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Central and peripheral contributions of endogenous opioid systems to nutrient selection in rats

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Abstract. The contribution of central and peripheral sites to opioid mediation of energy intake and dietary self-selection of the three macronutrients, protein, fat, and carbohydrate, was examined in male rats. Animals given access to either Purina Chow or a self-selection regime were injected with either the opioid antagonist, naltrexone (0.0, 0.1, 1.0, and 5.0 mg/kg, IP), or quarternary naltrexone (0.0, 0.1, 1.0, and 5.0 mg/kg, IP), an opioid antagonist that does not readily enter the central nervous system. Animals received injections at the beginning of an 8-h feeding period, and nutrient intakes were measured at 1, 2, 4, and 8 h postinjection. Naltrexone and its quarternary analogue differed in their effects both on total energy intake and macronutrient selection. Naltrexone led to significant decreases in total energy intake in animals on both dietary regimes, whereas quarternary naltrexone did not modify energy intake of animals given access to either diet. Naltrexone produced a sustained reduction in fat intake and initial decreases in carbohydrate and protein intakes. Quarternary naltrexone did not modify overall energy intake but did lead to modifications in nutrient choice. In contrast to naltrexone, quarternary naltrexone resulted in increased fat intake, decreased carbohydrate intake, and a small reduction in protein intake. These data suggest that both peripheral and central sites contribute to opioid effects on patterns of nutrient choice.

Key words: Dietary self-selection – Protein – Fat – Carbohydrate – Naltrexone – Quarternary naltrexone – Endorphins

Recent experimental evidence has led to the hypothesis that the endogenous opioid peptides play a significant role in the regulation of feeding behavior (for review see Morley et al. 1983). Support for this hypothesis comes from studies demonstrating that in a variety of species the administration of opiate agonists leads to increased energy intake (Jalowiec et al. 1981; Kumar et al. 1971; Sanger and McCarthy 1981), whereas administration of opiate antagonists leads to reduction in energy intake (Cooper 1980; Frenk and Rogers 1979; Holtzman 1974, 1979; Thornhill et al. 1982).

Most investigators examining the relationship between opioid peptides and feeding behavior have only measured total energy intake. However, it recently has been reported that opiate agonists and antagonists also differentially affect intakes of the three macronutrients, protein, fat, and carbohydrate (Marks-Kaufman 1982; Marks-Kaufman and Kanarek 1980, 1981; Marks-Kaufman and Lipeles 1982). Morphine administration resulted in increased fat intake and decreased carbohydrate and protein intake (Marks-Kaufman 1982; Marks-Kaufman and Kanarek 1980). In contrast, administration of naloxone, the prototypic opiate antagonist, was found to produce a suppression in fat intake with no major modifications in either carbohydrate or protein intake (Marks-Kaufman and Kanarek 1981).

While it appears evident that the endogenous opioid peptides play a role in the regulation of food intake and nutrient selection, it has not been established if the effects of these substances are mediated at sites within the central nervous system (CNS) or at peripheral locations. Support for central regulation of opioid effects on food intake comes from the observation that administration of opioid agonists directly into the CNS leads to an increase in food intake (Grandison and Guidotti 1977; McKay et al. 1981; Tepperman and Hirst 1982; Tepperman et al. 1981). Injection of β-endorphin directly into the ventromedial hypothalamus stimulates food intake in satiated rats. This effect is blocked by the administration of the opiate antagonist, naltrexone (Grandison and Guidotti 1977). In contrast to opiate agonists, opiate antagonists when centrally administered lead to a suppression in food intake (Jones and Richter 1980; Thornhill et al. 1982). For example, Jones and Richter (1980) found that 15 μg/rat of naloxone injected into the lateral ventricles of food-deprived rats resulted in decreased feeding, while the same dose had no effect when given systemically.

While there is much support for CNS involvement in opioid regulation of food intake, there is also evidence implicating peripheral involvement of these peptides in feeding behavior. First, endogenous opioid peptides are not only found within the CNS, but are also found in the gastrointestinal tract and pancreas (Bruni et al. 1979; Grube et al. 1978; Polak et al. 1977). Additionally, it has been found that both genetically obese mice *(ob/ob)* and rats *(fa/fa)* have elevated levels of plasma and pituitary β-endorphin relative to their lean littermates (Margules et al. 1978). Both *ob/ob* mice and *fa/fa* rats exhibit an increased responsiveness to naloxone, decreasing food intake to a greater extent than lean animals following naloxone injections (Margules et al. 1978; Thornhill et al. 1982). These data suggest that excess levels of peripheral β-endorphin may contribute to the hyperphagia observed in

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genetically obese animals. Further support for peripheral involvement of opioid peptides in feeding behavior, comes from the demonstration that administration of loperamide, a peripherally effective opiate agonist that does not pass through the blood-brain barrier, elevates food intake to the same extent as morphine (Yim et al. 1980).

As indicated above, one method of investigating the extent of peripheral and central mediation of opiate effects, is to compare feeding behavior following the administration of centrally and peripherally acting opioid agonists and antagonists. This method was employed in the present experiment. Energy intake and patterns of macronutrient selection of rats injected with the opiate antagonist, naltrexone, were compared to those of animals injected with quarternary naltrexone, an opiate antagonist that does not readily enter into the CNS (Valentino et al. 1981).

Materials and methods

Animals and diets. Twenty-six male Sprague-Dawley rats (CD outbred, Charles River Breeding Laboratories, Wilmington, MA) weighing approximately 250 g at the beginning of the experiment were used. Animals were housed individually in hanging wire-mesh cages in a temperature-controlled room (23° ± 1° C) maintained on a 12-h light-dark cycle (lights on 08:00 h).

Animals were randomly divided into two dietary groups. Animals in the first group (n = 12) received ground Purina Rodent Chow 5001 (caloric density 3.6 kcal/g). Animals in the second group (n = 14) were given access to three separate dietary rations: a protein ration, a carbohydrate ration, and a fat ration. The protein ration (caloric density 3.76 kcal/g) was composed of 960 g 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 (caloric density 3.76 kcal/g) was composed of 580 g corn starch (Teklad Test Diets), 100 g commercial-grade sucrose, 40 g minerals (U.S.P. XIV Salt Mixture), and 22 g vitamins (Vitamin Diet Fortification Mixture). The fat ration (caloric density 7.85 kcal/g) was composed of 912 g hydrogenated vegetable fat (MFB Shortening, Wesson), 48 g safflower oil (Teklad Test Diets), 90 g minerals (U.S.P. XIV Salt Mixture), and 49.5 g vitamins (Vitamin Diet Fortification Mixture). Vitamins and minerals were added to the components so that the three dietary rations contained equal amounts of these micronutrients 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. All animals had continuous access to water throughout the experiment.

Drugs. Naltrexone hydrochloride (generously provided by Endo, Garden City, NY) and the quarternary naltrexone antagonist, naltrexone methobromide (MRZ 2663-BR, generously supplied by Dr. H. Merz, C. H. Boehringer-sohn, D-6507 Ingelheim, Federal Republic of Germany) were dissolved in 0.9% saline to concentrations that allowed studied doses to be injected in volumes of 0.1 ml/100 g body weight.

Procedure. Animals were given 2 weeks to adapt to their respective dietary conditions. Access to the nutrients was then restricted to an 8-h feeding period during the light portion of the 24-h cycle (09:00–17:00 h). Following a 2-week adaptation period to the restricted feeding schedule, testing for the effects of quarternary naltrexone and naltrexone on caloric intake and diet selection was initiated.

Animals within each dietary condition were divided into two groups, one that received naltrexone; and the other, quarternary naltrexone. On test days, half the animals in the diet selection group and half the animals in the chow group received (IP) injections of naltrexone at the beginning of the feeding period. Three doses of naltrexone were used: 0.1, 1.0, and 5.0 mg/kg body weight. Each animal received each dose of naltrexone with approximately 7 days intervening between drug injections. To minimize temporal order effects, drug injections were given in a counterbalanced order to animals within each dietary condition. Nutrient intakes measured at 1, 2, 4, and 8 h following naltrexone injections were compared to intakes after IP injections of physiological saline given 2–4 days prior to each naltrexone injection.

The remaining animals in each dietary condition received IP injections of quarternary naltrexone (0.1, 1.0, and 5.0 mg/kg body weight). The same procedure was followed as outlined above for animals receiving naltrexone.

Statistical analysis. Data were analyzed at each time point using one-way analysis of variance followed by a posteriori multiple comparison tests. Data reported as significant have a P-value of 0.05 or less. As no differences were observed either in total caloric intakes or intakes of the individual macronutrients among the three injections of saline, mean values for the three injections were used in the data analysis.

Results

Total energy intake

Animals given Purina Chow. Naltrexone and quarternary naltrexone differed in their effects on total energy intake. Relative to saline injections, administration of the two higher doses of naltrexone led to significant decreases in cumulative energy intake at 1, 2, 4, and 8 h after injections (Fig. 1). In contrast to the suppression in food intake seen following naltrexone administration, no differences in chow intake were observed as a function of quarternary naltrexone injections (Fig. 2).

Animals given the self-selection regime. The effects of naltrexone and quarternary naltrexone on total energy intake of animals maintained on the self-selection regime were similar to those observed in animals given Purina Chow. Naltrexone administration reduced energy consumption. Total cumulative caloric intake (calculated as the sum of caloric intakes from the three macronutrient sources) was significantly reduced at all time points following the administration of 5.0 mg/kg naltrexone and at 2, 4, and 8 h following administration of 1.0 mg/kg naltrexone (Fig. 1). In contrast to naltrexone, administration of quarternary naltrexone did not modify total caloric intake of animals maintained on the self-selection regime (Fig. 2).

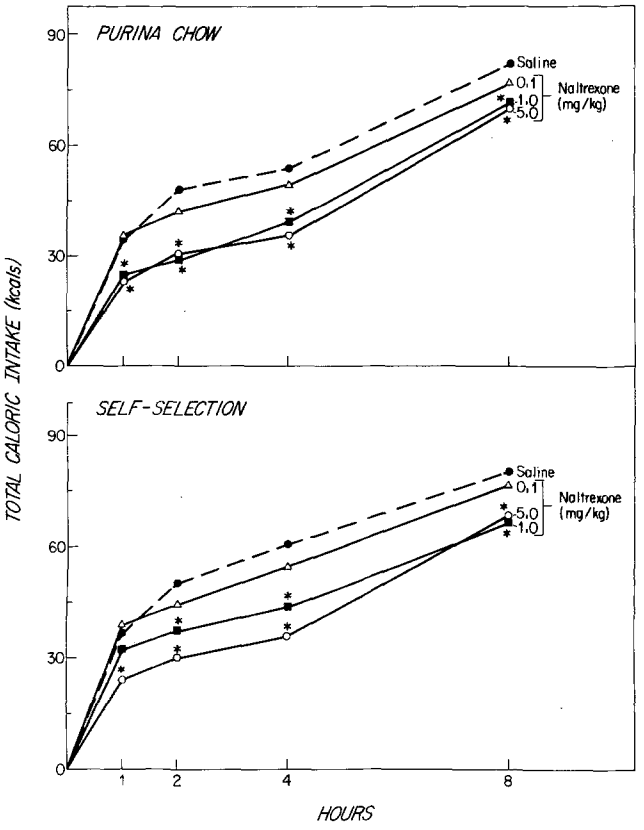

Fig. 1. Mean total cumulative caloric intakes across an 8-h feeding period of animals maintained on Purina Chow (*upper*) or a self-selection (*lower*) regime (cumulative caloric intakes calculated as the sum of caloric intakes from each of the three macronutrients) following injections of saline and 0.1, 1.0, and 5.0 mg/kg naltrexone. *Asterisks* indicate values significantly different from saline injections, $P < 0.05$

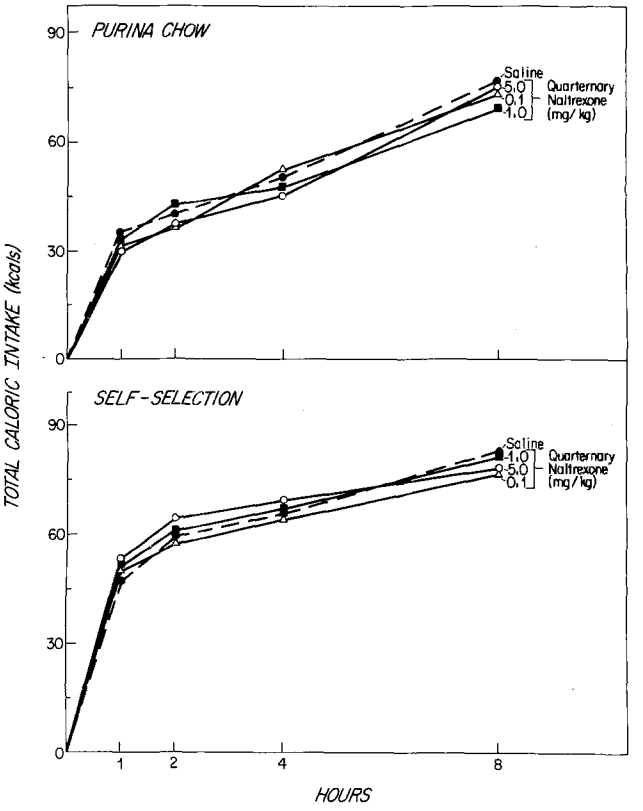

Fig. 2. Mean total cumulative caloric intakes across an 8-h feeding period of animals maintained on Purina Chow (*upper*) or a self-selection (*lower*) regime (cumulative caloric intakes calculated as the sum of caloric intakes from each of the three macronutrients) following injections of saline and 0.1, 1.0, and 5.0 mg/kg quarternary naltrexone. *Asterisks* indicate values significantly different from saline injections, $P < 0.05$

Macronutrient selection

Effects of naltrexone on individual macronutrient intakes. Fat intake was suppressed at 2 and 4 h after the injection of 5.0 mg/kg naltrexone (Fig. 3). Although the difference was not significant, animals did consume less fat across the 8-h feeding period following injections of the two higher doses of naltrexone than following saline.

Cumulative intakes of carbohydrate were significantly lower at 2 h after all doses of naltrexone than after saline administration. Carbohydrate intake was also suppressed at 4 h after injection of the highest dose of the drug (Fig. 3).

Significant reductions in protein intake were observed at 1 and 2 h following injection of the highest dose of naltrexone (Fig. 3).

Effects of quarternary naltrexone on individual macronutrient intaktes. In contrast to naltrexone, quarternary naltrexone (5.0 mg/kg) led to a significant elevation in fat intake (Fig. 4). For all postinjection times, fat intake was significantly greater after the administration of the highest dose of quarternary naltrexone (5.0 mg/kg) than after saline administration. Administration of 1.0 mg/kg quarternary naltrexone led to a significant increase in fat intake at 1 h postinjection.

Carbohydrate intakes were significantly decreased at 1, 2, 4, and 8 h following the highest dose, and at 2 h after injection with the intermediate dose of quarternary naltrexone (Fig. 4).

Injection of the two higher doses (1.0 and 5.0 mg/kg) of quarternary naltrexone led to a slight decrease in protein consumption, which was significant at 8 h postinjection (Fig. 4).

Discussion

Naltrexone and quarternary naltrexone differed in their effects on both total energy consumption and macronutrient selection. With respect to energy intake, naltrexone administration led to significant decreases in caloric intake both in animals fed Purina Chow and those maintained on a self-selection regime. These data are consistent with previous results demonstrating reductions in ingestive behaviors in animals following the administration of centrally active opioid antagonists, but not following administration of quarternary naltrexone, an opioid antagonist that does not readily cross the blood-brain barrier (e.g., Brown and Holtzman 1981; Carr and Simon 1983; Cooper 1980; Cooper and Turkish 1983).

Patterns of nutrient selection following naltrexone administration also were similar to those found after

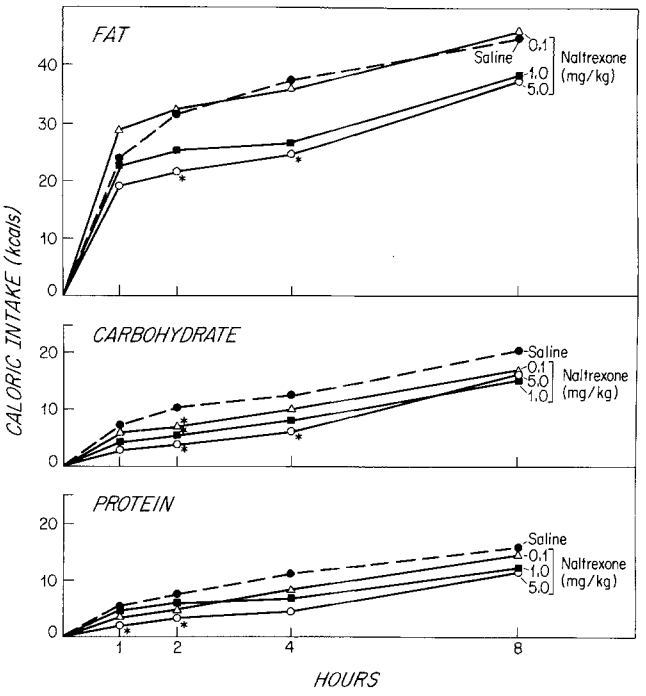

Fig. 3. Mean cumulative caloric intakes across an 8-h feeding period of fat, carbohydrate, and protein of animals maintained on a self-selection regime following injections of saline, 0.1, 1.0, and 5.0 mg/kg naltrexone. *Asterisks* indicate values significantly different from saline injections, $P < 0.05$

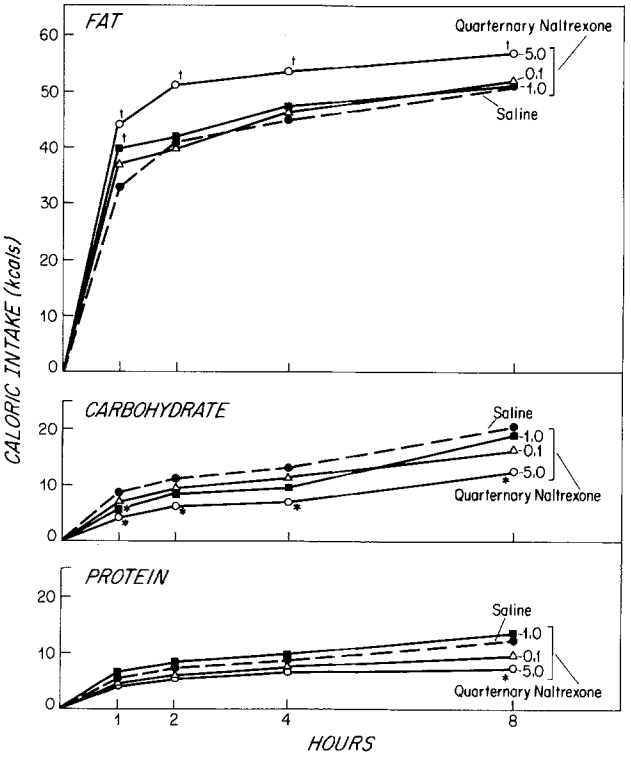

Fig. 4. Mean cumulative caloric intakes across an 8-h feeding period of fat, carbohydrate, and protein of animals maintained on a self-selection regime following injections of saline, 0.1, 1.0, and 5.0 mg/kg quarternary naltrexone. *Asterisks* indicate values significantly different from saline injections, $P < 0.05$

injections of other opioid antagonists (Marks-Kaufman and Kanarek 1981). Both naltrexone and naloxone produced a sustained reduction in fat intake, an initial decrease in carbohydrate intake and little effect on protein consumption. In contrast to the effects of these drugs on nutrient choice, administration of the quarternary analogue of naltrexone resulted in increased fat consumption, decreased carbohydrate intake, and a small reduction in protein intake. It is interesting to note that the pattern of nutrient intake observed following the administration of quarternary naltrexone is similar to that found after the administration of the opioid agonist, morphine (Marks-Kaufman 1982).

Examination of only data on energy intake would suggest that the effects of opiate antagonists on feeding behavior are mediated within the CNS. Administration of an opiate antagonist that can act within the CNS led to a significant suppression in energy intake, whereas administration of an antagonist whose actions are concentrated in the periphery had no effect on energy consumption. The addition of data on patterns of nutrient selection, however, indicates that peripheral, as well as central sites, may be involved in opioid regulation of feeding behavior. Although quarternary naltrexone did not affect overall energy intake, the drug did lead to alterations in the intake of individual macronutrients.

The selective effects of opioid agonists and antagonists on nutrient intake may be mediated in the periphery by endocrine mechanism. Endorphinlike immunoreactivity has been observed in the glucagon (α) cells of the pancreas, suggesting that the opiates may have a direct effect on this organ (Grube et al. 1978). Moreover, opioids appear to influence the release of the pancreatic hormones, insulin and glucagon (Ipp et al. 1978), which are important in the regulation of energy balance. For example, β-endorphin infused into healthy volunteers led to a rise in glucagon, which was followed by a rise in plasma glucose levels. Increases in plasma levels of insulin were also observed (Feldman et al. 1983). Both insulin and glucagon have profound effects on food intake (de Castro et al. 1978; Jacobs 1958; Richter 1942). Moreover, exogenously administered insulin has been shown to modify patterns of nutrient choice (Kanarek et al. 1980; Richter 1942). In addition, morphine and naloxone act in opposition on the release of a number of pituitary hormones associated with the regulation of food intake (e.g., Bruni et al. 1977; Meites et al. 1979; Shaar et al. 1977) including growth hormone, prolactin, and thyroid stimulating hormone. While it seems likely that these hormones, which are influenced by opioid agonists and antagonists, may play a role in diet choice, it becomes difficult to clarify their role with present available data.

More detailed examination of dietary patterns of self-selection following the administration of opioid agonists and antagonists may provide a greater understanding of the mechanisms involved in the regulation of food intake.

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