Pharmacology Biochemistry & Behavior, Vol. 38, pp. 681–684. © Pergamon Press plc, 1991. Printed in the U.S.A. 0091-3057/91 $3.00 + .00

BRIEF COMMUNICATION

Dietary Influences on Morphine-Induced Analgesia in Rats[1]

ROBIN B. KANAREK,[1] ERIC S. WHITE, MATHEW T. BIEGEN AND ROBIN MARKS-KAUFMAN*

*Department of Psychology, Tufts University, Medford, MA and *Institute of Human Nutrition, Columbia University, New York, NY*

Received 27 June 1990

KANAREK, R. B., E. S. WHITE, M. T. BIEGEN AND R. MARKS-KAUFMAN. *Dietary influences on morphine-induced analgesia in rats.* PHARMACOL BIOCHEM BEHAV **38**(3) 681–684, 1991.—Morphine-induced analgesia was examined using a tail-flick apparatus in 36 adult male Sprague-Dawley rats. Rats were given ad lib access to Purina Chow alone (N = 9) or given a choice of Purina Chow and either a 0.15% saccharin solution (N = 9), a 32% sucrose solution (N = 9), or hydrogenated vegetable fat (Crisco) (N = 9). Analgesic testing was conducted immediately preceding and at 30, 60 and 90 minutes following intraperitoneal administration of morphine sulfate (0.0, 2.5, 5.0 and 10.0 mg/kg). No differences in analgesic responsiveness were observed as a function of diet preceding morphine administration. However, dietary variables did alter morphine-induced analgesia. At 30 minutes following injections of the highest dose of morphine, animals fed saccharin, sucrose or Crisco had significantly longer tail-flick latencies than rats given only Purina Chow. Sixty minutes following injections, rats fed Crisco continued to display a significantly longer tail-flick latency than rats fed only Chow. These data demonstrate that palatable substances can enhance the analgesic properties of exogenous opioids.

Morphine Analgesia Sucrose Fat Saccharin Tail-flick

PREVIOUS research has suggested a connection between the intake of palatable foods and the endogenous opioid peptide system. For example, sweet-tasting substances such as sucrose and saccharin can interact with endogenous opioid systems to alter pain sensitivity (1–3, 7, 9, 11, 12). In an earlier study, we observed that rats fed a 32% sucrose solution in addition to a standard laboratory diet (Purina Chow) showed a decreased latency on a tail-flick apparatus than rats given Purina chow alone (11). However, when injected with 10 mg/kg morphine sulfate, rats consuming the sucrose solution showed an increased tail-flick latency as compared to those given only chow. Similarly, Roane and Martin (12) recently found that rats given a 20% sucrose solution in addition to their standard diet demonstrated an increased analgesic response when injected with morphine. Moreover, Lieblich and colleagues (9) have reported that ingestion of saccharin decreased the latency of the pain response to morphine in certain genetically selected strains of rats.

It should be noted, however, that animals may not respond to all sweet-tasting solutions in the same fashion. Preliminary data from our laboratory revealed that rats given access to a 32% sucrose solution for a two-week period displayed a much shorter tail-flick latency when compared to rats given a 0.15% saccharin solution to drink. However, when injected with morphine, rats drinking the sucrose solution exhibited a significantly longer latency to respond on a tail-flick test than rats drinking the saccharin solution (11).

Although evidence suggests that highly palatable substances interact with endogenous opioid systems, most of this research has been conducted utilizing sweet-tasting fluids or foods. Very little has been reported on the effects of other foods which rats avidly consume. Initial data provided by Shide and Blass (13), however, suggest that intake of fats may also alter pain sensitivity. These researchers found that intraoral administration of corn oil significantly increased paw-lick latency in 10-day-old rat pups.

To examine the effects of palatable substances on morphine-induced analgesia in more detail, the present experiment investigated the effects of two sweet-tasting, highly palatable solutions (saccharin and sucrose), and one nonsweet food (Crisco) on response latency using a tail-flick apparatus. Analysis of the data reaffirmed the prospect that sweet-tasting substances are not a criterion for increased opioid-induced analgesia.

[1]Requests for reprints should be addressed to Robin B. Kanarek, Department of Psychology, Research Building, 490 Boston Avenue, Tufts University, Medford, MA 02155.

METHOD

Animals

Thirty-six drug-naive male Sprague-Dawley rats (CD outbred, Charles River Breeding Laboratories, Wilmington, MA), weighing between 225 and 250 g at the beginning of the experiment, were used. Animals were housed individually in standard stainless-steel cages in a temperature-controlled room (21 ± 1°C) with a 12–12-h light-dark cycle (lights on: 0100–1300 h).

Diets

Animals were randomly assigned to one of four groups. Animals in each group (N = 9) had ad lib access to ground Purina Rodent Chow No. 5001. In addition to Purina chow, animals in group two had access to a 0.15% saccharin solution, animals in group three access to hydrogenated vegetable fat (Crisco), and animals in group four access to a 32% sucrose solution. The concentration for the saccharin solution was chosen on the basis of previous data demonstrating that under the conditions used in this study, rats drank equivalent amounts of a 0.15% saccharin solution and a 32% sucrose solution (11).

Purina chow was presented in Wahmann (Timonium, MD) LC-306A nonspill food cups. The fat ration was presented in 75 ml glass cups. The sucrose and saccharin solutions were presented in glass water bottles with rubber stoppers and nonleaking stainless-steel drinking spouts. All animals had ad lib access to foods and tap water throughout the experiment.

Drugs

Morphine sulfate, generously provided by the National Institute on Drug Abuse, was dissolved in 0.9% saline to concentrations that allowed studied doses to be administered in volumes of 2.5, 5.0, and 10.0 mg/ml.

Procedure

Animals were given 20 days to adapt to dietary conditions, and were handled on a daily basis during that period to adapt them to handling conditions. Body weights and nutrient intakes were measured three times per week. All nociceptive testing was done in the afternoon, within four hours after the onset of the dark cycle. Pain thresholds were assessed by the radiant heat tail-flick method.

To adapt animals to injections and testing procedures, all animals were first given an intraperitoneal (IP) injection of physiological saline. Animals were then placed on the platform of the tail-flick apparatus, with tails smoothed into the tail groove. All animals were gently held by the same experimenter during all tests. A light source was focused on the tail with the intensity of the light adjusted with a rheostat to obtain a control tail-flick latency of 2 to 4 seconds. To prevent tissue damage, if animals did not respond within 9 seconds, the light source was automatically turned off.

To examine the effects of dietary variables on morphine-induced analgesia, animals were injected with three doses of morphine sulfate (2.5, 5.0, and 10.0 mg/kg body weight). Reaction times on the tail-flick apparatus were measured immediately preceding and at 30, 60 and 90 minutes following drug injections. To control for order effects, a Latin square procedure was used to administer drug injections. All animals received each dose of morphine once, with a minimum of seven days intervening between injections.

Statistical Analyses

Data were analyzed using one-way analyses of variance followed by a posteriori multiple comparisons using Scheffe's method.

TABLE 1

DAILY CALORIC INTAKE AND BODY WEIGHT GAIN

Purina Chow	Saccharin	Sucrose	Crisco
	Daily Caloric Intake (kcal ± SD)		
88.9^{a} ± 6.3	91.0^{a} ± 6.4	99.5^{b} ± 7.5	94.1^{ab} ± 7.1
	Body Weight Gain (g ± SD)		
164.2 ± 22.3	158.3 ± 23.6	172.1 ± 18.4	176.1 ± 30.5

For daily caloric intake, numbers not sharing a common superscript are significantly ($p<0.05$) different from each other.

RESULTS

Food Intake and Body Weight

Daily caloric intake averaged across the experiment differed significantly as a function of dietary conditions, $F(3,32) = 3.64$, $p<0.02$ (Table 1). Rats given Purina Chow and the sucrose solution consumed significantly more calories than rats given either Purina Chow alone, or Purina Chow and the saccharin solution.

Although animals given either Crisco, or the sucrose solution in addition to Purina Chow gained more weight than rats given only Purina Chow, or Purina Chow and the saccharin solution, these differences were not significant (Table 1).

Analgesic Responses

Analgesic responses were obtained by subtracting premorphine baseline tail-flick latencies from latencies recorded at each time interval following morphine administration. No differences in tail-flick latency were observed as a function of dietary conditions when animals received saline injections (Table 2). Additionally, premorphine baseline tail-flick latencies did not differ as a function of dietary conditions.

Injections of morphine led to dose-related increases in tail-flick latency. No differences in analgesic responsiveness were observed as a function of diet when rats when injected with 2.5 mg/kg morphine. When the 5.0 mg/kg dose was administered, tail-flick latencies of animals fed either sucrose or Crisco in addition to Purina Chow were longer than those of rats fed only Purina Chow or Purina Chow and saccharin. These differences, however, failed to reach statistical significance (Table 3). Diet did significantly affect morphine-induced analgesia when 10 mg/kg morphine was injected. At 30 minutes following the administration of 10 mg/kg morphine, rats given either sucrose, saccharin,

TABLE 2

MEAN TAIL-FLICK LATENCIES (s) AFTER PHYSIOLOGICAL SALINE INJECTIONS

	Minutes After Injections			
	0	30	60	90
Purina Chow	2.7	2.5	2.5	2.5
Purina Chow + Saccharin	2.6	2.3	2.6	2.3
Purina Chow + Sucrose	2.7	2.2	2.4	2.4
Purina Chow + Crisco	2.4	2.3	2.4	2.4

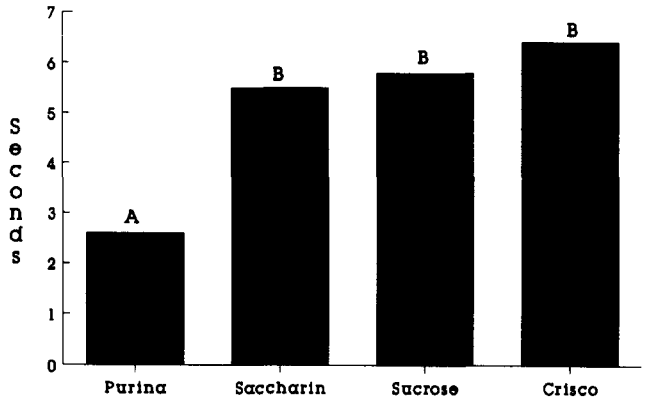

FIG. 1. Analgesic responses (tail-flick latencies at 30 minutes following injections of 10 mg/kg morphine sulfate − tail-flick latencies immediately preceding morphine injections) for rats fed either Purina Chow alone, or given a choice of Purina Chow and either a 0.15% sodium saccharin solution, a 32% sucrose solution, or hydrogenated vegetable fat (Crisco). Groups not sharing a common letter are significantly different from each other.

or Crisco in addition to Purina Chow displayed significantly longer tail-flick latencies than rats given only Purina Chow, $F(3,32) = 9.78$, $p<0.0001$ (Fig. 1). At 60 minutes following injections, rats given Crisco and Purina Chow continued to show significantly greater tail-flick latencies than rats given Purina Chow alone, $F(3,32) = 3.54$, $p<0.03$. Additionally, at 60 minutes following injections, tail-flick latencies of rats fed Crisco and Purina Chow was significantly greater ($p<0.05$) than latencies for rats fed saccharin and Purina Chow.

DISCUSSION

The results of the present experiment demonstrate that dietary variables can play a significant role in morphine-induced analgesia. Rats given either a sucrose solution, a saccharin solution or hydrogenated vegetable fat in addition to Purina Chow displayed

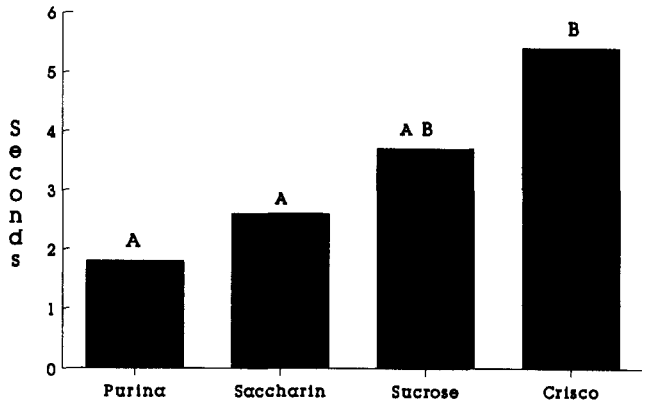

FIG. 2. Analgesic responses (tail-flick latencies at 60 minutes following injections of 10 mg/kg morphine sulfate − tail-flick latencies immediately preceding morphine injections) for rats fed either Purina Chow alone, or given a choice of Purina Chow and either a 0.15% sodium saccharin solution, a 32% sucrose solution, or hydrogenated vegetable fat (Crisco). Groups not sharing a common letter are significantly different from each other.

TABLE 3

MEAN TAIL-FLICK LATENCIES (s ± SEM) 30 MINUTES FOLLOWING INJECTIONS OF 2.5 OR 5.0 mg/kg MORPHINE

	Dose of Morphine (mg/kg body weight)	
	2.5	5.0
Purina Chow	3.98 ± 0.91	3.39 ± 0.38
Purina Chow + Saccharin	3.73 ± 0.39	4.08 ± 0.30
Purina Chow + Sucrose	3.26 ± 0.59	5.68 ± 0.95
Purina Chow + Crisco	3.86 ± 0.78	5.07 ± 0.65

significantly longer tail-flick latencies following morphine administration than rats given only Purina Chow.

These results support previous work showing that access to palatable sweet-tasting solutions for prolonged periods of time increase the analgesic effects of morphine (3, 11, 12). Additionally, these data illustrate that the effects of dietary variables on morphine-induced analgesia are not limited to palatable carbohydrates, but also extend to fats. Indeed, at 60 minutes following injections of 10 mg/kg morphine, rats fed fat continued to display an elevated tail-flick latency relative to animals given only Purina Chow, while rats fed sucrose did not.

Although the mechanism for the increased analgesia in rats given a choice of a palatable-tasting substance and a standard laboratory diet is unknown, there are several potential alternatives. First, it is possible that sucrose and/or fat alter the ability of the body to metabolize morphine, thus leading to higher levels of plasma morphine and increased morphine analgesia (14).

A second feasible alternative is that dietary variables interact directly with endogenous opioid systems to influence pain sensitivity. There is substantial evidence to propose that intake of palatable sweet-tasting foods leads to the release and/or breakdown of endogenous opioid peptides. In support of this proposal, Dum and colleagues (4,5) reported that intake of palatable sucrose-containing foods results in an increase in the amount of beta-endorphin occupying hypothalamic receptors in rats.

Differences in opiate receptor binding also have been observed as a function of both sucrose and fat feeding. For example, Marks-Kaufman et al. (10) reported that opiate receptor binding was significantly greater in genetically obese mice given access to sugar than in obese animals given access only to Purina Chow. Similar findings have been found in rats (Marks-Kaufman, unpublished data). While less well studied, there are data to suggest that dietary fats also increase opiate receptor binding, at least in some strains of animals (8). An increase in opiate receptor binding could accentuate the effects of exogenous morphine on pain sensitivity.

In the present study, both sucrose and saccharin increased morphine-induced analgesia. This result can be contrasted with the results of previous work in which we found that after morphine injections rats given a 32% sucrose exhibited a longer latency to respond on the tail-flick test, and rats given a 0.15% saccharin solution, a shorter latency to respond on the test than rats given only Purina Chow (11). Several differences between these studies could contribute to these discrepant results. First, differences in the intensity of the light source used in the tail-flick apparatus could have produced varied response. Second, while in both studies, Sprague-Dawley rats were used, in our original study these animals were virus and antibody free (VAF). There are data indicating that VAF rats respond differently to saccharin than non-VAF animals (6). Third, it should be noted that in the present study, there was a tendency for rats given saccharin to display

slightly lower tail-flick latencies than rats given sucrose or fat.

Another difference between this and previous studies [e.g., (11,12)] is that in this study, diet did not affect analgesic responsiveness in premorphine tests. Both Roane and Martin (12) and Marks-Kaufman et al. (11) found that chronic dietary supplements of sucrose increased pain sensitivity in rats. One reason for this discrepancy may relate to the intensity of the light source and baseline tail-flick latencies. In this study, rats rapidly removed their tails from the light source during premorphine testing. It may be that sucrose could not produce a further reduction in tail-flick latency.

In conclusion, the present data provide further evidence for an important role for dietary variables in determining the effects of exogenous opioids on pain sensitivity. Additional research is required to determine if this role is related directly to the nutritional value of the food, or to some more general characteristic such as palatability.

ACKNOWLEDGEMENT

This research was supported in part by Grant No. DA 04132 to R.M.K. and R.B.K. from the National Institute on Drug Abuse.

REFERENCES

1. Bergmann, F.; Lieblich, I.; Cohen, E.; Ganchrow, J. R. Influence of intake of sweet solutions on the analgesic effect of a low dose of morphine in randomly bred rats. Behav. Neural Biol. 44:347–353; 1985.
2. Blass, E. M.; Fitzgerald, E. Milk-induced analgesia and comforting in 10-day-old rats: Opioid mediation. Pharmacol. Biochem. Behav. 29:9–13; 1988.
3. Davis, W. M.; Miya, T. S.; Edwards, L. D. The influence of glucose and insulin pretreatment upon morphine analgesia in the rat. J. Am. Pharm. Assoc. 45:60–62; 1956.
4. Dum, J.; Herz, A. Endorphinergic modulation of neural reward systems indicated by behavioral change. Pharmacol. Biochem. Behav. 21:259–266; 1984.
5. Dum, J.; Gramsch, CH.; Herz, A. Activation of hypothalamic β-endorphin pools by reward induced by highly palatable food. Pharmacol. Biochem. Behav. 18:443–447; 1983.
6. Kanarek, R. B.; Marks-Kaufman, R. Factors influencing the effects of nutritive and nonnutritive sweeteners on energy intake and body weight. Appetite 11:16–19; 1988.
7. Klein, S. P.; Green, K. F. Tolerance to morphine analgesia from brief exposure to a palatable solution. Brain Res. Bull. 21:963–965; 1988.
8. Lee, C-R.; Marks-Kaufman, R.; Hamm, M. W. Effect of dietary fat on opiate receptor binding and body composition in mice. Nutr. Res. 7:1269–1280; 1987.
9. Lieblich, I.; Cohen, E.; Blass, E. M.; Bergmann, F. Morphine tolerance in genetically selected rats induced by chronically elevated saccharine intake. Science 221:871–873; 1983.
10. Marks-Kaufman, R.; Hamm, M. W.; Barbato, G. F. The effects of dietary sucrose on opiate receptor binding in genetically obese (ob/ob) and lean mice. J. Am. Coll. Nutr. 8:9–14; 1989.
11. Marks-Kaufman, R.; Kanarek, R. B.; Delanty, S. N. Sweet-tasting solutions modify the analgesic properties of morphine in rats. FASEB J. 2(5):A1567; 1988.
12. Roane, D. S.; Martin, R. J. Continuous sucrose feeding decreases pain threshold and increases morphine potency. Pharmacol. Biochem. Behav. 35:225–229; 1990.
13. Shide, D. J.; Blass, E. M. Opioid-like effect of intraoral infusions of corn oil and polycose on stress reactions in 10-day-old rats. Behav. Neurosci. 103:1168–1175; 1989.
14. Wade, A. E.; Wu, B.; Lee, J. Nutritional factors affecting drug-metabolizing enzymes of the rat. Biochem. Pharmacol. 24:785–789; 1974.