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Continuous Sucrose Feeding Decreases Pain Threshold and Increases Morphine Potency

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ROANE, D. S. AND R. J. MARTIN. *Continuous sucrose feeding decreases pain threshold and increases morphine potency.* PHARMACOL BIOCHEM BEHAV **35**(1) 225–229, 1990.—Although an opioid-mediated mechanism appears to be involved in the alteration of pain perception during feeding behavior, little is known about macronutrient effects on nociception. In this report we show that prolonged sucrose feeding alters responsiveness to painful stimuli and the analgesic potency of morphine. Male Sprague-Dawley rats maintained on ad lib laboratory chow with continuous access to a 20% sucrose solution displayed a significant decrease in tail-flick latency as early as 20 hours after introduction of the sucrose. The differences in pain threshold were naloxone sensitive. After 25 days on the diet, morphine sulfate, 8 mg/kg administered IP, proved to be significantly more potent in the sucrose-fed animals. The results indicate that sucrose feeding alters endogenous opioid-mediated nociception.

Sucrose Feeding Pain Morphine Analgesia Tail-flick Nociception Diet

PREVIOUS research has strongly indicated an opioid link between food intake and the perception of pain. Induction of feeding behavior by the tail-pinch method simultaneously elicits analgesia and both the feeding and analgesia are blocked by the opioid antagonist, naloxone (12). Concomitant elevations of pain threshold and feeding are also seen following injections of the antimetabolite 2-deoxy-D-glucose, with the analgesia being cross-tolerant to morphine (10). Food deprivation has been shown to alter central levels of endogenous opioids (6,9) and diminish sensitivity to nociceptive stimuli (14). McGivern and Berntson have reported that the well-known naloxone-reversible diurnal variations in pain threshold in rats are actually a function of diurnal feeding patterns (15).

The ingestion of sweet and highly palatable substances seem to interact with endogenous opioid systems. Rats fed sweet foods shortly before sacrifice showed a decrease in hypothalamic beta-endorphin and a decrease in opioid binding (3). The authors of this study attributed these phenomena to a release of hypothalamic beta endorphin following a "pleasurable stimulus." In addition to the reports on animals, obese humans have shown an increase in plasma levels of beta-endorphin following glucose ingestion (5).

Opioid interactions with sweet foods have been reported to affect pain sensitivity. Sucrose infused directly into the mouths of 10-day-old rats has the immediate effect of increasing pain threshold (1). This single meal effect is consistent with the work of Wurtman (25) which demonstrates that an insulin stimulating meal increases brain tryptophan and, hence, brain serotonin which then may facilitate a state of hypogesia. It should be noted that the increase in brain tryptophan and serotonin following high carbohydrate feeding appears to be a single meal effect and is not seen with extended high carbohydrate feeding when even small amounts of protein are included in the diet (4).

Very little has been reported on the effects of longer-term sugar-feeding as it relates to the perception of pain and opioid-mediated analgesia. One preliminary report has noted that continued sucrose feeding lowers pain threshold and enhances morphine analgesia (16). We now report similar findings: sustained sucrose feeding in rats results in an apparent opioid-mediated decrease in pain threshold, while increasing the potency of morphine analgesia.

METHOD

Forty male Sprague-Dawley rats weighing approximately 150 g each were sorted by weight into two groups and were individually housed under standard conditions with 12-hour photoperiods and were allowed ad lib access to Purina Rat Chow and water. After one week of accommodation to housing and handling conditions, and for the remainder of the testing period, one of the groups was

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given ad lib access to a 20 percent sucrose solution. All drug and nociceptive testing was done in the morning, beginning two hours after the onset of the light cycle. Pain thresholds were assessed by the radiant heat tail flick method, immediately prior to the introduction of the sucrose solution and again 20 hours later. Tail flick tests were performed again on days 7, 10, 12, 16, 18 and 25. One days 26 and 34 all animals were injected with morphine sulfate, 8 and 6 mg/kg respectively. In order to prevent the occurrence of tissue damage, predetermined limits (10 sec) were set on maximal allowable exposure to the noxious stimulus. Percent analgesia was determined by comparing each animal's tail flick latency obtained 30 min postinjection to the animal's tail flick latency obtained on day 25 according to the formula: percent analgesia = 100 × (drug value − control value)/(10 − control value). To test the effect of sucrose on endogenous opioid function in baseline pain threshold all animals were injected on day 32 with naloxone HCl (5 mg/kg) 30 minutes prior to tail flick testing.

In an attempt to examine the effects of sucrose feeding on stimulation-induced analgesia, on day 30, all animals were subjected to centrifugal rotation approximating the method described by Hayes *et al.* (8). In this procedure control tail flick latencies were established in three trials prior to centrifugation. The animals were placed individually in a cloth bag and centrifuged at 120 rpm (8 G's) for one min. Tail flick latencies were measured and percent analgesia was calculated using the same formula used for calculating morphine analgesia with the control value being the mean of the three latencies measured just prior to the centrifugation.

At the end of the testing period, the unanesthetized animals were sacrificed by rapid decapitation. Trunk blood was collected for the determination of serum insulin and glucose. The brains were rapidly removed and sectioned into two small blocks containing the periaqueductal gray (PAG) and nucleus raphe magnus (NRM) by a modified method of Segal and Kuczenski (21). Location of the PAG and NMR were made according to the areas shown in the stereotaxic atlas of Paxinos and Watson (19). The remaining carcasses were saved for body composition determination by the methods previously described (7). Serum insulin values were obtained by RIA (kit from Cambridge Medical Technology, Billerica, MA) and the serum glucose values were measured by the glucose oxidase method (Glucose Trinder, Sigma Chemical Co., St. Louis, MO).

NMR serotonin values were determined from supernatants of tissue homogenized and centrifuged in 0.1 N perchloric acid. Serotonin was quantified by peak heights following HPLC-EC with mobile phase being 0.5 M sodium acetate, 0.5 M octane sulfonic acid, 5% (v/v) methanol and 1.8% (v/v) tetrahydrofuran. Flow rate was 1 ml/min. The working electrode was glassy carbon referenced to a Ag/AgCl electrode (Bioanalytical Systems), oxidation potential was 0.65 V.

Relative density of mu opioid receptors in PAG were measured with 250 pM [^{125}I]-DAGO ([D-Ala^2,N-Me-Phe^4,Gly^5-ol]-enkephalin) at single concentrations due to the limited amount of tissue. The DAGO iodination procedure was performed with the chloramine-T method according to the conditions described by Zadina and Kastin (26). Purification of the monoiodinated peptide was achieved by HPLC. The binding conditions have been previously described (26). Briefly, tissues from the PAG dissections were weighed and homogenized in 10 volumes of a 0.32 M sucrose–50 mM Hepes buffer in a glass/Teflon mortar and pestle. This suspension was centrifuged 5 min at 1000 × g at 4°C. The supernatant containing the membrane fraction was carefully decanted and brought up to 50 volumes in 50 mM Hepes, pH 7.4 and centrifuged 20 min at 30,000 × g. The resulting pellet was washed twice in 50 mM Hepes and resuspended finally in 50 volumes of Hepes. Membranes were preincubated 30 minutes at 23°C. Binding tubes, in triplicate, consisted of 500 μl of the membrane suspension and 500 μl buffer containing 250 pM [^{125}I]-DAGO, ± 1 μM cold DAGO for nonspecific and total binding. Tubes were allowed to incubate for 90 minutes at 23°C. Membranes were collected by vacuum filtration over glass fiber filters using a Skatron cell harvester. Filters were then counted on a Beckman gamma counter. Specific binding was determined as total minus NSB.

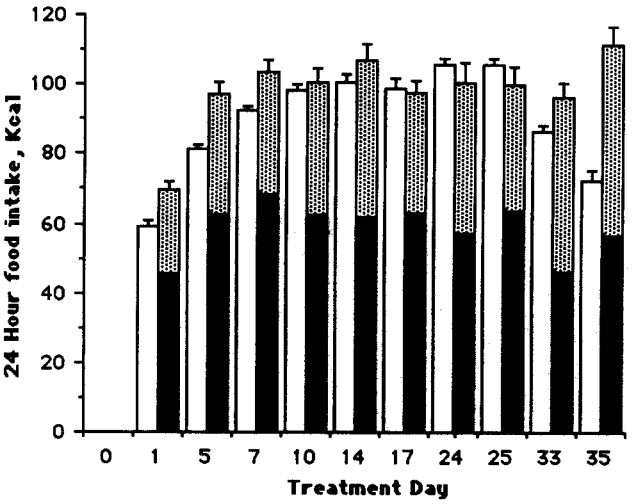

FIG. 1. This graph shows the average 24-hour calorie consumption over the course of the experiment. The stacked bars indicate the portion of calories consumed in the form of chow (solid bar) and sucrose (stippled bar) as compared to the calories consumed by the chow-fed animals (open bars).

RESULTS

The data presented in Fig. 1 show the average 24-hour kcal consumption from rat chow and sucrose. The sucrose-fed rats consumed an average of 40.8 percent of their daily calories from the sucrose solution. Analysis of variance with repeated measures indicates that the over the first 25 days of diet treatment the sucrose-fed animals were hyperphagic compared to the chow-fed controls with daily mean kcal intakes of 97.94 vs. 94.32, $F(1,532) = 4.17$, $p<0.05$. Body weight was not significantly affected by the diet treatment though there was a trend toward the sucrose-fed animals being heavier.

The body composition measures (Fig. 2) are all expressed as percentage of total carcass weight. Statistical analysis of these measures was performed by the general linear models procedure, least squares means. According to these measures the sucrose-fed rats had signficantly elevated fat stores, 11.53 vs. 8.16 ± 0.469 percent of carcass content, $p<0.001$. Carcass water contents were slightly, but significantly lower in the sucrose-fed group, 63.9 vs. 67.0 ± 0.76, $p<0.011$. No differences were seen in carcass protein content, 21.9 vs. 22.2 ± 0.41, sucrose vs. chow, and ash weight, 2.60 vs. 2.62 ± 0.12, sucrose vs. chow.

The results of the tail flick testing (Fig. 3) show a significant decrease in the latency of response in the sucrose-fed animals after twenty hours of sucrose feeding and these differences remained evident throughout the testing period ($p<0.05$, unpaired Student's *t*-test). The subsequent injection of naloxone abolished the nociceptive differences between the treatment groups. Following the morphine injections of 8 mg/kg (Fig. 3), the tail flick latencies for

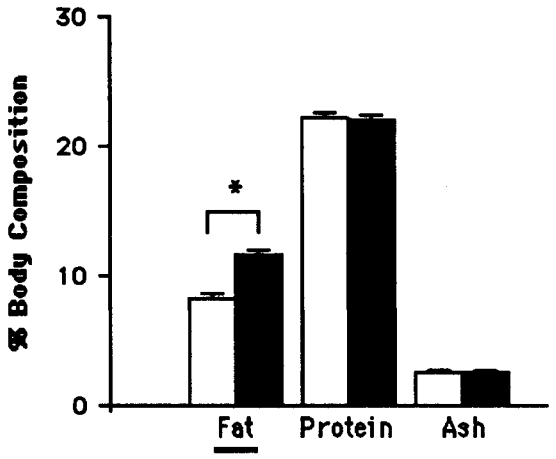

FIG. 2. The bar graphs show the body composition measures for body fat, protein and ash. Chow-fed group = open bars, sucrose-fed = solid bars. The values are expressed as percent of carcass weight. Sucrose feeding resulted in a significant increase in fat, 11.53 vs. 8.16 ± 0.469 percent, $*p<0.001$.

the sucrose-fed animals significantly surpassed those of the control animals ($*p<0.05$, t-test). Significant differences in analgesia were not found following 6 mg/kg morphine or centrifugal rotation.

Analysis of serum insulin and glucose, NRM serotonin and PAG DAGO binding are shown in Table 1. No significant differences due to the diet treatment were found with regard to (values listed as chow vs. sucrose-fed, means ± s.e.) serum glucose 135.5 ± 3.74 vs. 138.2 ± 2.5 mg/dl, serum insulin 24.8 ± 0.51 vs. 26.26 ± 0.47 uU/ml, NRM serotonin 23.9 ± 5.5 vs. 31.9 ± 6.6 pg/mg tissue, or [^{125}I]-DAGO binding 6396 ± 786 vs. 6906 ± 1205 dpm/mg protein.

DISCUSSION

Our interpretations of these results are that sustained ad lib sucrose feeding in rats interferes with endogenous opioid mediation of pain thresholds as assessed by the tail flick method. This conclusion is supported by the findings that a) sucrose-fed rats showed consistently lower tail flick latencies when compared to the control animals and, b) opioid blockade following the administration of naloxone eliminated the differences in tail flick latencies.

The finding of an increased potency of morphine at a selected dose in sucrose-fed rats is consistent with a previous report (16). The mechanism of this phenomenon is not known, but one possible explanation is that long-term sucrose feeding diminishes the capacity of the liver to metabolize morphine, thus leading to higher levels of plasma morphine and increased morphine analgesia. An earlier report (24) has shown that high levels of dietary sucrose in rats results in lower levels of hepatic cytochrome p-450, an enzyme critical to the metabolism of morphine.

An alternate explanation also seems plausible. Pain threshold reduction by sucrose feeding seems to be attributable to some alteration in endogenous opioid function. It may be that sucrose-feeding initiates a mechanism that inhibits the tonic release of endogenous opioids. The increase in morphine potency may be due to an increase in opioid receptors which could result from receptor up-regulation following a lack of exposure of the receptors to the endogenous ligand. This postulation is not confirmed by the PAG mu receptor binding data. However, the studies by Millan *et al.* (17,18) involving a rat model of altered pain perception show that pain thresholds are decreased, morphine analgesia is heightened, but no changes are seen in mu (DAGO-labelled) opioid receptor binding. These features are consistent

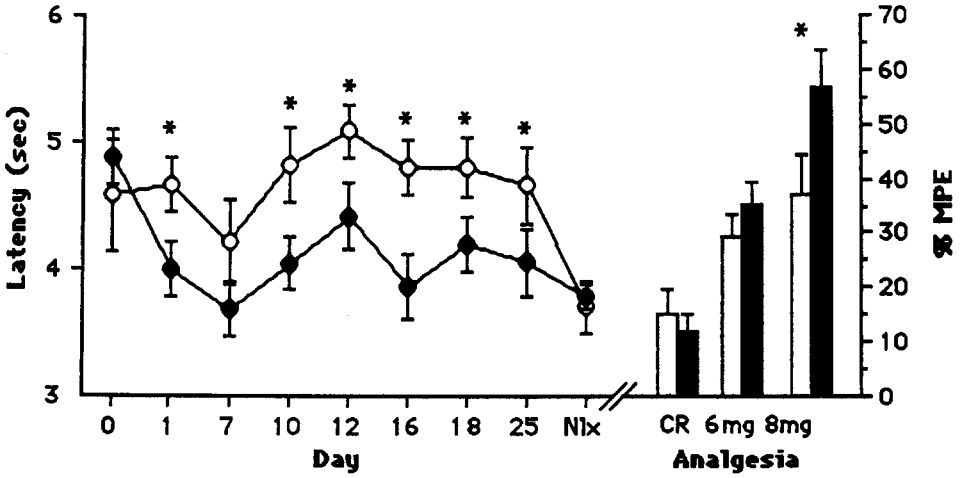

FIG. 3. The line graph on the left-hand side of this figure shows the latency of response (seconds) of chow-fed (open circles) and sucrose-fed (closed circles) rats. The measurement at the first time-point was made immediately prior to the introduction of sucrose. The last point shows the tail-flick latencies 30 min after the injection of naloxone HCl (5 mg/kg). The bar graph on the right-hand side shows the percent maximal analgesia (% MPE) that results following centrifugal rotation (CR) or morphine (6 and 8 mg/kg). Chow-fed = open bars, sucrose-fed = solid bars. Comparisons are made between sucrose-fed and controls at the same time-point. ($*p>0.05$, unpaired Student's t-test.)

TABLE 1

SERUM GLUCOSE AND INSULIN VALUES, NRM SEROTONIN CONTENT AND DAGO BINDING TO PAG

	Chow-Fed	Sucrose-Fed
Glucose (mg/dl)	135.5 ± 3.74 (20)	138.2 ± 2.5 (20)
Insulin (Uu/ml)	24.8 ± 0.51 (20)	26.26 ± 0.47 (19)
NRM serotonin (pg·mg tissue)	23.9 ± 5.5 (9)	31.9 ± 6.6 (8)
DAGO binding (dpm/mg protein)	6396 ± 786 (8)	6906 ± 1205 (9)

Statistical analysis was performed by unpaired Student's *t*-test. The numbers in parentheses are the n/group. No significant differences were found due to diet treat in any of these measures.

with the data we present here. Interestingly, the model described by Millan shows an increase in PAG kappa opioid receptor binding and a reduced level of beta-endorphin in the same area. While we did not measure these parameters in our study, we certainly plan to do so in future studies with sucrose feeding.

A previous report (8) indicated that centrifugal rotation elicits analgesia that is not opioid mediated in that it is not reversed by naloxone. In repeating this procedure in our sucrose-fed rats, we found that centrifugal rotation did induce a mild level of analgesia (presumably nonopioid) which was not found to be statistically different with respect to the diets.

We assayed serum glucose and insulin because a fair number of studies have shown that treatments that produce alterations in these substances concomitantly alter opioid analgesia. These studies, in mice and Sabra rats, have shown that forced alterations in plasma glucose levels achieved by streptozotocin pretreatment or by IP infusion of carbohydrate result in a decrease in morphine potency (13, 20, 22). These reports represent a potential source of conflict with our current data if sucrose feeding elevates plasma glucose. However, our measurements of serum glucose and insulin revealed no differences between the two groups of animals (Table 1) and these values are similar those reported earlier in animals on similar diets (23).

The reward component of sucrose feeding may play an important role in the alteration of pain threshold and opioid function. In studying the interactions between aversion, pain sensitivity and sapid solutions, LeMagnen *et al.* have suggested the hypothesis that reward and pain modulating systems belong to a biochemical continuum in a functionally unique brain mechanism (11). Further, Carr (2), who has extensively studied lateral hypothalamic stimulation-mediated reward, aversion and analgesia, has postulated that "opioids potentiate reward processes in the service of an appetitive motivational state" that "may concurrently diminish emotional responsiveness to aversive stimuli." We support these conclusions which, together with an earlier report stating that direct oral infusion of a sucrose solution caused an immediate increase in pain threshold (1), bolster the hypothesis that endogenous opioid activity subserving the mediation of response to noxious stimuli is elevated immediately after sucrose feeding. We conclude that the data presented here allows for the expansion of this hypothesis to include a refractory period of endogenous opioid activity following longer-term sucrose feeding and that sustained consumption of sucrose sustains the diminution of pain threshold by a unknown mechanism.

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