Role of lipid type on morphine-stimulated diet selection in rats

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Glass, Michael J., Charles J. Billington, and Allen S. Levine. Role of lipid type on morphine-stimulated diet selection in rats. Am. J. Physiol. 277 (Regulatory Integrative Comp. Physiol. 46): R1345–R1350, 1999.—Administration of morphine is said to increase fat consumption among rats allowed to self-select nutrients. However, fats represent a diverse group of molecules, differing in metabolic and sensory properties. Despite this, lipid has yet to be manipulated as a variable in drug-stimulated nutrient selection studies. To determine whether lipid source can impact daily and morphine-stimulated (1, 3, and 10 mg/kg) diet intake, rats were provided with a choice between a high-fat and high-carbohydrate diet in three regimens in which the source of fat was varied between vegetable shortening, lard, or corn oil. Daily and morphine-stimulated diet selections were determined under all conditions. Under daily feeding conditions, rats ate more of the high-lipid diet compared with the high-carbohydrate diet when vegetable shortening or lard was the main lipid alternative, but lipid and carbohydrate intake did not differ when corn oil was the main lipid alternative. When rats were stimulated with morphine, the percentage of lipid increased relative to baseline intake only when the lipid diets were the preferred alternatives (i.e., vegetable shortening or lard). When preference between lipid and carbohydrate diets was neutral (i.e., corn oil condition), morphine did not enhance lipid consumption. These results indicate that morphine increases consumption of total energy or preferred diets and not lipid per se.

One problem with hypotheses favoring selective macronutrient regulation is related to the nature of macronutrient taxonomic categories themselves. As a macronutrient class, fats, or more precisely lipids, represent a diverse group of nutrients that can differ in both physical (i.e., chemical composition, length of carbon chains, viscosity) and sensory (taste, texture) properties. For example, lard is highly saturated and solid at room temperature, whereas corn oil is highly unsaturated and liquid at room temperature. Furthermore, lard and corn oil are known to taste different to rats and humans. Thus the concept of fats or lipids as a monolithic macronutrient class obscures important differences between lipid types, and the potential significance of those differences on morphine-stimulated diet intake has not yet been adequately evaluated. There is

THE PREFERENTIAL MU OPIOID receptor agonist morphine influences food intake as a function of metabolic, temporal, and dietary factors. Morphine has a biphasic effect on food intake, mildly increasing eating (2–3 g) in nondeprived rats (28) after the sedation has waned (i.e., 2 or more hours postinjection) (23), while inhibiting feeding in deprived or restricted animals (28). Additionally, morphine potently stimulates consumption of foods high in fat or sucrose when only one food is presented during testing conditions (6).

One of the earliest hypotheses concerning the behavioral mechanisms underlying morphine-stimulated food intake suggested that this compound stimulated selection of the macronutrient fat (21, 23). This claim was based on findings derived from use of the nutrient self-selection paradigm in which rats are presented with a choice between separate jars of the macronutrients fat, carbohydrate, or protein, each fortified with micronutrients (for reviews see Refs. 4, 16, 19, 26). When morphine was given to rats under these testing conditions, it was reported that lipid intake was stimulated, with no change in protein and suppression of carbohydrate consumption (21, 23). Furthermore, in rats provided with a choice between high-lipid and high-carbohydrate diets, the preferential kappa-receptor agonist butorphanol tartrate or ketocyclazocine both stimulated intake of the high-lipid diet (27). Along with reports that general opioid antagonists inhibited fat selection in rats tested in the nutrient selection paradigm (22, 24), these findings suggested that the opioid system played a role in consumption of lipids.

Subsequent reports have called into question the specificity of the relationship between opioids and lipid ingestion. For example, others have found that in rats allowed to self-select nutrients, morphine treatment resulted in elevated protein consumption (2, 30). Still more recent findings indicate that morphine stimulates selection of the preferred nutrient or diet (8, 14), and, similarly, opioid antagonists have been shown to influence intake of the preferred nutrient (12, 17). For example, in a previous study, daily preference was used as a covariate to determine the impact of preference on morphine-stimulated diet intake (33). Whereas preference was shown to have a significant impact on nutrient or diet intake, morphine-injected rats did eat more fat than carbohydrate (33). That a special relationship may exist between opioid stimulation and fat ingestion is also suggested by a more recent report that showed that stimulation of mu receptors in the nucleus accumens resulted in elevated intake of fat, independent of preference (34). Thus the status of the role of morphine in fat selection remains unclear.

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reason to believe this to be an important issue. In a previous report examining a similar problem with respect to the putative carbohydrate regulator neuropeptide Y (NPY) (31, 32), it was shown that its effect on selection between high-fat and high-carbohydrate diets was a function of the carbohydrate source used in the high-carbohydrate diet (11). When rats were given a choice between a high-fat diet and a sucrose- or polycose-based high-carbohydrate diet, carbohydrate intake was stimulated by NPY. However, when offered a corn starch-based high-carbohydrate diet, NPY stimulated intake of the high-fat diet. Furthermore, NPY-stimulated diet selection patterns tended to parallel daily diet selection patterns (11). Such findings pose a serious challenge to the specificity of the effect of NPY on carbohydrate consumption. Within this context, the present experiment attempted to address the specificity of the relationship between morphine and lipids by manipulating the main source of lipid in high-lipid diets offered to rats along with a cornstarch-based high-carbohydrate diet. It was hypothesized that, as a specific stimulator of lipid, morphine would increase intake of the high-fat relative to the high-carbohydrate diet, independent of the source of lipid.

METHODS

Animals

Male Sprague-Dawley rats (Charles River, MA) weighing 275–350 g were maintained on a 12:12-h light-dark cycle in a temperature-controlled room. Animals were individually housed in stainless steel wire cages with ad libitum food and water access, except where noted. Rats were fed a standard laboratory diet (Certified Rodent Chow, Teklad, Indianapolis, IN) before exposure to the experimental diets. Animals were presented with a choice between a high-carbohydrate diet and one of three isocaloric and isonitrogenous high-lipid diets (77% of kilocalories derived from lipid) that differed only in the source of lipid (vegetable shortening, corn oil, or lard) in two separate regimens (detailed in Morphine regimen). Rats were adapted to the diets for 10–14 days. The composition of the diets is shown in Table 1. Placement of food jars was rotated to avoid any placement preference, and intake was corrected for spillage.

Procedure

Morphine regimen. The effect of lipid type on diet selection was examined through the substitution of different lipid sources in high-lipid diets presented to morphine-stimulated rats. All subjects (n = 12) were exposed to all combinations of diets (vegetable shortening-based high-lipid and high-carbohydrate diets, corn oil-based high-lipid and high-carbohydrate diets, and lard-based high-lipid and high-carbohydrate diets) presented in counterbalanced order in two phases: 1) spontaneous feeding and 2) morphine stimulation. Spontaneous feeding is defined as a consistent pattern of diet choice over 3 days following a 10- to 14-day adaptation period for each diet combination. After daily diet intake measurement, rats were given morphine (1, 3, and 10 mg/kg subcutaneously) or vehicle (0.9% saline) in counterbalanced order. During this phase, food was removed from home cages at 1000–1100, and the animals were immediately injected with morphine or vehicle. All subjects received both morphine and saline (within-groups design). Then, after 15 min, preweighed food jars, one high fat and the other high carbohydrate, were returned to home cages. Food intake was quantified and corrected for spillage at 1, 2, and 4 h postinjection. Due to the small amount of food ingested during the first 2 h, only the 4-h data are presented.

Food deprivation regimen. Under the food deprivation regimen, tested in a separate group of rats, all subjects (n = 12) were exposed to all diet combinations presented in counterbalanced order. First, spontaneous diet selection was determined, as described in Morphine regimen, and then rats were food deprived for 24 h. After this period, preweighed food jars, one containing high lipid and the other high-carbohydrate diet, were returned to home cages. Food intake was quantified and corrected for spillage.

Statistical Analyses

Data were analyzed with one- and two-way repeated-measures ANOVA. Differences between means were tested for significance with Fisher’s protected least significance difference test. When comparisons were made based on percentages, data were transformed (arc sine, used for comparison of proportions) before ANOVA.

RESULTS

Lipid type impacted both daily fat [kcal: F(2,69) = 11.3, P = 0.0001] and carbohydrate [kcal: F(2,69) = 12.5, P = 0.0001] consumption when measured in kilocalories or grams. Rats ate significantly less of the corn oil diet (53.9 kcal) relative to the vegetable shortening (77.8 kcal) or lard (92.2 kcal) diets, whereas intake of the vegetable shortening or lard diets was not significantly different (Fig. 1). Conversely, rats ate significantly more high-carbohydrate diet when the corn oil diet (44.2 kcal) was the alternative compared with vegetable shortening (30.2 kcal) or lard (20.6 kcal), whereas high-carbohydrate consumption did not significantly differ when vegetable shortening or lard was the lipid option (Fig. 1). Relative selection between lipid and carbohydrate diets differed under particular lipid-carbohydrate diet pairing conditions. Under both the lard and vegetable shortening conditions, rats ate more of the high-fat diet relative to the cornstarch diet.
MORPHINE AND LIPID TYPE

Table 2. Effect of morphine administration (0, 1, 3, 10 mg/kg) on intake of a high-fat (lard, vegetable shortening, or corn oil) or a high-CHO diet

<table>
<thead>
<tr>
<th>Regimen, mg/kg</th>
<th>Fat Intake, g</th>
<th>CHO Intake, g</th>
<th>Fat Intake, kcal</th>
<th>CHO Intake, kcal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lard</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0.5 ± 0.2</td>
<td>0.01 ± 0.01</td>
<td>2.8 ± 1.1</td>
<td>0.03 ± 0.03</td>
</tr>
<tr>
<td>1</td>
<td>1.6 ± 0.5*</td>
<td>0.2 ± 0.1</td>
<td>9.6 ± 3.2*</td>
<td>0.8 ± 0.5</td>
</tr>
<tr>
<td>3</td>
<td>2.3 ± 0.5*</td>
<td>0.3 ± 0.1</td>
<td>14.0 ± 3.1*</td>
<td>1.0 ± 0.5</td>
</tr>
<tr>
<td>10</td>
<td>3.6 ± 0.6*</td>
<td>0.08 ± 0.04</td>
<td>21.6 ± 3.8*</td>
<td>0.3 ± 0.1</td>
</tr>
<tr>
<td>Vegetable shortening</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0.4 ± 0.3</td>
<td>0.5 ± 0.2</td>
<td>2.6 ± 1.5</td>
<td>1.8 ± 0.7</td>
</tr>
<tr>
<td>1</td>
<td>0.9 ± 0.3</td>
<td>0.6 ± 0.3</td>
<td>5.4 ± 1.7</td>
<td>2.2 ± 1.2</td>
</tr>
<tr>
<td>3</td>
<td>2.0 ± 0.4*</td>
<td>0.7 ± 0.5</td>
<td>12.2 ± 2.6*</td>
<td>2.8 ± 1.8</td>
</tr>
<tr>
<td>10</td>
<td>3.2 ± 0.4*</td>
<td>0.7 ± 0.3</td>
<td>19.5 ± 2.7*</td>
<td>2.7 ± 1.3</td>
</tr>
<tr>
<td>Corn oil</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0.2 ± 0.1</td>
<td>0.5 ± 0.2</td>
<td>1.3 ± 0.8</td>
<td>2.2 ± 0.9</td>
</tr>
<tr>
<td>1</td>
<td>0.6 ± 0.2</td>
<td>0.9 ± 0.3</td>
<td>3.8 ± 1.1</td>
<td>3.3 ± 1.3</td>
</tr>
<tr>
<td>3</td>
<td>0.4 ± 0.1</td>
<td>1.6 ± 0.3*</td>
<td>2.9 ± 0.9</td>
<td>6.3 ± 1.4*</td>
</tr>
<tr>
<td>10</td>
<td>1.2 ± 0.3*</td>
<td>1.6 ± 0.5*</td>
<td>7.1 ± 1.6*</td>
<td>6.2 ± 1.8*</td>
</tr>
</tbody>
</table>

Values are means ± SE measured in either g/4 h or kcal/4 h. CHO, carbohydrate. *P < 0.05 compared with "0" dose.

There were main effects of lipid type [g: F(2,33) = 13.7, P = 0.0001; kcal: F(2,31) = 11.7, P = 0.0002] and morphine [g: F(2,62) = 12.2, P = 0.0001; kcal: F(2,62) = 5.5, P = 0.007], but no lipid × morphine interaction [g: F(2,4) = 2.3, P = 0.07; kcal: F(2,4) = 0.9, P = 0.44] on the percentage of fat consumed. Under both the lard and vegetable shortening conditions, rats increased the percentage of fat eaten relative to daily feeding (Fig. 2).

Whereas there was no difference between fat and carbohydrate consumption under the corn oil regimen when measured in kilocalories or grams (Fig. 1).

In the 12 rats tested with morphine, food intake was significantly stimulated under the lard [g: F(3,33) = 13.0, P = 0.0001], vegetable shortening [g: F(3,33) = 13.96, P = 0.0001], and corn oil regimens at 4 h postinjection [g: F(3,33) = 4.85, P = 0.006; Table 2]. Collapsed across all treatments, there was a main effect of diet type [g: F(1,66) = 15.2, P = 0.0002] and morphine [g: F(3,198) = 30.72, P = 0.0001], but not of lipid type [g: F(2,66), P = 0.6] on total food intake, whereas there were significant diet type × lipid type [g: F(2,66) = 12.1, P = 0.0001], diet type × morphine [g: F(3,198) = 13.7, P = 0.0001], and diet type × lipid type × morphine interactions [g: F(6,198) = 4.75, P = 0.0002].

To determine the relationship between morphine-stimulated and baseline diet selection, the percentage of total food intake derived from fat consumption under baseline conditions was compared with that of morphine stimulation under the 3- and 10-mg/kg doses. Because baseline intake was determined over a 3-day period and stimulated eating was measured over a 4-h period, it was necessary to convert data to a percentage. In addition, the 1-mg/kg dose was excluded from this analysis based on low stimulation of eating at this dose.
at both the 3.0- and 10.0-mg/kg doses when intake was measured in grams and kilocalories under the lard regimen and at the highest dose under the vegetable shortening regimen (Fig. 2). However, under the corn oil regimen, there were no significant differences in the percentage of fat consumed between daily and morphine-stimulated feeding when food intake was measured either in grams or kilocalories.

In food-deprived rats, there was a main effect of lipid type \( [g: F(2,33) = 6.8, P = 0.003; \text{kcal: } F(2,33) = 6.5, P = 0.004] \) and food deprivation \( [g: F(1,33) = 28.0, P = 0.0001; \text{kcal: } F(1,33) = 24.8, P = 0.0001] \), but no lipid \( \times \) deprivation interaction \( [g: F(1,2) = 0.17, P = 0.84; \text{kcal: } F(1,2) = 0.2, P = 0.81] \) on the percentage of fat consumed when food intake was measured in grams or kilocalories. There were significant increases in the percentage of food intake derived from the high-fat diet under the lard \( [g: F(1,23) = 14.28, P = 0.003; \text{kcal: } F(1,23) = 13.49, P = 0.004] \), vegetable shortening \( [g: F(1,23) = 9.5, P = 0.01; \text{kcal: } F(1,23) = 8.4, P = 0.01] \), and corn oil \( [g: F(1,23) = 7.9, P = 0.01; \text{kcal: } F(1,23) = 7.0, P = 0.02] \) conditions (Fig. 3).

**DISCUSSION**

When rats were offered a choice between a cornstarch-based high-carbohydrate diet and one of three high-lipid diets, both daily and morphine-stimulated diet selection were significantly impacted by the type of lipid used in the high-lipid diets. When lard was the main lipid type, rats ate more of the high-lipid diet compared with the carbohydrate diet under daily feeding, and morphine significantly increased high-lipid diet intake but not high-carbohydrate intake. A similar pattern emerged in the case of the vegetable shortening-based high-lipid diet. However, the opposite pattern was seen when corn oil was the main lipid source in the high-lipid diet: carbohydrate was eaten in greater amounts during daily food intake, whereas morphine stimulated intake of the carbohydrate diet to a greater degree than the high-fat diet.

As a whole, the pattern of results reviewed is inconsistent with the view that morphine influences food intake by selectively affecting lipid consumption. Instead, there appears to be a relationship between daily and morphine-stimulated diet selection patterns. Overall, morphine increased the proportion of food eaten as the high-lipid diet under both the lard and vegetable shortening regimens compared with daily intake. Lipid consumption was elevated beyond what would have been expected based on observed patterns of daily feeding, but lard and vegetable shortening were also highly preferred foods. Because there was no preference for the corn oil-based diet over the high-carbohydrate diet under daily conditions, this is a dietary situation in which food preference is removed as a factor. Under this condition, consumption of the corn oil diet under daily and morphine-stimulated feeding were not different. Taken together, these findings suggest that morphine does not increase fat intake but does stimulate either total energy or preferred food consumption. These results agree with previous studies demonstrating that daily preferences play an important role in the effects of opioid agonists and antagonists on diet or nutrient intake (8, 12, 14, 17).

Diet preference may be related to the relative palatability of diets. The finding that morphine induces intake of preferred diets would be consistent with the hypothesis that opioids affect food consumption by modulating hedonic reactions to foods (6, 10). Opioid agonists have been shown to stimulate intake of preferred foods when offered alone, to increase consumption of sucrose, saccharine (5), or saline (15) solutions relative to water, and to increase hedonic reactions to bitter-sweet solutions (7).

On the other hand, food deprivation in this study appeared to stimulate lipid consumption independently of daily feeding patterns. Others have shown that energy deprivation or restriction may stimulate fat consumption (25, 29), whereas we have suggested the importance of preference in deprivation-induced diet intake (33).

The present findings are consistent with a similar study evaluating the relationship between carbohydrate type and the putative carbohydrate regulator NPY (11). It was shown that daily diet consumption varied as a function of the carbohydrate source used in the high-carbohydrate diet and that NPY-stimulated diet selection followed daily intake patterns. For example, when cornstarch served as the main carbohydrate source, rats selected a higher proportion of daily energy from the high-fat diet, and, after treatment with NPY, rats still chose a higher proportion of energy from the high-fat diet, whereas the converse was true in
situations in which sucrose or polyose were the main carbohydrate sources of the high-carbohydrate diet. Along with the present results, the previous findings with NPY show that preferences for different nutrient types appear to be more significant than macronutrient class with respect to morphine- or NPY-stimulated diet intake patterns. Moreover, these results suggest that neither morphine nor NPY specifically affect consumption of a particular macronutrient class.

Perspectives

Whereas the present results suggest that macronutrient class is less influential than diet preference in opioid-stimulated diet intake patterns, the precise nature of opioid-nutrient relationships still remains unclear. Diet selection may be impacted by food palatability and the energy needs of the organism rather than simply the type of macronutrients present in the diet. Furthermore, feeding stimulated by energy needs or palatability may be mediated by differing neural systems. For example, the hypothalamic paraventricular nucleus (PVN) has been hypothesized to coordinate energy balance, and the central nucleus of the amygdala (CeA) (3) may mediate sensory and/or affective events (1). Given the functional heterogeneity of neural feeding circuitry, peripheral administration of drugs, commonly used in nutrient-intake studies, would be expected to affect multiple feeding-related processes involved in nutrient intake. Opioid receptors have been localized within a variety of brain regions such as the PVN and CeA (20). Significantly, blockade of opioid receptors in each of these regions has been shown to decrease food intake (9, 13, 18). Together, these findings indicate that opioids may influence multiple food-intake control systems. To elucidate the nature of connections between opioids and nutrient consumption, future research will require manipulation of specific populations of receptors within the context of behavioral tests that measure factors important in nutrient consumption, such as energy regulation or the sensory control of eating.

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