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Combined Naloxone and Fluoxetine on Deprivation-Induced Binge Eating of Palatable Foods in Rats

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HAGAN, M. M., F. D. HOLGUIN, C. E. CABELLO, D. R. HANSCOM, AND D. E. MOSS. *Combined naloxone and fluoxetine on deprivation-induced binge eating of palatable foods in rats.* PHARMACOL BIOCHEM BEHAV **58**(4) 1103–1107, 1997.—Opioid antagonism and serotonergic stimulation is associated with macronutrient-specific hypophagia in animals. In the present study we evaluated their systemic effect alone, and in combination, at various doses, on the intake of sweet carbohydrate-rich and sweet fat-rich foods, tastes, and nutrients that are typical of binge-food items. Low-dose (1 mg/kg) naloxone, alone, preferentially suppressed fat-rich intake while low-dose (2.5 mg/kg) fluoxetine, alone, preferentially suppressed carbohydrate-rich intake. Each drug at these doses, combined with various doses of the other (2.5–10 mg/kg fluoxetine; 0.01-1 mg/kg naloxone) additively suppressed both kinds of the sweet foods. Naloxone and fluoxetine have therapeutic potential in treating binge-eating disorders. This animal study suggests what shortcomings and benefits might be expected when combining these two agents. © 1997 Elsevier Science Inc.

Naloxone Fluoxetine Carbohydrate Fat Palatable food Rats Binge eating Bulimia Hyperphagia Food intake Animal model

NALOXONE (NAL), an opioid antagonist, and fluoxetine (FLU), a serotonin-selective reuptake inhibitor, have each been shown to reduce spontaneous and deprivation-induced food intake (5,26,34) as well as the powerful feeding response to peptide YY (13) in animals. The nature of an interaction between these two systems, if any, remains unclear but there is evidence that an interaction is likely. For example, Fernandez-Tome and Del Rio (7) have shown that a dose of NAL (2 mg/kg), which had no effect by itself, reduced deprivation-induced chow intake when combined with a dose of 5-HTP that also had no effect by itself. In addition, Beczkowska and Bodnar (2) found that serotonin agonists can either potentiate or attenuate the anorectic effect of NAL, depending upon the 5-HT receptors being activated. NAL and FLU have also been tested separately in patients with bulimia nervosa and binge-eating disorder (a binge-eating syndrome not accompanied by compensatory purging against weight-gain (1,22,23,31). Despite the well-known anorexic effects of these two drugs, their combined pharmacological effect has not been tested in animals or humans. In light of well-established opioid and serotonergic effects on food intake, as well as the existence of opioid and serotonin receptors at common brain sites (12,28,33), combinations of naloxone and fluoxetine may exert an interactive suppression on food intake.

Therefore, the purpose of this study was to assess the efficacy of a NAL–FLU combination on deprivation-induced binge eating in rats. In Experiment 1, various doses of FLU were tested alone and in combination with a moderate but intake-suppressing dose of NAL. In Experiment 2, a low but effective dose of FLU was tested alone and in combination with various doses of NAL. The outcome was measured by intake of palatable carbohydrate-rich and fat-rich foods.

METHOD

Animals

Forty-eight male Sprague–Dawley rats (weighing 340–440 g and 370–460 g at the onset of Experiments 1 and 2, respec-

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tively), were kept under standard light conditions (12 L:12 D cycle, light on at 0700 h) with ad lib Purina rat chow and water in group cages. Periodically, the animals were given the palatable foods to overcome xenophobia prior to the feeding tests.

Drugs

Naloxone hydrochloride (Sigma Chemical Co., St. Louis, MO) was injected SC in a vehicle (VEH) of physiological saline. Fluoxetine hydrochloride (Eli Lilly Co., Indianapolis, IN) was injected IP in a VEH of deionized water. Sham injections consisted of corresponding VEHs.

Food

Kellogg's Froot Loop cereal (4.0 kcal/g; 88% of kcals from carbohydrate, 3% from fat, and 6% from protein) was given as the palatable carbohydrate-rich item. Mars Almond M&Ms chocolate candy (5.48 kcal/g; 57% of kcals from carbohydrate, 30% from fat, and 8% from protein) was used as the palatable fat-rich item. These items did not represent isolated macronutrients (e.g., the chocolate candy contained a considerable amount of carbohydrate in addition to fat). Instead, these items were chosen to represent mixtures of macronutrients more commonly encountered by humans. For example, rarely is fat eaten in its purest form. Instead, most highly palatable sweet items have a carbohydrate base with some items containing considerably more fat than others. Therefore, although no strict macronutrient comparisons can be made, differences due to food type could, in part, be attributed to differences in fat content. During eating tests only the cereal and chocolate items (no rat chow) were available along with ad lib water.

Procedures

Experiment 1. The animals were deprived for 24 h before testing to induce feeding during the light phase (1100–1300 h). All animals were initially randomly assigned to be pretreated with either 1 mg/kg NAL or VEH followed 10 min later by an injection of one of four doses of FLU (0, 2.5, 5, and 10 mg/kg; $n = 12$ per FLU dose condition) in a randomized counterbalanced design with rats weight matched into each of the four groups. After an additional 20 min, the animals were presented with a plentiful, premeasured amount of chocolate and cereal. Intake was measured after 30 min and 2 h of feeding. Immediately after all animals were tested, the procedure was repeated only this time animals that had been injected with NAL received VEH and visa versa.

Experiment 2. After 3 months without testing, the same procedure was conducted with the same animals in Experiment 2. They were all pretreated with one of four doses of NAL (0, 0.01, 0.1, and 1 mg/kg; sham dose consisting of VEH; $n = 12$ per NAL dose condition) in a randomized counterbalanced design. Ten minutes later they were given 2.5 mg/kg FLU or VEH and presented 20 min later with the palatable food. This dose of FLU was chosen because it was effective in suppressing intake, yet was also a low dose compared to the other doses that were tested in Experiment 1. The procedure was repeated with FLU-treated rats now receiving VEH and visa versa.

Statistical Analyses

In Experiment 1, 1 mg/kg NAL and VEH was a within-subjects variable and the four levels of FLU dose was a between-group variable (four different dose groups). Therefore, although all rats received NAL and VEH, no rat received more than one dose of FLU. For Experiment 2, the opposite was true (i.e., 2.5 mg/kg FLU and VEH was the within-subjects variable and the four levels of NAL dose was a between-group variable).

Data were analyzed by separate repeated measures analysis of variance (ANOVA) to assess the effect of NAL or FLU (four dose levels), time (30 min and 2 h), and 2.5 mg/kg FLU or 1 mg/kg NAL, respectively, on intake of cereal and intake of chocolate. Results are expressed as mean ± SEM kilocalories.

RESULTS

An analysis of the order in which the animals received 1.0 NAL or VEH showed it was not significant, $F(1, 40) = 0.79$, $p > 0.05$. Therefore, order of drug administration was not included in any further analyses.

Experiment 1: Combinations of 1 mg/kg NAL With Doses of FLU

As shown in Fig. 1, the main effects of FLU and NAL show that each of these agents reduced intake of cereal, $F(3, 44) = 37.35$, $p < 0.001$, and $F(1, 44) = 204.00$, $p < 0.001$, respectively. There was also an interaction between FLU and NAL,

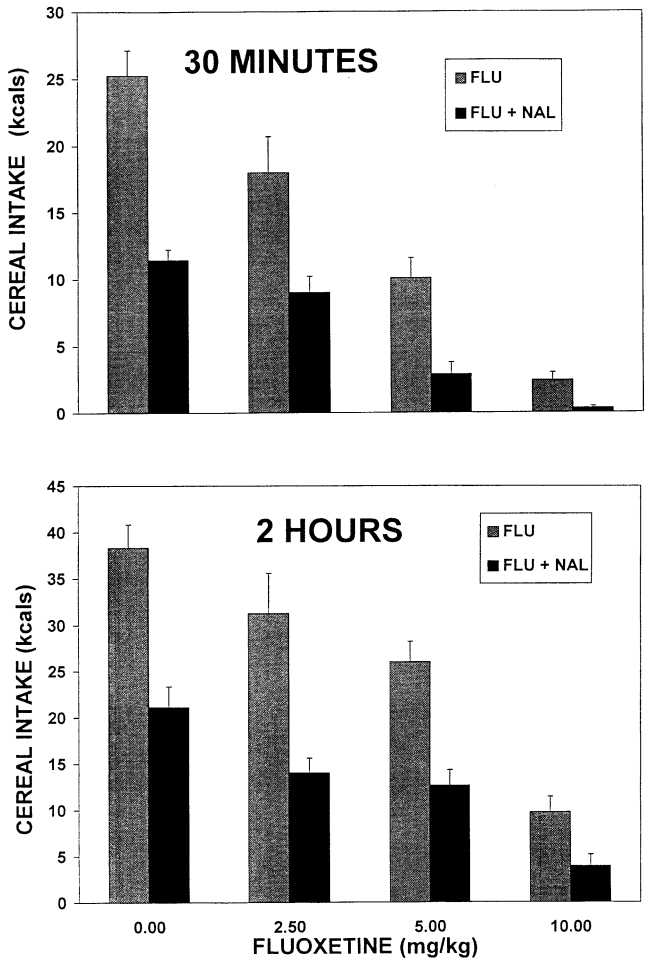

FIG. 1. IP fluoxetine doses alone and in combination with 1 mg/kg SC naloxone reduced ($p < 0.01$) kilocalorie intake of sweetened cereal, a carbohydrate-rich food, across time.

which indicates that the food suppression by various doses of FLU was increased by including NAL, $F(3, 44) = 5.58$, $p < 0.01$. However, this apparent interaction may have been due to a floor effect on intake suppression at the higher doses of FLU. Overall, there was a powerful FLU-induced dose-dependent suppression of cereal intake to about 10% of the control condition and, in addition, NAL clearly enhanced the FLU-induced suppression at every FLU dose level (Fig. 1).

As shown in Fig. 2, 1 mg/kg NAL significantly reduced chocolate intake, $F(1, 44) = 46.41$, $p < 0.001$, and there was no overall main effect of FLU on suppression of chocolate, $F(3, 44) = 1.63$, NS. There was no interaction between NAL and FLU, $F(3, 44) = 1.82$, NS.

Experiment 2: Combinations of 2.5 FLU With Low Doses of NAL

Unlike Experiment 1, the effect of low doses of NAL on cereal intake was marginal, $F(3, 44) = 2.84$, $p = 0.05$, however, as expected on a carbohydrate-rich food, the effect of FLU was highly significant, $F(1, 44) = 85.79$, $p < 0.001$ (Fig. 3). Furthermore, there was no interaction between FLU and NAL, $F(3, 4) = 1.89$, N.S.

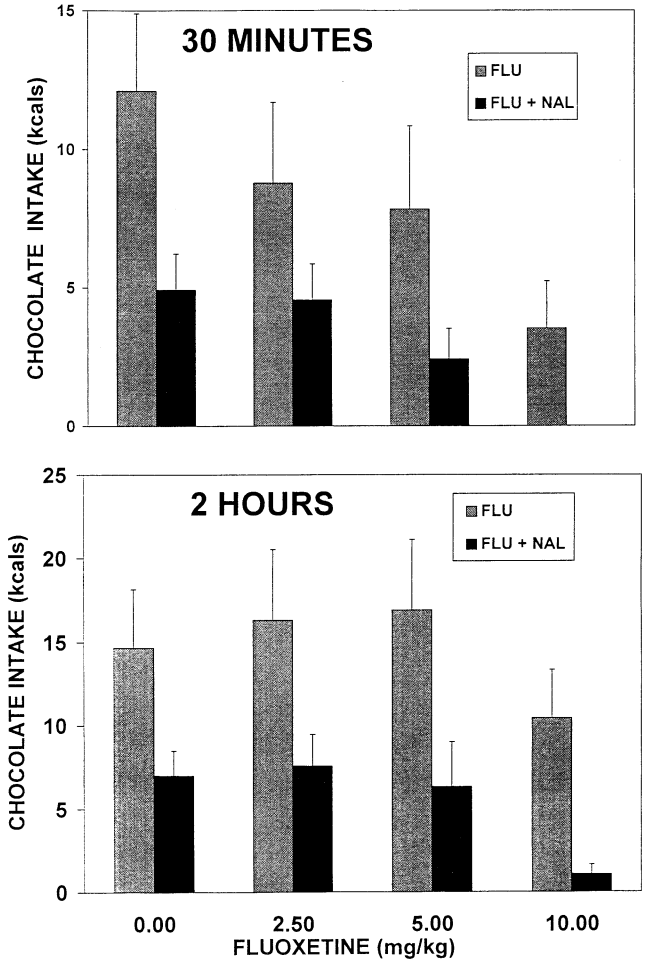

FIG. 2. IP fluoxetine with 1 mg/kg SC naloxone reduced ($p < 0.01$) kilocalorie intake of chocolate, a fat-rich food, across time. The effect of fluoxetine alone was not significant.

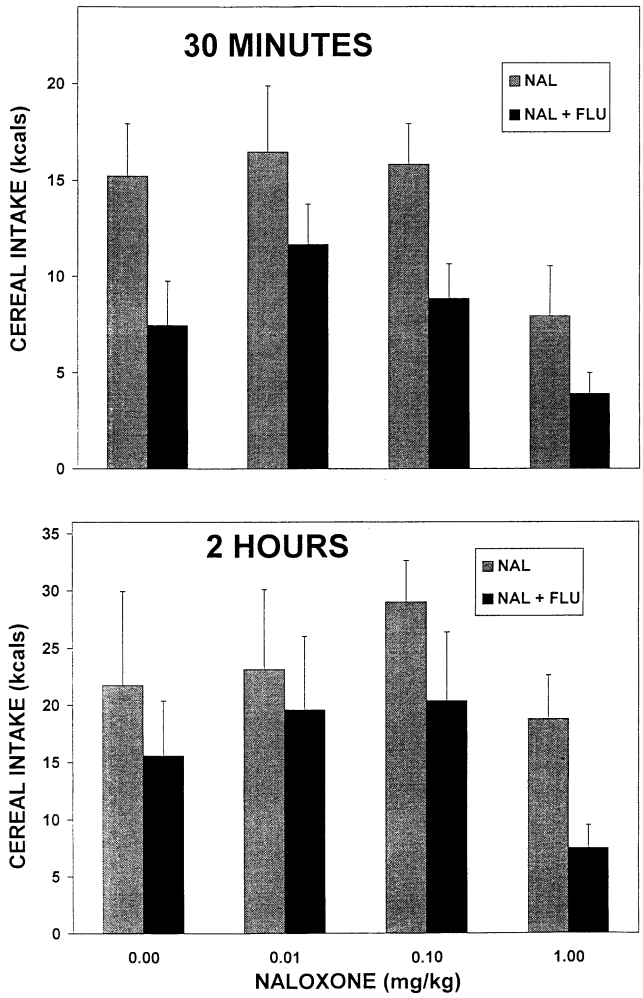

FIG. 3. The effect of SC naloxone doses alone ($p < 0.05$) and in combination with 2.5 mg/kg IP fluoxetine reduced ($p < 0.01$) kilocalorie intake of sweetened cereal, a carbohydrate-rich food, across time.

As in Experiment 1, FLU and NAL suppressed intake of chocolate, $F(1, 44) = 39.13$, $p < 0.001$, and $F(3, 44) = 3.17$, $p < 0.05$, respectively (Fig. 4). No interaction between FLU and NAL was found, $F(3, 44) = 1.95$, NS.

DISCUSSION

Results from Experiment 1 indicate that NAL and FLU clearly reduced intake of sweetened cereal, a palatable carbohydrate-rich food (Fig. 1), to about 50% at each dose of FLU. This indicates that there may be an additive advantage of combining NAL and FLU to reduce intake of these food aspects, commonly found in binge-food items. On the other hand, however, only NAL had a significant effect on chocolate, a fat-rich item (Fig. 2). Therefore, there appears to be an advantage to adding NAL to FLU treatment for the suppression of carbohydrates, but no probable advantage to adding FLU to NAL, at least at these doses, for intake of fat-rich food.

An overview of the results from Experiment 2 suggests that NAL did not produce meaningful suppression on carbohydrate-rich food intake (Fig. 3). On the other hand, a rela-

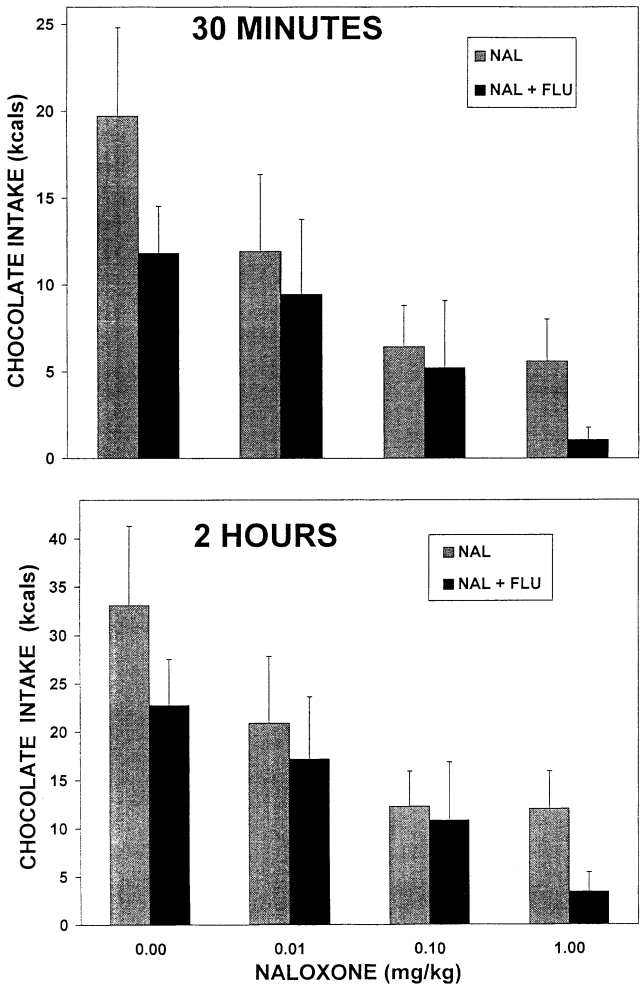

FIG. 4. The effect of SC naloxone doses alone ($p < 0.05$) and in combination with 2.5 mg/kg IP fluoxetine reduced ($p < 0.01$) kilocalorie intake of chocolate, a fat-rich food, across time.

tively low dose of FLU produced a strong and clear reduction of carbohydrate-rich food without the aid of NAL. In contrast to its effects on carbohydrate-rich food, low doses of NAL produced a dose-dependent suppression of fat-rich food (Fig. 4). A low dose of FLU, although it produced a statistically significant additive effect with NAL, did not appear to produce a meaningful additive anorectic effect.

We have replicated these results in our laboratory with a smaller number of female rats. In that study, as in the present study, 2.5 mg/kg FLU reduced cereal intake by 50%, 1 mg/kg NAL had no significant effect, and a combination of both reduced cereal intake to only 26%. With chocolate intake, NAL was most effective, reducing intake to 44%. FLU, on the other hand, had no effect, and a combination of both did not add to NAL's anorectic effect on fat. Therefore, it appears that the different effects of NAL and FLU on carbohydrate and fat-rich sweet food generalizes across sexes in animals.

The role of NAL and FLU in selection of specific macronutrients cannot be absolutely ascertained without utilizing pure macronutrient diets. However, taken together, the present results support the idea that FLU, in general, suppresses intake of sweet carbohydrate-rich food and NAL reduces consumption of sweet fat-rich food. The results also support previous findings attributing opioid function with a selective macronutrient feeding for fats (4,15,21), although these effects may be confounded with a baseline preference for fats (11) or prior ingestive conditions (17). Our results also corroborate a role of serotonergic mechanisms in the selective feeding of carbohydrates (16,18,20,27).

Because the present study involved systemic injections, sites of possible serotonin and opioid interactions cannot be ascertained. However, much evidence has supported a central system of serotonin–opioid interaction. Although systemic opioid-induced anorexia is modified by systemic serotonergic treatments (2,7), central alterations of serotonin levels can produce changes in brain opioid levels, depending on the opioids tested (28). The striatum and hypothalamus are proposed as common sites of central action (12,28,33). In particular, medial nuclei of the hypothalamus appear to be highly responsive to both the carbohydrate suppression of several serotonin agents, including fluoxetine (19,30), as well as the hyperphagic and hypophagic actions of opioids (12,32). Although acting in concert at common brain sites, opioids and serotonin may be activating heterogeneous functions related to food intake.

One such role for opioids that is currently being explored involves their function in the palatability of preferred foods (6,17). Although we did not focus on testing the effect of NAL on preferred food, in Experiment 1 the preferred food was recorded for each rat a few days prior to drug tests. As many as 75% of the animals preferred cereal over chocolate. In those animals receiving 1.0 NAL, when tested without FLU, we observed that the effect of NAL in the first 30 min of feeding, regardless of the preferred food, was to suppress intake of chocolate, the more fatty choice. This does not necessarily mean that opioid blockade does not affect intake of a preferred food as reported by others testing palatable food against less palatable and even aversive foods (6,9). Instead, it may mean that opioid-induced selection is affected by sweetness so that a nonsweet is preferred. Thus, in a choice between two highly palatable and sweet foods, as in the present study, initial preference may be irrelevant and NAL may be acting primarily to decrease the more fatty food.

The only condition under which the combination of NAL and FLU produced any robust advantage over either drug alone is the condition in which NAL increased FLU-induced suppression of cereal. When lower doses of NAL were tested, they were combined with a relatively small dose of FLU (2.5 mg/kg). Because intake of cereal was only effectively reduced with a higher dose of NAL (1 mg/kg), a higher dose (than 2.5 mg/kg) of FLU may have been more effective as shown when NAL and FLU combination reduced fat-rich food at the highest dose of FLU. Overall, although additive anorectic effects were obtained, the results did not support the original hypothesis that low doses of NAL and FLU might produce a powerful anorectic interaction effect.

Perhaps what our data most strongly support is that NAL was effective under all conditions tested, and that opioid antagonists such as NAL should still be viewed as very viable candidates in the treatment of binge-eating disorders. Because of the serious nature of reported side effects with high dose NAL and naltrexone, an alternate opioid antagonist such as nalmefene, shown to be equipotent to NAL doses (10) but void of dose-dependent liver toxicity effects (24) may be promising. Besides eating behavior, opioids mediate reward processes and serotonin is well known to affect mood. Binge eating in humans is associated with depression and has been described as addictive in nature (3,8,14,22,23,25,29). Further

clarification of the relationship between the endogenous opioid and serotonergic systems may lead to a better understanding of how these symptoms are neurochemically interrelated and may lead to improved research and treatment strategies of eating disorders.

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