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BRIEF COMMUNICATION

Diet and Estrous Cycle Influence Pain Sensitivity in Rats

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FRYE, C. A., C. A. CUEVAS AND R. B. KANAREK. *Diet and estrous cycle influence pain sensitivity in rats.* PHARMACOL BIOCHEM BEHAV 45(1) 255–260, 1993.—Effects of estrous cycle and acute and chronic access to palatable fluids on tail-flick latency and opiate-induced analgesia were assessed in 124 female Long Evans rats. Following three consistent cycles, rats were water deprived for 8 h and then given ad lib access to 20 ml of either water, a 32% sucrose solution, or corn oil for 5 h. Nociceptive testing was conducted immediately preceding and 30, 60, and 90 min following an SC injection of morphine sulfate (7.5 mg/kg). Diestrus rats had prolonged premorphine tail-flick latencies compared to rats in proestrus. Rats that consumed corn oil had longer tail-flick latencies preceding and 30 min following morphine injections than rats that drank water or the sucrose solution. Rats were retested after they had ad lib access to the same fluid for 3 weeks. No estrous cycle differences were noted following chronic consumption. Rats with chronic access to sucrose showed increased baseline pain sensitivity and increased morphine-induced analgesia at 30, 60, and 90 min postinjection. These data support the notion that palatable fluid consumption attenuates estrous cycle-dependent differences in pain sensitivity.

Hormones Estrogen Progesterone Nociception Tail-flick latency Opiates Morphine Sucrose consumption Fat consumption

THE steroid hormones, estradiol (E) and progesterone (P), separately and synergistically influence pain sensitivity. Evidence indicates that E is involved in the regulation of analgesia. For example, opioid analgesia in ovariectomized rats is restored by E replacement; however, further increases in E suppress analgesia (13). Additional support for a role of E in opioid-mediated behavior comes from data demonstrating that central levels of β-endorphin and met-enkephalin fluctuate with the estrous cycle (16). Estrogen treatment reduces sensitivity to opioid antagonists (21), and 17-α-estradiol and its metabolites show selective affinity for opiate receptors (14).

Progesterone also has well-documented effects on pain sensitivity. For example, P and its reduced analogs produce analgesia and sedation in experimental animals independent of E (1,20,28). When P is administered in conjunction with E, in a manner consistent with the endogenous hormonal milieu, dose-dependent alterations in nociception are noted (10, 11). However, the relative roles that endogenous E and P have on pain sensitivity and whether this may contribute to sex differences in opiate-induced analgesia (13) are not known.

Consumption of palatable foods and fluids also influences pain sensitivity. The relationship between opioids and feeding is reciprocal, such that opioid agonists promote feeding, particularly of palatable foods, (4,17,18,22) and consumption of sweet, highly palatable foods can alter nociception and opioid-mediated analgesia (2,12,23). Similar to morphine administration, the effects of palatable foods on nociception vary depending upon whether exposure is acute or chronic. Acute intake of sweet-tasting fluids produces increases in analgesia and tolerance to morphine-induced analgesia (2), while chronic intake results in increases in pain sensitivity and a potentiation of morphine-induced analgesia (10,12,23).

Preliminary results from our laboratory suggest that chronic access to sucrose may attenuate hormonally induced differences in pain sensitivity in repeatedly tail-flicked female rats (10). The purpose of the present experiment was to confirm these earlier findings in experimentally naive rats and extend them to determine whether acute access to palatable fluids can also influence hormonally induced differences in pain sensitivity. A between-subjects design was used to compare pain sensitivity of rats that had acute and chronic access to sucrose, corn oil, or water on each day of the cycle.

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METHOD

Animals

One-hundred and 24 virus antibody free (VAF), naive, adult, female Long Evans rats (Charles River, Raleigh, NC), weighing between 200 and 225 g at the beginning of the experiment, were used. Animals were individually housed in standard stainless steel cages in a temperature- (21 ± 1°C) and humidity- (30–40%) controlled room, with a 12 L : 12 D cycle (lights on 2145 h).

Cycling

Samples of vaginal epithelium were obtained from all animals by daily lavage between 0730 and 0930 h to verify the day of the estrous cycle (6). Vaginal epithelium were examined with low-power light microscopy by an uninformed, independent observer and classified as being characteristic of diestrus 1, diestrus 2, estrus, metestrus, or proestrus. Diestrus 1 smears were distinguished from metestrus smears by the absence of leukocytic cells.

Drugs

Morphine sulfate, generously provided by the National Institute on Drug Abuse, was dissolved in 0.9% saline at a concentration of 7.5 mg/ml.

Nociceptive Testing

Rats were placed on the platform of the tail-flick apparatus and their tails smoothed into the groove. All animals were held gently by the same experimenter during all tests. A light source was focused halfway up the tail with the intensity of the light adjusted before the study began with a rheostat to obtain a control tail-flick latency of 2–4 s. If animals did not respond within 9 s, the light source was automatically turned off to prevent tissue damage. Rats were tested three times preceding morphine administration, and the median tail-flick response was used as a baseline measure.

To examine the effects of dietary variables on morphine-induced analgesia, animals were injected with morphine sulfate (7.5 mg/kg body weight, SC). Reaction times on the tail-flick apparatus were measured 30, 60, and 90 min following drug injection.

Procedure

Animals were randomly assigned to one of three groups. Animals in each group had ad lib access to ground Purina Rodent Chow No. 5001. All animals had ad lib access to food and tapwater throughout the experiment except for an 8-h water deprivation period prior to presentation of sucrose, corn oil, or water. Animals with three consistent estrous cycles were considered for testing. Experimental subjects were water deprived from 2200–0600 h on the night prior to testing. Twenty milliliters of a 32% sucrose solution (n = 42), corn oil (n = 42), or water (n = 40) were presented 5 h prior to nociceptive testing. Purina Chow was presented in Wahmann (Timoniun, MD) LC-306A nonspill food cups. Sucrose, corn oil, and water were presented in glass water bottles with rubber stoppers with nonleaking stainless steel drinking spouts. Rats given either the sucrose solution or water consumed almost all of the fluid in the 5-h access period, while rats given corn oil consumed only 3–4 ml of the fluid. There are a number of possible reasons for this difference in fluid intake, including the palatability and/or caloric density of the fluids. In fact, on a caloric basis, rats given the sucrose solution, which contains 1.28 kcal/ml, and rats given corn oil, which contains 9 kcal/ml, consumed approximately the same number of kilocalories.

All animals were tested for nociceptive responses beginning at 1100 h, 1.5 h after lights out. Animals were tested so that there were eight or nine rats in each stage of the estrous cycle in each dietary group.

Following acute testing, animals were provided ad lib access to the solution they had consumed in the acute test in addition to chow and water. Animals were continually monitored for day of the estrous cycle; however, no attempt was made to match day of the cycle for acute and chronic testing. After a minimum of 3 weeks, most animals (n = 69) were retested for nociceptive responses. Unfortunately, climate control problems in the animal housing facility prior to the conclusion of the experiment precluded retesting all animals in the chronic condition.

Statistical Analysis

Tail-flick latencies following morphine were expressed as the percent maximum possible effect (% MPE), using the following equation:

$$\%\,\text{MPE} = 100 \times \frac{(\text{postmorphine response latency}) - (\text{baseline latency})}{(\text{cut-off time-9 s}) - (\text{baseline latency})}$$

Pain sensitivity data were analyzed using two-way analyses of variance (ANOVAs) followed by multiple comparisons using Scheffe's method for posthoc t-tests. One-way ANOVA was used to examine representative daily energy intake from chow and the auxiliary fluids during the 3 weeks of chronic access to the palatable foods.

RESULTS

Acute Testing

Two-way ANOVAs were used to examine diet (water, sucrose, or corn oil) and day of cycle (diestrus 1, diestrus 2, proestrus, estrus, metestrus) effects on baseline tail-flick latency and MPE 30, 60, and 90 min after morphine injections. There were main effects of diet, $F(2, 123) = 3.14$, $p < 0.05$, and day of cycle, $F(4, 123) = 2.68$, $p < 0.05$, as well as an interaction, $F(8, 123) = 2.07$, $p < 0.05$, between diet and day of cycle at premorphine baseline. Posthoc comparisons revealed tail-flick latency was significantly lower during proestrus relative to diestrus 1 and 2 (Fig. 1). At baseline, rats that had acute access to corn oil had significantly elevated mean tail-flick latency (χ = 3.06 ± 0.09 s) compared to those who had sucrose (χ = 2.71 ± 0.09 s) or water (χ = 2.91 ± 0.10 s). This diet effect was also borne out in testing 30 min after morphine. There was a trend for a main effect of diet, $F(2, 123) = 2.66$, $p = 0.07$, but no cycle effect or interaction. Posthoc t-tests indicate that 30 min following morphine injections rats consuming corn oil had significantly ($p < 0.05$) elevated MPEs compared to those with water. Sucrose was less effective at potentiating morphine-induced analgesia. No main effects or interactions were noted 60 or 90 min after morphine (Fig. 2).

Chronic Testing

There was a main effect of diet, $F(2, 68) = 12.56$, $p <$ 0.05, noted in premorphine testing. Those animals with

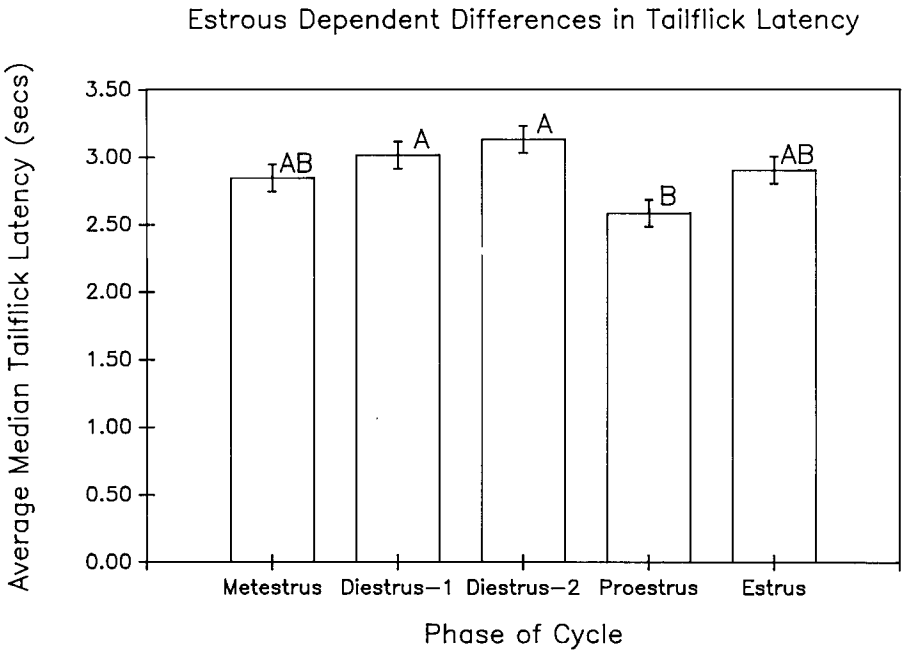

FIG. 1. Estrous-dependent differences in mean baseline tail-flick latency ± SE (seconds) averaged across dietary conditions. Bars with different letters are significantly different from one another ($p < 0.05$).

chronic sucrose had decreased tail-flick latencies (χ = 2.53 ± 0.08 s) compared to the water (χ = 3.08 ± 0.12 s) or corn oil animals (χ = 3.35 ± 0.13 s). In assessing cycle effects subsequent to chronic fluid consumption, diestrus 1 and 2 were combined because there were no differences between these 2 days; this allowed all cycle × diet groups to have a minimum of five members. There were no main effects of cycle noted after chronic consumption at baseline, but posthoc *t*-tests revealed that animals in proestrus that had access to fat showed significantly elevated tail-flick latencies (Fig. 3).

Main effects of diet were also noted 30, $F(2, 68) = 4.55$, $p < 0.05$, 60, $F(2, 68) = 5.82$, $p < 0.05$, and 90 min after

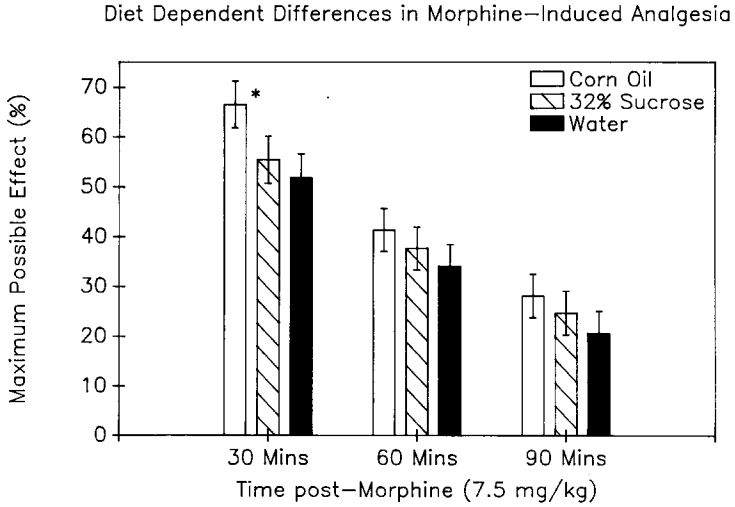

FIG. 2. Acute diet-dependent differences in morphine-induced analgesia measured by tail-flick latency 30, 60, and 90 min after 7.5 mg/kg morphine. Morphine-induced analgesia is expressed as maximum possible effect (MPE) or percent possible increase over baseline ± SE. *MPE for rats consuming corn oil and chow significantly greater than MPE for rats consuming only chow.

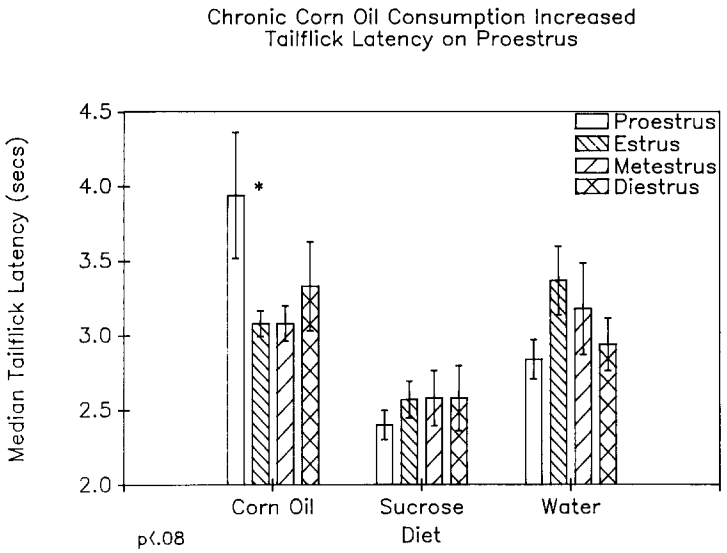

FIG. 3. Chronic diet-dependent differences in mean baseline tail-flick latency ± SE. *Tail-flick latencies for animals tested on proestrus with chronic access to corn oil significantly ($p < 0.08$) greater than tail-flick latencies for all other groups.

morphine injections, $F(2, 68) = 3.06$, $p < 0.05$. Posthoc tests revealed that animals that had access to sucrose had increased MPEs 30, 60, and 90 min after morphine injections (Fig. 4).

Consumption

One-way ANOVA was used to compare representative total calorie consumption and calories consumed from chow during the 3-week period between testing for the acute and chronic effects of diet on morphine-induced analgesia. No differences were noted in total calories consumed between animals fed corn oil ($\chi = 77.6 \pm 3.4$) or sucrose ($\chi = 77.6 \pm 3.0$); however animals with access to water consumed fewer calories ($\chi = 58.7 \pm 13.7$). Sucrose animals consumed significantly fewer calories from chow (20.5 ± 1.6) than corn oil (33.9 ± 2.4) or water only (58.7 ± 13.7) animals.

DISCUSSION

The results of the present experiment demonstrate that estrous cycle and acute and chronic dietary variables can significantly affect pain sensitivity in female rats. Rats given acute corn oil had longer tail-flick latencies before and after morphine. Access to sucrose revealed varying effects on pain sensitivity depending upon duration of consumption and morphine administration. For example, following acute intake of sucrose, animals were more sensitive to pain in the baseline condition. However, acute intake of sucrose did not affect morphine-induced analgesia. After chronic sucrose consumption, animals had decreased tail-flick latencies before morphine, but after morphine the opposite was found. Chronic sucrose consumption increased morphine-induced analgesia 30, 60, and 90 min after injection. These dietary differences did not override the premorphine increase in pain sensitivity during proestrus after acute consumption but they did after chronic consumption. This suggests that morphine and chronic dietary variables may override estrus-dependent differences in pain sensitivity.

These findings support previous reports indicating that access to palatable sweet-tasting solutions or high-fat foods for prolonged periods of time increase the analgesic effects of morphine (3,12,19,23,27). In addition, these data illustrate that acute fat intake can reduce pain sensitivity. Further, access to palatable foods affects pain sensitivity in females, as has been noted previously in males (12).

As well, these findings support previous work showing increases in nociception during proestrus and estrus. Pain sensitivity measured by flinch and jump threshold after electric foot-shock (7) and tail-flick (10,13) is increased during proestrus/estrus. The postmorphine increases in analgesia during proestrus (for corn oil animals) presently noted confirms proestrus increases in tail-flick latency and jump threshold 60 min postmorphine (13). Significant postmorphine elevations in proestrus may be related to the sufficient "room" for analgesia; normal increase in pain sensitivity are noted on this day, which coincides with fluctuating low levels of E and P (10,29). Alternately, refractory increases in morphine-induced analgesia may be due to alterations in metabolism over the cycle (5) and/or hormonally induced alterations in affinity for the opiate receptor (14).

Sex differences in continuous and intermittent cold water swimming before and after morphine (24,26) and after morphine in tail-flick and jump tests (13) have been reported. Females appear to be more sensitive to pain and show less morphine-induced analgesia. In the present experiment, acute and chronic fat consumption attenuated estrous-dependent differences in pain sensitivity. It is interesting that oil injections similarly override sex differences in tail-flick latency and jump tests after cold water swim-induced analgesia (25). Together, this suggests that intake of different fats may alter the efficiency of promoting analgesia (31).

The mechanism for the increase in analgesia in rats given a

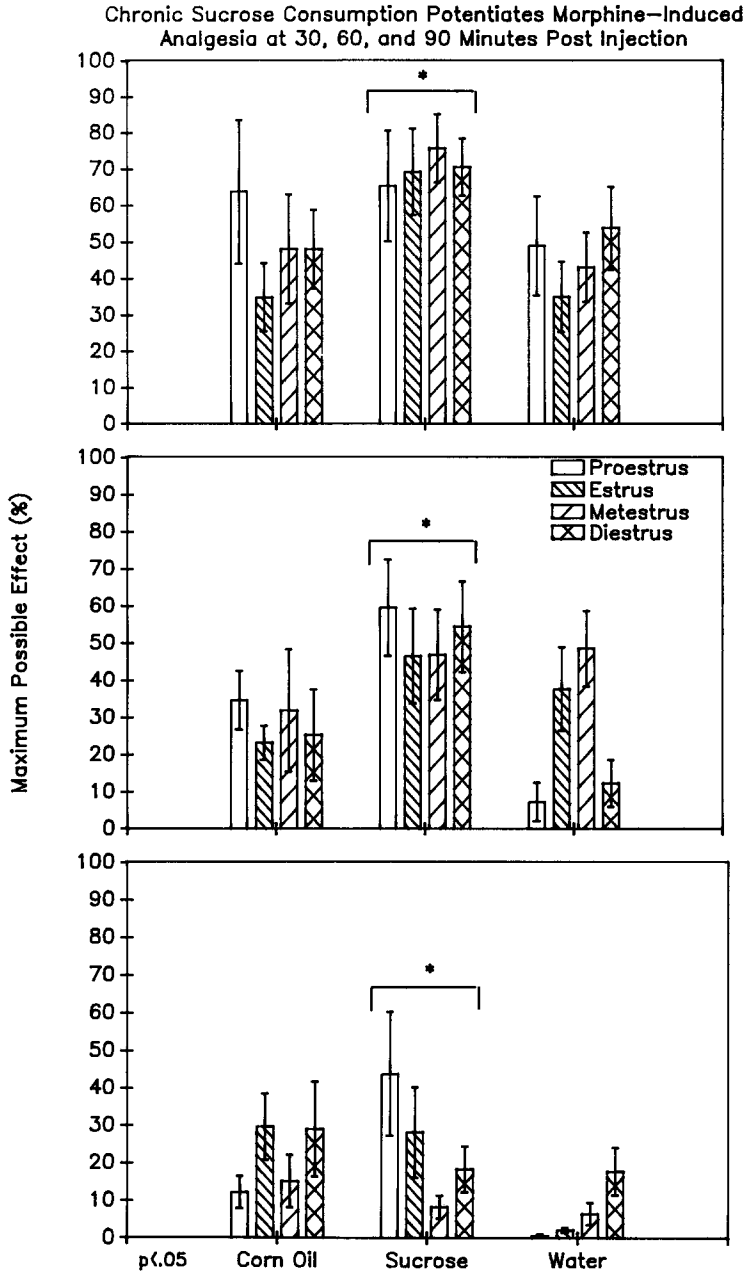

FIG. 4. Chronic diet-dependent differences in morphine-induced analgesia measured by tail-flick latency 30 (top), 60 (middle), and 90 (bottom) min after 7.5 mg/kg morphine. Morphine-induced analgesia is expressed as maximum possible effect (MPE) or percent possible increase over baseline ± SE. *MPEs for rats chronically consuming sucrose significantly ($p < 0.05$) greater than MPEs for animals fed corn oil or chow alone at 30, 60, and 90 min after morphine administration.

choice of palatable-tasting substances in addition to a standard laboratory diet are unknown. It is possible that sucrose and/or fat alter morphine metabolism and lead to higher levels of plasma morphine and increased morphine analgesia (30). However, recent work demonstrating that rats consuming sucrose and fat show increased analgesia relative to rats fed only chow following ICV administration of morphine (Kanarek, Frye, and Marks-Kaufman, unpublished data) make this possibility unlikely.

It is also possible that dietary variables interact directly with the endogenous opioid system to effect pain sensitivity. Palatable foods may lead to the release or breakdown of endogenous opioids. For example, in rats, sucrose consumption increases the number of hypothalamic receptors occupied by

β-endorphin (8,9). Dietary access to sucrose (19) and fat (15) also increases opiate binding. An increase in opiate receptor binding could accentuate the effects of exogenous morphine on pain sensitivity.

In conclusion, the present data provide further evidence for hormonally induced differences in pain sensitivity while reinforcing the notion that dietary variables are important factors effecting exogenous opioid's impact on pain sensitivity. The overriding effects of palatable foods on pain sensitivity are of interest because nociception and food intake normally fluctuated over the cycle. The extent and mechanism by which altered consumption of foods may normally attenuate hormonally induced increases in pain sensitivity is presently under investigation.

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