; Weiss et al., 1990) suggest that naloxone has a greater inhibiting effect on the EtOH consumption of "high-alcohol-preferring" animals. Both of these hypotheses were supported by our meta-analysis, although there were not enough data points to draw definitive conclusions.

Potentially confounding variables in the studies in the meta-analysis were the calorie content and the sweetness of the control liquid. There were not enough data points to permit a meaningful analysis of the effects of these variables. Therefore, we designed the following experiment to provide an extensive test of the effects of naloxone on the consumption of four test liquids: (a) 10% EtOH in water, (b) an isocaloric solution of sucrose and water, (c) an "equally sweet" solution of saccharin in water, and (d) plain tap water.

A naloxone dose of 1.0 mg/kg was selected to represent a moderate dose. It was both the median and the modal dose used in the subset of the rat studies we examined that were used to represent the limited-access EtOH drinking studies.

One of our hypotheses, based on the meta-analysis, was that naloxone decreases many kinds of consummatory behavior. Some of the prior studies failed to find significant decreases in the consumption of the control liquids due primarily to insufficient statistical power. To avoid this problem, we conducted a power analysis that suggested a much larger sample size ($n = 60$) than was typical in the prior studies. In addition, each animal served as its own control by being tested multiple times while consuming each of the four test liquids both under the influence of naloxone and not.

Many studies are concerned with the issue of drinking preference rather than with the quantity consumed. These studies present two competing liquids to the animals. The preferred liquid is, by definition, the one that is consumed in greater quantity. However, when a sweetened liquid is presented in competition with an unsweetened one, often so little of the unsweetened one is consumed under baseline conditions that there is no room for analysis of suppression effects. Even some high-alcohol-drinking rats (HAD rats), which will consume more EtOH solution than plain water in a two-bottle choice test, will dramatically reduce their EtOH consumption when they are simultaneously given a sweet alternative (Lankford and Myers, 1994). To counter this problem, we presented each of the test liquids individually (i.e., in a one-bottle test) and repeatedly to each of the animals. This procedure led to measurable consumption of all of the liquids and allowed the suppression of consumption following pretreatment with naloxone to become visible.

Our primary hypotheses were:

(a) the consumption-suppressing effects of naloxone are not unique to EtOH, and they will be evident for all the control liquids and
(b) there will be a greater percentage suppression of consumption for the high-drinking animals of each liquid than for the low-drinking animals.

Two additional hypotheses followed from these major ones. Given that the animals were expected to prefer sweetened liquids to nonsweetened ones and to prefer caloric liquids to noncaloric ones,

(a) there will be a greater suppression of consumption of caloric liquids (EtOH and sucrose solutions) than of noncaloric liquids (saccharin solutions and plain water) and
(b) there will be a greater suppression of consumption of sweet liquids (sucrose and saccharin solutions) than of nonsweet liquids (EtOH solutions and plain water).

2. Methods

2.1. Subjects

Sixty male Sprague–Dawley rats were purchased from Charles River Laboratories. They weighed between 250 and 330 g upon arrival and between 450 and 760 g by the end of the experiments. They were individually housed and maintained on a reversed 12-h light/dark cycle with lights off at 10:45 a.m. Rat chow was available ad libitum; water was available ad libitum except during the daily limited-access sessions described below. The experimental protocol was approved by the Temple University Institutional Animal Care and Use Committee.

2.2. Drugs

Naloxone hydrochloride, purchased from Sigma, was dissolved in isotonic saline daily to a concentration of 1 mg/ml. EtOH solutions were made from 100% EtOH and tap water by volume. Sucrose solutions were made from table sugar and tap water. Saccharin solutions were made from Sweet 'N Low artificial sweetener and tap water. (See Appendix A for the calculations of the proportions used.)

2.3. The limited-access drinking paradigm

All of the animals were trained to drink gradually increasing solutions of EtOH in water via 3 weeks of a limited-access paradigm (MacDonall and Marcucella, 1979; Marcucella and Munro, 1987). Each weekday (Monday–Friday), the normal water bottles were removed at approximately 11:00 a.m. (15 min after the start of the dark cycle) and a single bottle of EtOH and water was placed on the front of the cage for 30 min. At the end of this period, the EtOH bottle was removed and the normal water bottle was returned. The EtOH bottle was weighed before and after the drinking session to determine the amount consumed. The initial concentration of EtOH was 2% (v/v) and it was gradually increased to 10% on the following schedule: 3 days at 2%, 2 days at 3%, a 2-day break, 2 days at 4%, 1 day at 5%, 2 days at 6%, a 2-day

break, 1 day at 7%, 1 day at 8%, 1 day at 9%, 2 days at 10% for a total of 15 exposure days.

Next, the animals were exposed to (a) 2 days of limited access to a sucrose and water solution that was isocaloric to the 10% EtOH solution, (b) 2 days of a saccharin and water solution that was equal in sweetness (according to the manufacturer of Sweet 'N Low) to the sucrose solution, and (c) one more day of a 10% EtOH solution. For each of these 5 days, the animals received an injection of saline (0.5 ml) just before the limited-access session to acclimate them to the injection process.

The drinking data gathered during these first 4 weeks were not used as baseline data, although they were examined to determine that all of the animals had sampled all of the solutions and that, for each solution, at least some of the animals drank substantial amounts of it.

2.4. Procedures

Over an elapsed time of 16 weeks, each animal was tested a total of 36 times (12 weeks, three times per week). The tests were conducted on Monday, Wednesday, and Friday, and they followed the pattern established during the baseline/training period.

Each test day, all the normal water bottles were removed at approximately 11:00 a.m. (15 min after the start of the

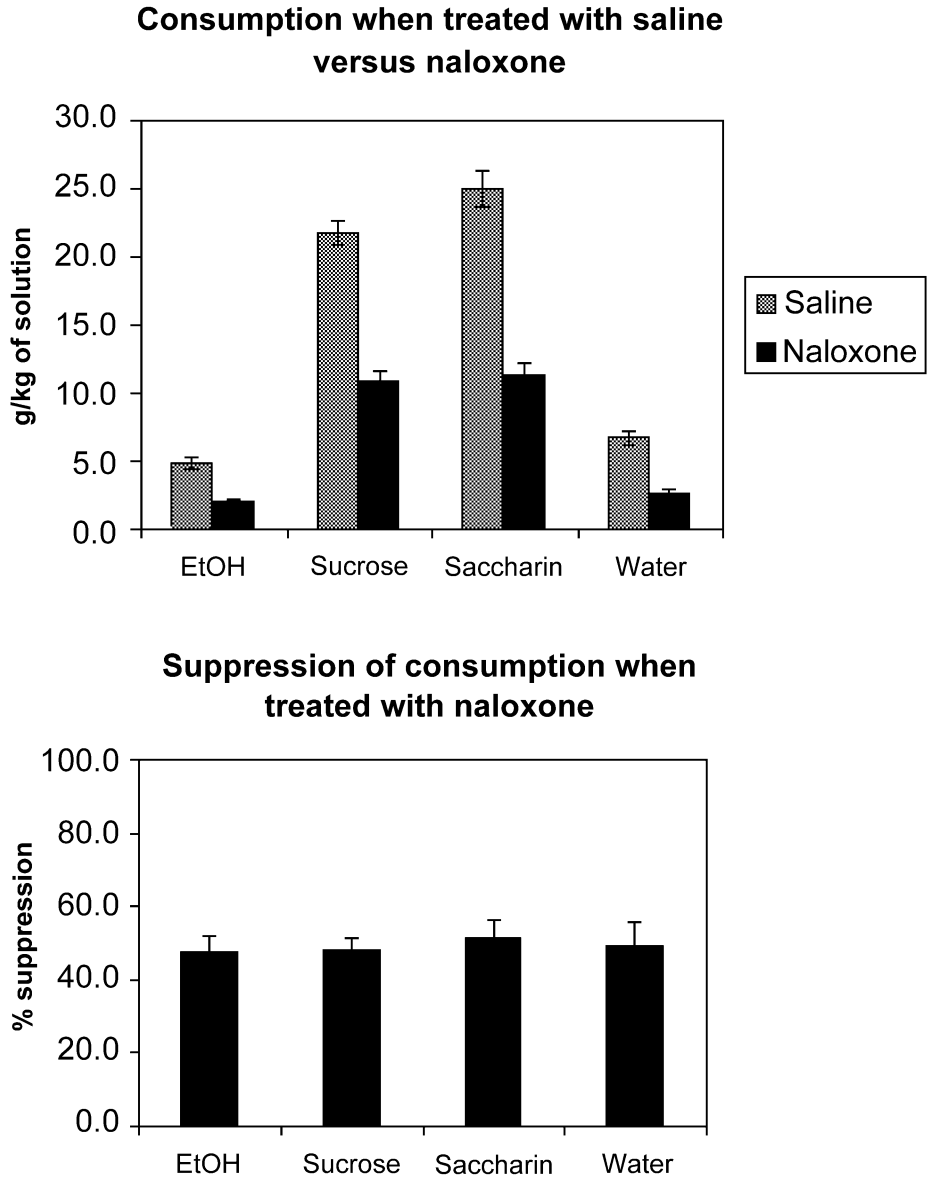

Fig. 1. Top: Mean consumption figures, in grams of solution per kilogram of body weight during saline trials and during naloxone trials for each of the four liquids tested. Bottom: Mean percentage suppression during naloxone trials for each of the four liquids. Note that these are the means of individual suppression percentages computed separately for each animal.

dark cycle). Rat chow remained available at all times. Each animal then received an injection of either saline (0.5 ml) or naloxone (1 mg/ml/kg). Fifteen minutes after each animal's injection (giving enough time for the animal to recover from any trauma due to the injection while still leaving a sufficiently long window for the naloxone to be active during the entire test), a single bottle of a test solution was placed on the front of the cage for 30 min. At the end of the 30 min, the test bottle was removed and the normal water bottle was returned. The test bottle was weighed before and after the drinking session to determine the amount consumed.

Over the duration of the experiment, for each of the four test liquids, each animal was tested three times following a naloxone injection (once on a Monday, once on a Wednesday, and once on a Friday) and six times following a saline injection (on two Mondays, two Wednesdays, and two Fridays). The order of these 36 tests for each animal was randomized across the 60 animals to minimize any order effects of either test solution or drug presentation.

3. Results

The mean consumption figures in the saline condition and in the naloxone condition, for the four substances, are shown in the top graph of Fig. 1 and in the second line of Table 1. For each liquid, there is a significant difference between the saline condition consumption and the naloxone condition consumption.

Much larger quantities of sucrose and of saccharin were consumed than of either water or EtOH. However, when percentage suppressions during the naloxone trials are compared, there is no difference between them for the four liquids, as shown in the bottom graph of Fig. 1 and in Table 1. An ANOVA of the four suppression percentages for each of the 60 animals found no effect [$F(3,236) = 0.10$, $P = .958$].

Table 1 summarizes the consumption figures and the results of several analyses for all four substances. To make a comparison of the consumption figures for all the liquids easier, they are all reported in grams of the solution per kilogram of animal body weight. The table also indicates the factor by which these figures should be multiplied to report grams per kilogram of the pure substance (EtOH, sucrose, or saccharin).

For each animal, a suppression percentage was calculated using that animal's mean consumption during saline trials as the denominator and the difference between the animal's mean consumption during saline trials and its mean consumption during naloxone trials as the numerator. The mean of these suppression percentages is reported in Table 1 for each liquid.

3.1. Analysis by drinking level

There were positive correlations, for each liquid, between consumption during saline trials and the percent suppression during naloxone trials. These correlations are shown in the first line of Table 2.

To pursue this relationship, separately for each liquid, the animals were divided for analysis into three categories based on their mean consumption during saline trials: "high drinkers" with a mean consumption in the saline trials greater than or equal to the group mean plus one standard deviation, "low drinkers" with a mean consumption in the saline trials less than or equal to the group mean minus one standard deviation, and "medium drinkers" with mean consumption figures in between these extremes.

Table 2 includes a summary of the analysis of the drinking patterns for these groups. It includes the criteria for "high" and "low" drinking for each liquid and the number of animals that fell into each category as well as the mean consumption during saline trials and during naloxone trials and the mean percentage suppression for each category.

For EtOH and for water, linear contrasts, hypothesizing that the percent suppression during naloxone trials is lower

Table 1
Mean consumption figures for all four liquids

	EtOH	Sucrose	Saccharin	Water
Multiply by this factor to convert consumption to grams per kilogram of pure substance	0.10	0.1525	0.0006	1
Mean consumption during saline trials (g/kg)	4.8	21.7	25.0	6.7
n	60	60	60	60
(S.E.M.)	(0.62)	(0.88)	(1.33)	(0.45)
Mean consumption during naloxone trials (g/kg)	2.1	10.8	11.3	2.7
n	60	60	60	60
(S.E.M.)	(0.19)	(0.76)	(0.90)	(0.22)
Significance of difference paired *t* test (*P*)	8.8×10^{-14}	5.43×10^{-18}	3.8×10^{-21}	2.0×10^{-15}
One-tailed effect size *r*	.78	.85	.88	.81
Mean percentage suppression (%)	47.5	48.1	51.2	49.4
n	60	60	60	60
(S.E.M.)	(4.40)	(3.50)	(5.00)	(6.56)

Table 2
Analysis by drinking level

	EtOH	Sucrose	Saccharin	Water
Correlation (r) of consumption during saline trials to percentage suppression during naloxone trials	.42	.25	023	.39
P value	.0007	.0577	.0801	.0019
Criterion for "high" drinking (g/kg)	≥ 8.0	≥ 28.5	≥ 35.3	≥ 10.2
Mean consumption of high drinkers during saline trials (g/kg)	9.4	32.6	44.5	11.8
n	12	9	5	12
(S.E.M.)	(0.47)	(1.37)	(1.07)	(0.51)
Mean consumption of high drinkers during naloxone trials (g/kg)	3.8	13.6	22.7	4.2
n	12	9	5	12
(S.E.M.)	(0.32)	(1.73)	(3.01)	(0.47)
Mean percentage suppression of high drinkers (%)	59.1	58.5	48.8	64
n	12	9	5	12
(S.E.M.)	(3.74)	(5.01)	(6.63)	(3.91)
Mean consumption of medium drinkers during saline trials (g/kg)	4.8	21.5	25.8	6.3
n	33	43	47	39
(S.E.M.)	(0.30)	(0.56)	(0.90)	(0.29)
Mean consumption of medium drinkers during naloxone trials (g/kg)	2.0	11.2	11.4	2.6
n	33	43	47	39
(S.E.M.)	(0.23)	(0.89)	(0.83)	(0.25)
Mean percentage suppression of medium drinkers (%)	60.2	47.3	55.7	56.2
n	33	43	47	39
(S.E.M.)	(3.34)	(4.03)	(3.15)	(4.73)
Criterion for "low" drinking (g/kg)	≤ 1.7	≤ 14.9	≤ 14.7	≤ 3.2
Mean consumption of low drinkers during saline trials (g/kg)	35.62	11.0	7.6	1.7
n	15	8	8	9
(S.E.M.)	(0.11)	(1.25)	(1.39)	(0.33)
Mean consumption of low drinkers during naloxone trials (g/kg)	0.9	5.7	3.8	35.62
n	15	8	8	9
(S.E.M.)	(0.13)	(1.28)	(1.36)	(0.18)
Mean percentage suppression of low drinkers (%)	10.4	40.7	25.9	0.5
n	15	8	8	9
(S.E.M.)	(11.43)	(13.82)	(32.87)	(35.62)
Significant linear contrast of percentage suppression by drinking levels				
F	35.35	no significant l inear contrast	no significant l inear contrast	4.7
P	1.76×10^{-7}			.0006
Lambda weights	1, 1, −2			1, 1, −2
Effect size r	.62			.41

for the "low-drinking" animals than for the other two groups, were significant. For sucrose and saccharin, although this same pattern was seen, the linear contrasts were not significant. For each liquid, there were a few animals demonstrating negative suppression (i.e., they drank more in the naloxone trials than in the saline trials). These animals were always "low" drinkers or some of the lowest of the "medium" drinkers.

3.2. Comparisons by animal across the four liquids

To investigate whether the same animals were the high drinkers of each liquid, correlations between the mean consumption amounts during saline trials across animals for each pair of liquids were computed. These correlations and their significance values are listed in Table 3.

There are significant correlations between all of the liquids with the highest ($r = .54$) between EtOH consumption and saccharin consumption. The lowest ($r = .32$) is between EtOH consumption and sucrose consumption.

Table 3
Correlations between the mean consumption amounts during saline trials across animals

	EtOH	Sucrose	Saccharin
Sucrose			
r	.32		
P	.0018		
Saccharin			
r	.54	.42	
P	7.0082×10^{-6}	.0008	
Water			
r	.37	.30	.46
P	.0033	.0195	.0002

Correlations between the mean percent suppression values during naloxone trials across animals for each pair of liquids were also computed. These correlations and the significance values are listed in Table 4.

The only significant correlations were between sucrose suppression and saccharin suppression and between sucrose suppression and water suppression (both correlations were the same, r=.26).

3.3. Analysis of order effects

For each liquid separately, an ANOVA was performed for the six saline condition consumption figures for all 60 animals and also for the three naloxone condition consumption figures. Table 5 reports the mean consumption figures for each saline trial and for each naloxone trial in order for each animal. (Remember that the trials for any given liquid were intermingled with trials for other liquids and the saline and naloxone trials were in a randomized order. This table reports the results of the saline trials and then the naloxone trials as though there were no other intervening trials for each animal.)

The results of the ANOVAs are shown in Table 5.

No effect was found across the six saline trials for EtOH, saccharin, or water. There was a significant effect found for sucrose, but there is no obvious interpretation. Consumption in Saline Trial 2 was higher than the others and consumption in Trial 6 was lower than the others.

An effect is evident across the three naloxone trials for each liquid. Consumption was highest in the first trial and lowest in the third trial.

Each animal was tested 12 times with naloxone (three times with each of the four liquids in a randomized order). We cannot compare actual consumption figures between the different liquids across the 12 trials, but we can compare percent suppression figures. For each animal, a percent suppression figure was computed for each naloxone trial in order equal to the difference between the mean saline trial consumption for this animal for this liquid and the particular naloxone trial consumption divided by the mean saline trial consumption for the animal for the liquid. The mean percent suppressions for each of the 12 trials are shown in Table 6. A linear contrast, hypothesizing an ever-increasing suppres-

Table 5
Analysis of order effects within each liquid

	EtOH	Sucrose	Saccharin	Water
Mean consumption (g/kg)				
Saline Trial 1	4.8	22.9	21.0	7.2
(S.E.M.)	(0.48)	(1.60)	(1.81)	(0.74)
Saline Trial 2	4.8	27.1	27.2	7.3
(S.E.M.)	(0.57)	(1.48)	(2.11)	(0.73)
Saline Trial 3	4.2	21.5	24.8	5.8
(S.E.M.)	(0.49)	(1.65)	(2.37)	(0.66)
Saline Trial 4	4.7	20.8	24.4	6.4
(S.E.M.)	(0.53)	(1.65)	(1.92)	(0.85)
Saline Trial 5	5.3	21.1	25.9	6.8
(S.E.M.)	(0.56)	(1.36)	(2.18)	(0.61)
Saline Trial 6	5.1	17.0	26.4	6.7
(S.E.M.)	(0.55)	(1.40)	(2.18)	(0.67)
ANOVA of six saline trials				
F	1.01	5.83	1.49	0.88
P	.413	3.71×10^{-5}	.193	.496
	no effect	see text	no effect	no effect
Mean consumption (g/kg)				
Naloxone Trial 1	2.6	12.9	11.7	3.1
(S.E.M.)	(0.30)	(1.13)	(1.15)	(0.33)
Naloxone Trial 2	1.9	10.9	13.3	2.9
(S.E.M.)	(0.26)	(1.05)	(1.54)	(0.39)
Naloxone Trial 3	1.8	8.7	8.8	2.2
(S.E.M.)	(0.21)	(0.79)	(0.96)	(0.28)
ANOVA of three naloxone trials				
F	4.18	6.96	4.76	2.48
P	.018	.001	.01	.088
Significant linear contrast				
F	8.13	13.92	8.38	4.70
P	.006	.0004	.005	.034
Lambda weights	+1, −0.5, −0.5	+1, 0, −1	0.5, 0.5, −1	0.5, 0.5, −1
Effect size r	.35	.44	.36	.28

Table 4
Correlations between the mean percent suppression values during naloxone trials across animals

	EtOH	Sucrose	Saccharin
Sucrose			
r	.16		
P	.23, n.s.		
Saccharin			
r	.12	.26	
P	.35, n.s.	.05	
Water			
r	.02	.26	.05
P	.90, n.s.	.05	.72, n.s.

Table 6
Analysis of order effects across all four liquids

Trial number	Mean % suppression	S.E.M.
1	14.3	15.9
2	21.9	11.0
3	29.5	9.2
4	43.1	7.7
5	48.6	6.0
6	47.2	4.2
7	64.0	4.6
8	57.9	4.3
9	68.6	3.3
10	61.1	3.7
11	57.4	4.2
12	67.5	4.4

sion across the trials, was significant [one-tailed t test, $t(708) = 3.00$, $P = .001$].

4. Discussion

Our major hypothesis was that the consumption-suppressing effects of naloxone are not unique to EtOH, and that they would be evident for all the control liquids. The data from this experiment support this hypothesis unequivocally. This is consistent with findings from the feeding literature (Brown and Holtzman, 1979; Cooper and Kirkham, 1990; Mitchell et al., 1986) and should not be surprising. This lends support to the idea that naloxone may act to suppress EtOH consumption, not by interfering with any aspect of the intoxicating effects of EtOH (e.g., the euphoria or ''high''), but by interfering with consummatory behavior in general.

We also hypothesized that there would be a greater suppression of caloric liquids (EtOH and sucrose solutions) than of noncaloric liquids (saccharin solutions and plain water). This was not supported by the data. The percentage suppression of all four liquids was the same. These results also contradicted the hypothesis that there would be a greater suppression of sweet liquids (sucrose and saccharin solutions) than of nonsweet liquids (EtOH solutions and plain water). The animals definitely consumed larger quantities of the sweet liquids than of the nonsweet ones in both the saline and naloxone test conditions, but percentage suppression (of the entire sample of animals as a whole) during the naloxone sessions was not affected by sweetness.

Our final hypothesis for this experiment was that there would be a greater suppression of each substance for the high-drinking animals of that substance than for the low-drinking animals. The current experiment demonstrated that, for each of the test liquids, the high-drinking animals for that liquid were affected differently by naloxone pretreatment than were the low-drinking animals. For EtOH and for water, the greatest percentage suppression was seen in the high-drinking and/or medium-drinking animals. For sucrose and for saccharin, this same trend was seen although it was not statistically significant. The mean percentage suppression was the lowest for the low-drinking animals for each liquid (this difference was statistically significant only for EtOH and for water). The variability in suppression was also the greatest for the low drinkers with some animals demonstrating negative suppression (i.e., they drank more of the liquid following naloxone pretreatment than in the saline test condition). This is a paradoxical finding that also has some support in the feeding literature (Giraudo et al., 1993). It is not clear whether this is a true effect, or a result of a combination of a floor effect (due to low consumption of the food or liquid) and inaccuracies of measurement of these low consumption amounts. Further research, perhaps using more accurate measuring equipment such as lickometers, might resolve the issue.

It was not the case that the same animals were the highest drinkers for all four liquids. Nevertheless, there were significant moderate correlations between the saline trial consumption figures for the four liquids. The highest correlation was found between EtOH drinking and saccharin drinking. This relationship has been described before (e.g., Gahtan et al., 1996), but we cannot offer an explanation for it. The second highest correlation was found between saccharin drinking and water drinking. There are many unanswered questions about these relationships.

When all the animals and all the trials were analyzed statistically, the suppressive effects of naloxone pretreatment increased across repeated naloxone trials. This could suggest sensitization to the effects of naloxone or it could be the result of learning by the animals. There were many examples, in the detailed data for individual rats, that did not follow this precise pattern. This is a case where we apparently do not have all of the variables identified and controlled (as evidenced by the large amount of variability). This study attempted to minimize these unknown order effects by randomizing the order of the trials. Additional research, specifically addressing the nature of these variables, is obviously needed.

In summary, the suppressive effects of naloxone are general (i.e., nonselective with regard to the liquids tested, sweetness, or calories) and somewhat selective between high and low consumers of each liquid.

Appendix A. Formulae for test solutions

EtOH:

1 l of 10% EtOH (v/v) = 100 ml of 100% EtOH + 900 ml of tap water;

100 ml of EtOH contains approximately 100 g of EtOH;

100 g of EtOH contains 590 kcal.

Sucrose:

100 g of granulated table sugar contains 387 kcal;

590 kcal → 152.5 g of granulated sugar;

1 l of solution = 152.5 g of sugar + water to make a liter;

concentration = 152.5 g/1000 ml = 15.25% (w/v).

Saccharin:

one packet of Sweet 'N Low granulated sugar substitute weighs 1 g and contains 3.6% (34 mg) calcium saccharin;

the manufacturer claims that ''1 packet contains the sweetness of 2 teaspoons of sugar'';

the sweetness of 152.5 g (or 36.3 teaspoons) of granulated sugar = 18.15 packets of Sweet 'N Low;

1 l of solution = 18 packets (18 g) of Sweet 'N Low + water to make a liter;

saccharin in 1 l of solution=$(18 \times 34$ mg$) = 612$ mg = 0.612 g;

concentration = 0.612 g/1000 ml = 0.06% (w/v).

References

Brown DR, Holtzman SG. Suppression of deprivation-induced food and water intake in rats and mice by naloxone. Pharmacol, Biochem Behav 1979;11:567–73.

Cooper SJ, Kirkham TC. Basic mechanisms of opioids' effects on eating and drinking. In: Reid LD, editor. Opioids, bulimia and alcohol abuse and alcoholism. New York: Springer-Verlag, 1990. pp. 91–110.

Froehlich JC, Zweifel M, Harts J, Lumeng L, Li T-K. Importance of delta opioid receptors in maintaining high alcohol drinking. Psychopharmacology 1991;103:467–72.

Gahtan E, Labounty LP, Wyvell C, Carroll ME. The relationships among saccharin consumption, oral ethanol, and IV cocaine self-administration. Pharmacol, Biochem Behav 1996;53(4):919–25.

Giraudo SQ, Grace M, Welch C, Billington C, Levine A. Naloxone's anorectic effect is dependent upon the relative palatability of food. Pharmacol, Biochem Behav 1993;46:917–21.

Lankford MF, Myers RD. Genetics of alcoholism: simultaneous presentation of a chocolate drink diminishes alcohol preference in high drinking HAD rats. Pharmacol, Biochem Behav 1994;49(2):417–25.

MacDonall JS, Marcucella H. Increasing the rate of ethanol consumption in food- and water-satiated rats. Pharmacol, Biochem Behav 1979;10(2): 211–6.

Marcucella H, Munro I. Ethanol consumption of free feeding animals during restricted ethanol access. Alcohol Drug Res 1987;7(5–6):405–14.

Mehiel R. The effects of naloxone on flavor–calorie preference learning indicate involvement of opioid reward systems. Psychol Rec 1996;46: 435–50.

Mitchell JE, Laine DE, Morley JE, Levine AS. Naloxone but not CCK-8 may attenuate binge-eating behavior in patients with the bulimia syndrome. Biol Psychiatry 1986;21:1399–406.

Myers RD, Critcher EC. Naloxone alters alcohol drinking induced in the rat by tetrahydropapaverolilne (THP) infused ICV. Pharmacol, Biochem Behav 1982;16:827–36.

Pulvirenti L, Kastin AJ. Naloxone, but not Tyr-MIF-1, reduces volitional ethanol drinking in rats: correlation with degree of spontaneous preference. Pharmacol, Biochem Behav 1988;31:120–34.

Samson HH, Doyle TF. Oral ethanol self-administration in the rat: effect of naloxone. Pharmacol, Biochem Behav 1985;22:91–9.

Sandi C, Borrell J, Guaza C. Naloxone decreases ethanol consumption within a free choice paradigm in rats. Pharmacol, Biochem Behav 1988;29:39–43.

Weiss F, Mitchiner M, Bloom FE, Koob GF. Free-choice responding for ethanol versus water in alcohol preferring (P) and unselected Wistar rats is differentially modified by naloxone, bromocriptine, and methysergide. Psychopharmacology 1990;101:178–86.