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Diet Selection Following a Chronic Morphine and Naloxone Regimen

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MARKS-KAUFMAN, R. AND R. B. KANAREK. *Diet selection following a chronic morphine and naloxone regimen.* PHARMACOL BIOCHEM BEHAV **35**(3) 665–669, 1990.—Total caloric intake and patterns of dietary self-selection of the three macronutrients, protein, carbohydrate and fat, were examined in adult male rats maintained on a 6-hr feeding schedule following daily injections of morphine (10 mg/kg), naloxone (1 mg/kg), the two drugs together, and saline. Animals received drug injections for 10 consecutive days. All animals received saline injections for the 5 days preceding and 5 days following the experimental period. Naloxone injections led to a significant reduction in total caloric intake. Neither morphine nor morphine and naloxone together significantly affected total caloric intake. Each of the drugs had a distinct effect on macronutrient selection. Morphine produced a significant increase in fat intake and decrease in carbohydrate intake, while naloxone led to a slight reduction in fat intake. When the two drugs were given together, a significant elevation in carbohydrate intake and reduction in fat intake were observed. Protein intake was not affected by any of the drugs. These results are discussed with respect to the hypothesized role of the endogenous opioid system in the regulation of energy balance.

Morphine Naloxone Opiate-induced feeding Diet selection Fat Protein Carbohydrate

SUBSTANTIAL evidence has accrued to support a role for the endogenous opioid peptides in the control of energy intake and body weight regulation [for recent reviews see (14,17)]. The first hint that these peptides played a role in feeding behavior came from studies examining the effects of opioid agonists and antagonists on food intake. Opioid agonists enhance the activity of the endogenous opioid system and in general, increase food intake in experimental animals. For example, systemic injections of the classic opioid agonist, morphine, can elevate food intake in both freely feeding and mildly food-deprived rats [e.g., (7, 9, 22, 23)]. In contrast to opioid agonists, opioid antagonists suppress feeding behavior [e.g., (2, 4, 8, 10, 16, 25)].

Most investigators examining the relationship between the endogenous opioid system and feeding have used only single nutritionally complete diets. However, to maintain an adequate nutritional status, most animals must select a balanced diet from foods varying widely in nutritional value, as well as consume sufficient food to meet energy needs. By providing animals with a choice of nutrients rather than a single diet, both qualitative and quantitative adjustments in feeding can be examined (5). Using this approach, it has been demonstrated that opioid agonists and antagonists not only act in opposition on total food intake, but also differentially alter nutrient choice. Following morphine injections, rats given separate sources of the three macronutrients, protein, carbohydrate and fat, increase fat intake while suppressing carbohydrate intake (11, 13, 19). In contrast, administration of opioid antagonists leads to a suppression in fat intake with little modification in intake of the other nutrients (12,16).

While the preceding data suggest that the effects of opioid agonists and antagonists on diet selection are mediated directly at opiate receptor sites, they do not eliminate the possibility of an indirect effect of the drugs on nutrient choice. To clarify the mechanism of modifications in diet selection following opiate administration, in the present study animals were given naloxone in conjunction with morphine injections to determine if naloxone would block morphine's effects on patterns of diet choice.

METHOD

Animals

Twenty-four male Sprague-Dawley rats (CD outbred, Charles River Laboratories, Wilmington, MA), weighing between 250 and 300 g at the start of the experiment, were used. Animals were housed individually in standard stainless steel laboratory cages in a temperature-controlled room (21 ± 1°C) maintained on a 12:12 hour light-dark cycle (lights on: 0800–2000 hr).

Diets

All animals were given access to a self-selection regime with separate sources of the three macronutrients, protein, carbohydrate, and fat. The protein ration (3.8 kcal/g) contained 960 g

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casein (ICN Pharmaceuticals, Cleveland, OH), 40 g minerals (U.S.P. XIV Salt Mixture, ICN Pharmaceuticals) and 22 g vitamins (Vitamin Diet Fortification Mixture, ICN Pharmaceuticals). The carbohydrate ration (3.8 kcal/g) contained 580 g cornstarch (Teklad Test Diets, Madison, WI), 280 g dextrin (Teklad Test Diets), 100 g commercial grade sucrose, 40 g minerals and 22 g vitamins. The fat ration (7.9 kcal/g) was composed of 912 g hydrogenated vegetable fat (Crisco), 48 g safflower oil (Hollywood Health Foods), 90 g minerals and 49.5 g vitamins. Vitamins and minerals were added to the components so that the three dietary rations contained equal amounts of these macronutrients on a per kilocalorie basis. The protein and carbohydrate rations were provided in Wahmann (Timonium, MD) spill-proof LC 306-A food cups. The fat ration was provided in 75-ml glass cups. The position of the food cups was altered on a daily basis to prevent the development of position preferences. Fresh food was provided each day. All animals had ad lib access to water throughout the experiment.

Drugs

Morphine sulfate, generously provided by the National Institute on Drug Abuse, and naloxone hydrochloride, generously provided by Endo Laboratories (Garden City, NY) were dissolved in 0.9% saline to concentrations that allowed studied doses to be administered in volumes of 0.1 mg/100 g body weight.

Procedure

Animals were given ten days to adapt to the self-selection diets. Following this adaptation period, access to the macronutrients was restricted to a six-hour feeding period during the light portion of the 24-hour cycle (0900–1500 hr). This feeding schedule was used to allow direct comparisons with previous work examining the effects of opioid agonists and antagonists on nutrient selection (11–13, 16). After two weeks of experience with the restricted feeding schedule, animals were given intraperitoneal (IP) injections of physiological saline for five days at the beginning of, and three hours into the feeding period.

The rats then were divided into four groups matched on the basis of body weight and macronutrient intakes. Animals in the first group (N = 6) received an IP injection of morphine sulfate (10 mg/kg) at the beginning of the feeding period. This dose of morphine was chosen on the basis of previous studies which had demonstrated that it produced significant alterations in nutrient selection without apparent debilitation of the animals (11,13). Three hours after the morphine injection animals received an IP saline injection. Animals in the second group (N = 6) received an IP injection of naloxone hydrochloride (1.0 mg/kg) at the beginning of, and three hours into the feeding period. Animals in the third group (N = 6) received an IP injection of naloxone (1 mg/kg) followed immediately by an IP injection of morphine (10.0 mg/kg) at the beginning of the feeding period, and naloxone (1.0 mg/kg) alone three hours into the feeding period. Finally, animals in the fourth group (N = 6) continued to receive IP injections of saline. Two injections of naloxone were given due to the relatively short duration of action of the drug. Saline injections three hours into the feeding period served as controls for the second naloxone injection. Drug injections were given for 10 consecutive days. For the last five days of the experiment, all animals again received injections of physiological saline at the beginning of, and three hours into the feeding period. Nutrient intakes were measured to the nearest 0.1 g at 1, 3 and 6 hr postinjection throughout the experiment. Body weights were measured at the end of each 6-hr feeding period.

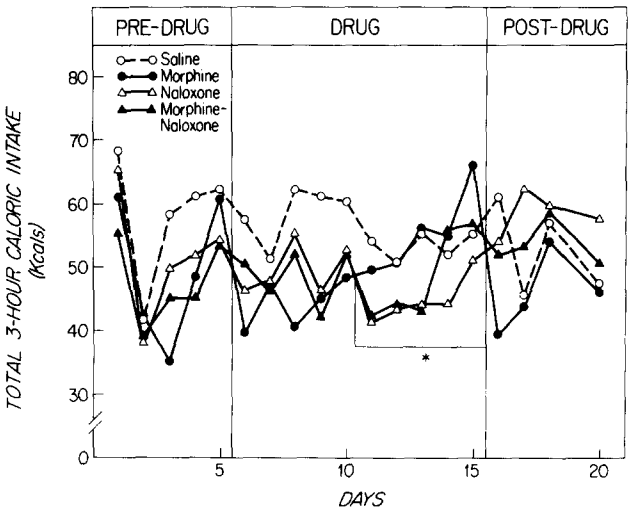

FIG. 1. Total caloric intake 3 hours following IP injections of saline (○), morphine sulfate (●), naloxone (△), or morphine and naloxone (▲) together during a 5-day predrug period, a 10-day drug period, and 5-day postdrug period. Six rats received each drug treatment. *Intake following naloxone significantly ($p<0.05$) less than intake following saline.

Statistical Analyses

Data were analyzed across five-day periods throughout the experiment using analyses of variance, followed by a posteriori multiple comparisons of within-group means using Scheffe's method.

RESULTS

Three-hour postinjection data are shown in the figures. In general, similar results were observed at 1 and 6 hours postinjection. Any differences in data among the measurement periods are mentioned in the text.

Total Caloric Intake

Total caloric intake was calculated as the sum of calories from the three macronutrient rations. No differences were observed among the groups prior to drug administration. At three hours following drug administration, no differences in caloric intake were observed among the groups during the first five days of the drug period (Fig. 1). During the second five days of the drug period, animals injected with naloxone consumed significantly ($p<0.05$) less calories than animals given either saline or morphine. Caloric intakes of animals given naloxone returned to control levels when saline injections replaced the drug administration. Similar results were observed at 1 and 6 hr following injections with the exception that at 1 hr postinjection, rats receiving morphine exhibited a significant ($p<0.05$) elevation in total caloric intake during the second five days of drug injections.

Protein Intake

No modifications in protein intake were observed as a function

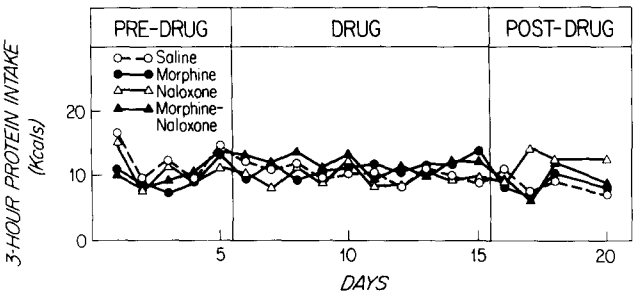

FIG. 2. Protein intake 3 hours following IP injections of saline (○), morphine sulfate (●), naloxone (△), or morphine and naloxone (▲) together during a 5-day predrug period, a 10-day drug period, and 5-day postdrug period. Six rats received each drug treatment.

of drug injections at 3 hr postinjections (Fig. 2). Similar results were found at 1 and 6 hours postinjections.

Carbohydrate Intake

No differences in carbohydrate intake were observed during the predrug period (Fig. 3). Throughout the drug injection period, at 3 hr postinjections, rats receiving morphine consumed significantly ($p<0.05$) less carbohydrate than rats receiving saline. During the postdrug period, no differences in carbohydrate intake were observed between rats injected with morphine and those injected with saline. Carbohydrate intake of animals given naloxone did not differ from that of rats injected with saline at any time during the study. In contrast, animals receiving both morphine and naloxone significantly ($p<0.05$) increased carbohydrate intake above saline levels during the last five days of the drug injection period. Carbohydrate intake of these animals remained significantly ($p<0.05$) elevated during the postdrug period. Similar results were observed at 1 and 6 hr postinjection.

Fat Intake

No differences in fat intake were observed during the predrug

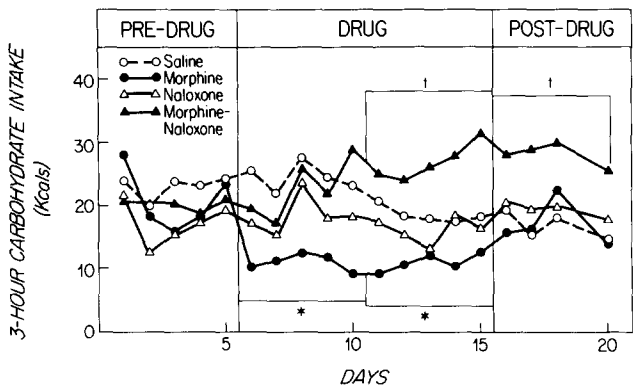

FIG. 3. Carbohydrate intake 3 hours following IP injections of saline (○), morphine sulfate (●), naloxone (△), or morphine and naloxone (▲) together during a 5-day predrug period, a 10-day drug period, and 5-day postdrug period. Six rats received each drug treatment. †Intake following morphine and naloxone injections given together significantly ($p<0.05$) greater than values following saline; *intake following morphine injections significantly ($p<0.05$) less than values following saline.

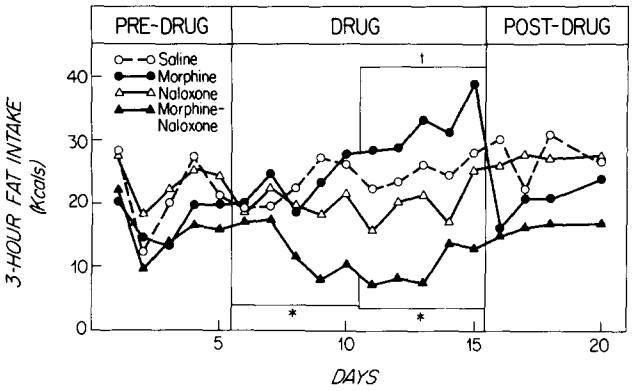

FIG. 4. Fat intake 3 hours postinjection following IP injections of saline (○), morphine sulfate (●), naloxone (△), or morphine and naloxone (▲) together during a 5-day predrug period, a 10-day drug period, and 5-day postdrug period. Six rats received each drug treatment. †Intake following morphine injections significantly ($p<0.05$) greater than values following saline; *intake following morphine and naloxone injections given together significantly ($p<0.05$) less than values following saline.

period. Animals given morphine consumed significantly ($p<0.05$) more fat than rats given saline during the second five days of the drug injection period (Fig. 4). Fat intake returned to saline levels when saline injections replaced morphine injections. Fat intake of rats injected with naloxone alone was slightly less than that of rats given saline. Fat intake of these animals returned to saline levels when naloxone injections were discontinued. Rats given concomitant injections of morphine and naloxone consumed significantly ($p<0.05$) less fat than saline-injected rats throughout the drug injection period. When saline injections replaced drug injections, fat intake of rats given morphine and naloxone increased slightly. No significant differences were found among the groups during the postdrug period. Similar results were observed at 1 and 6 hours postinjection.

Body Weight

Body weight gain did not vary among the groups during the five-day predrug period. Significant differences in body weight were observed as a function of drug administration. Animals given morphine (mean weight gain = 16.3 g), or morphine and naloxone (mean weight gain = 17.2 g) gained significantly ($p<0.05$) less weight during the 10-day drug period than rats injected with saline (mean weight gain = 38.8 g). Animals injected with naloxone alone gained an average of 31 g across the ten-day period. No differences in body weight gain were observed among the groups during the five-day postdrug period.

DISCUSSION

In the present study, naloxone led to a suppression in food intake, while morphine had minor effects on total caloric intake. This latter finding can be contrasted with the results of previous studies demonstrating morphine-induced feeding in rats [e.g., (1, 7, 22, 23)]. The most probable reason for our failure to observe morphine-induced feeding was the level of food deprivation employed in this study. Morphine readily elicits feeding in freely feeding and mildly deprived rats, but generally fails to do so in more severely deprived animals (22,23). Deprivation may induce

alterations in endogenous neurochemical systems which may affect the stimulatory effects of morphine on feeding behavior. However, it is as likely that differences in morphine's effects on total caloric intake reflect the variations in baseline feeding. Restricted animals have higher baseline levels of feeding, which may reduce morphine's stimulatory effects on energy intake. A second reason for the failure of morphine to stimulate overall caloric intake in the present study was the fact that a relatively high dose of the drug was used. High doses of morphine can produce nonspecific pharmacological effects, such as sedation, which could limit the drug's effectiveness in stimulating caloric intake.

In the present study, morphine and naloxone led to patterns of food intake and nutrient choice similar to those previously observed following acute (11, 13, 16, 23) or chronic administration (15,19) of these pharmacological agents. Animals receiving morphine injections increased fat intake and decreased carbohydrate intake relative to animals injected with saline. Cessation of morphine injections led to an immediate return to predrug patterns of nutrient choice. Rats injected with naloxone displayed a small decrease in fat intake relative to saline-injected animals. Giving naloxone in conjunction with morphine led to a pattern of nutrient choice directly opposed to that observed when morphine alone was injected. Animals given the two drugs together displayed significant reductions in fat intake and elevations in carbohydrate intake. This pattern of nutrient choice was maintained when saline injections replaced drug injections.

It has been hypothesized that morphine has a specific stimulatory effect on fat intake both when rats are food restricted (11, 13, 19) or given ad lib access to food (15). It is possible, however, that the increase in fat intake observed following morphine administration represents a more nonspecific effect of the drug. For example, morphine could be increasing intake of the macronutrient which is most consumed or most preferred by the animals. In this and most previous studies, prior to drug administration, rats consumed a larger proportion of their calories from the fat component of the diet than from either the protein or carbohydrate components (11, 15, 19, 23). Morphine may have acted to increase intake of this preferred food. In support of this idea is the one series of studies in which morphine administration was associated with a selective increase in protein rather than fat intake. In those experiments, rats given ad lib access to nutrients consumed more protein than fat prior to drug injections (23). Arguing against the idea that morphine increases intake of a preferred food, however, are the findings that animals given a fat component isocaloric to the protein and carbohydrate components, or a liquid fat component consumed only a small proportion of their calories as fat prior to drug administration, but significantly increased fat intake as a result of morphine injections (11,13). These data provide credence for the hypothesis that morphine specifically stimulates fat intake. Further support for the hypothesis comes from the observation that when the opioid antagonist, naloxone, was given in conjunction with morphine a specific reduction in fat intake was observed.

Under a variety of conditions, morphine consistently leads to a reduction in carbohydrate intake (1, 11, 13, 15, 19, 23). The suppressive effect of morphine on carbohydrate consumption occurs following a wide range of doses of the drug, administered either on an acute or chronic basis. The effect is observed in male and female rats maintained on a number of different feeding schedules and diet selection regimes. These data also indicate a specific drug-nutrient interaction.

In this study, naloxone alone had minimal effects on diet selection. However, when the opioid antagonist was given in conjunction with morphine, major modifications in diet selection were observed. Morphine and naloxone given together produced a pattern of nutrient selection directly opposed to that produced by morphine alone, i.e., a decrease in fat intake and an increase in carbohydrate intake. These findings are rather different from those of other studies examining the effects of naloxone on morphine-induced hyperphagia. Naloxone given in conjunction with morphine typically returns food intake to baseline levels rather than producing major alterations in feeding behavior [e.g., (10,22)]. The present data indicate that a complex interaction may exist between morphine and naloxone which only may be observed when feeding behavior is examined on a more detailed level. Opioid control of the individual macronutrients may be mediated at different opioid receptor sites (10,16). To tease out opioid modulation of nutrient selection, it may be necessary to employ specific receptor agonists and antagonists.

Morphine's effect on nutrient selection may reflect, at least in part, the drug's action on fat metabolism. Morphine and other opioid agonists stimulate lipolysis in adipose tissue [e.g., (18, 21, 26)]. Further, animals given morphine on a chronic basis are less efficient at using calories for weight gain than control animals (15) and demonstrate a reduction in adiposity (3). These data suggest that animals given morphine may have a reduced ability to store fats or a facilitation of fat breakdown. Following morphine administration, animals may choose fat as a result of the metabolic signals arising from increased fat catabolism. In support of this suggestion, diabetic rats, which also display increased lipolysis, choose a diet similar to that of animals given morphine. Diabetic animals select a diet containing more fat and less carbohydrate than nondiabetic animals (6, 20, 24).

Acute injections of morphine can lead to an increase in fat intake (13). However, continued drug administration produces a more pronounced increase in fat intake (11, 15, 19). In the present experiment, fat intake following morphine was not significantly greater than fat intake after saline until the second five days of drug injections. In conjunction with this finding, it can be noted that a similar pattern of behavior was observed in rats given morphine and naloxone together. Carbohydrate intake following the administration of the two drugs together was not significantly elevated above control values until the final five days of the drug period. These data suggest that tolerance may not develop to the stimulatory action of these drugs on nutrient choice. In contrast to the stimulatory actions, morphine led to an almost immediate decrease in carbohydrate intake, and morphine and naloxone, together, an immediate decrease in fat intake.

The present results point to the value of using a diet selection paradigm in studies of opioid modulation of feeding behavior. As in the present experiment, opioid agonists and antagonists given alone or together may have relatively minor effects on total caloric intake. However, these drugs can lead to major differences in nutrient selection. These patterns of selection can provide insights into the role of the endogenous opioid system in the control of ingestive behaviors.

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