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Duration of Sucrose Availability Differentially Alters Morphine-Induced Analgesia in Rats

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D'ANCI, K. E., R. B. KANAREK AND R. MARKS-KAUFMAN. *Duration of sucrose availability differentially alters morphine-induced analgesia in rats.* PHARMACOL BIOCHEM BEHAV **54**(4) 693–697, 1996.—The effects of duration of sucrose consumption on morphine-induced analgesia (MIA) were examined in 20 adult male Long–Evans rats. Ten rats were tested for MIA on a tail-flick apparatus following acute (5 h), chronic (3 weeks) intake, and subsequent removal of a 32% sucrose solution. Ten rats that never received the sucrose solution served as controls. Morphine sulfate was administered according to a cumulative dosing procedure beginning with 2.5 mg/kg morphine. The same dose was administered every 30 min until a total dose of 15 mg/kg was achieved. Tail-flick latencies were measured immediately prior to injections, and 30 min following each injection. After acute intake of sucrose, there was a trend for animals drinking the sugar solution to show suppressed MIA relative to animals drinking water. In contrast, after drinking the sucrose for 3 weeks, rats showed an enhanced MIA relative to rats drinking water. Three weeks after sucrose removal, there were no differences in MIA as a function of prior dietary conditions. The results support the hypothesis that length of exposure to sucrose influences morphine-induced analgesia and suggest that any change in physiology resulting from sucrose exposure may be reversible.

Morphine Analgesia Sucrose Tail-flick Pain Rats Opiates Palatability Sweet taste

RESEARCH over the past decade demonstrates an association between the endogenous opioid system and food intake. The administration of opiate agonists increases food intake, particularly intake of highly palatable or preferred foods, while administration of opiate antagonists decreases palatable food intake [for review, see (14)]. Further evidence of a link between the hedonic properties of foods and the endogenous opioid system comes from work illustrating that consumption of palatable foods produces a change in the analgesic potency of morphine [e.g., (2,10,11,13,15,20)]. Although previous research has demonstrated that intake of palatable sweet fluids can moderate morphine-induced analgesia (MIA) in experimental animals, there are conflicting reports of what actually occurs. Intake of sweet solutions has been reported to both decrease and increase sensitivity to morphine's analgesic effects (1–3,6,10–18,20). Examination of these studies suggests that length of exposure to sweet solutions prior to nociceptive testing may be important in determining whether these solutions diminish or enhance the analgesic properties of morphine. In experiments in which animals were given access to sweet solutions for a relatively short time (6–48 h), consumption of these solutions generally was associated with a decrease in the analgesic potency of morphine (1,10,12). In contrast, in experiments in which animals were allowed to drink sweet solutions for a longer period of time (2–4 weeks), intake of sweet substances enhanced MIA (11,15,18,20).

The present experiment is part of an ongoing body of work designed to resolve the apparently divergent results of previous research. It was hypothesized that animals that were tested for antinociception following acute (5 h) access to a 32% sucrose solution would display decreased MIA relative to rats drinking water. It was anticipated that the same rats given chronic exposure to the sucrose solution would show increased MIA relative to rats drinking water. Finally, to determine if dietary-induced changes in sensitivity to morphine were permanent or transient, MIA was assessed for a final time, 3 weeks after removal of the sucrose solution.

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METHOD

Animals

Twenty male Long–Evans VAF rats (Charles River, Portage, MI), weighing between 250–295 g at the beginning of the experiment, were used. Animals were individually housed in standard hanging stainless steel cages in a temperature-controlled room (22 ± 2°C), maintained on a 12 L:12 D reverse cycle (lights on at 2000 h). All manipulations were conducted under red lights during the middle of the dark phase (1330–1700 h).

Feeding and Diets

All animals were given ad lib access to ground Purina chow. Water was freely available throughout the experiment, except as noted. Rats were divided into two groups initially matched on the basis of body weight. During the acute and chronic phases of the experiment half of the animals (n = 10) were given ad lib access to a 32% sucrose solution (w/v) in addition to chow. Chow was presented in Wahman LC306A (Timonium, MD) stainless steel food cups with lids. The cups were clipped to the cage floors to prevent spillage. Both water and the sucrose solution were presented in glass bottles fitted with drip-proof stainless steel spouts. Chow and liquid intakes and body weights were measured every other day. The positions of the bottles were switched at the time of weighing to preclude the development of a side preference.

Drugs

Morphine sulfate, provided by the National Institute on Drug Abuse, was dissolved in physiological saline in a concentration of 2.5 mg/ml. Injections were administered IP at a volume of 1 ml/kg.

Nociceptive Testing

Pain thresholds were assessed by the radiant heat tail-flick method (5). All animals were taken into the procedure room and placed on the tail-flick apparatus (Endie Instrument Co., Montpelier, VT) with their tails smoothed into the tail groove. All rats were held gently in a clean cloth by the same experimenter. The light source was activated and remained focused on the tail until the rat moved its tail, thus switching the light off, or until 9 s had elapsed. A 9-s cutoff point was employed to prevent tissue damage to the tail.

A baseline measure was determined by using the median of three tail-flick tests, separated by approximately 15 s. Immediately after determining the baseline latency, the animals were injected with 2.5 mg/kg of morphine and returned to their cages for 30 min. Tail-flick latencies were again measured, and the animals were again injected and returned to their cages for another 30 min. This procedure was repeated a total of six times, yielding a final cumulative dose of 15 mg/kg.

Acute Presentation of Sucrose

All animals were water deprived overnight for a total of 15 h to encourage drinking. Ten animals were given the sucrose solution and 10 were given tap water. The animals were permitted to drink the respective liquids for 5 h before the nociceptive testing commenced.

Chronic Presentation of Sucrose

Once the nociceptive testing for the acute phase of the experiment was completed, the 10 animals that had been given sucrose were maintained on sucrose and water for 3 weeks before the second session of nociceptive testing. During this period, the other ten rats were given only water to drink.

Removal of Sucrose

The day following tail-flick testing for the chronic phase, the sucrose solution was removed and all rats given only water to drink. Nociceptive testing was conducted 3 weeks after the sucrose solution was removed.

Statistical Analysis

The data were analyzed with repeated measures two-factor ANOVAs (diet by dose) and repeated measures three-factor ANOVAs (diet by dose by phase of experiment). Post hoc comparisons were done using t-tests to determine differences between latencies for specific doses. Analyses were conducted on the percent maximal possible effect (%MPE), which was calculated as follows:

$$\%\text{MPE} = \left(\frac{\text{test latency} - \text{baseline latency}}{\text{maximal latency} - \text{baseline latency}}\right) \times 100$$

where maximal latency is the cutoff time of 9 s (7). All procedures were approved by the Tufts University Institutional Animal Care and Use Committee.

RESULTS

Acute Sucrose Exposure

All animals drank at least 10 ml of the liquid presented to them. Rats consuming the sucrose solution drank significantly less liquid (19.0 ± 5.1) than rats drinking water (30.4 ± 5.93), $t(17) = 4.48$, $p < 0.01$.

Mean baseline tail-flick latencies for animals drinking sucrose (3.50 s) and water (3.78 s) groups did not differ. % MPE varied directly as a function of the dose of morphine, $F(5,90) = 6.42$, $p < 0.01$. Across morphine doses, %MPEs were lower for rats that drank the sucrose solution than for those who drank water during the 5-h drinking period, $F(1,18) = 4.13$, $p < 0$ .05. Post hoc comparisons revealed that following 5.0 mg/kg morphine, %MPE of rats drinking the sucrose solution was significantly lower than that of rats drinking water, $t(18) = 2.16$, $p < 0.05$.

Chronic Exposure to Sucrose

Animals given the sucrose solution consumed 60% of their total caloric intake from sucrose on the day of testing. Although animals given sucrose gained more weight during the 3-week drinking period, mean body weights did not differ as a function of diet (sucrose = 401.7 ± 45.8 g; water only = 384.3 ± 28.4 g).

Baseline tail-flick latencies did not differ between dietary conditions (sucrose = 2.43 s; water only = 3.01 s). %MPE varied significantly as a function of morphine dose, $F(5, 90) = 10.29$, $p < 0.01$. Additionally, animals chronically drinking the sucrose solution and water had significantly greater %MPE than those drinking only water, $F(1, 18) = 4.79$, $p < 0.05$. There was a significant effect for the diet by dose interaction, $F(5,90) = 2.46$, $p < 0.05$.

Sucrose Removal

Body weights of rats in the two dietary conditions were not different following 3 weeks of sucrose abstinence (sucrose removed = 454.1 ± 42.3 g; water = 457.9 ± 38.7 g).

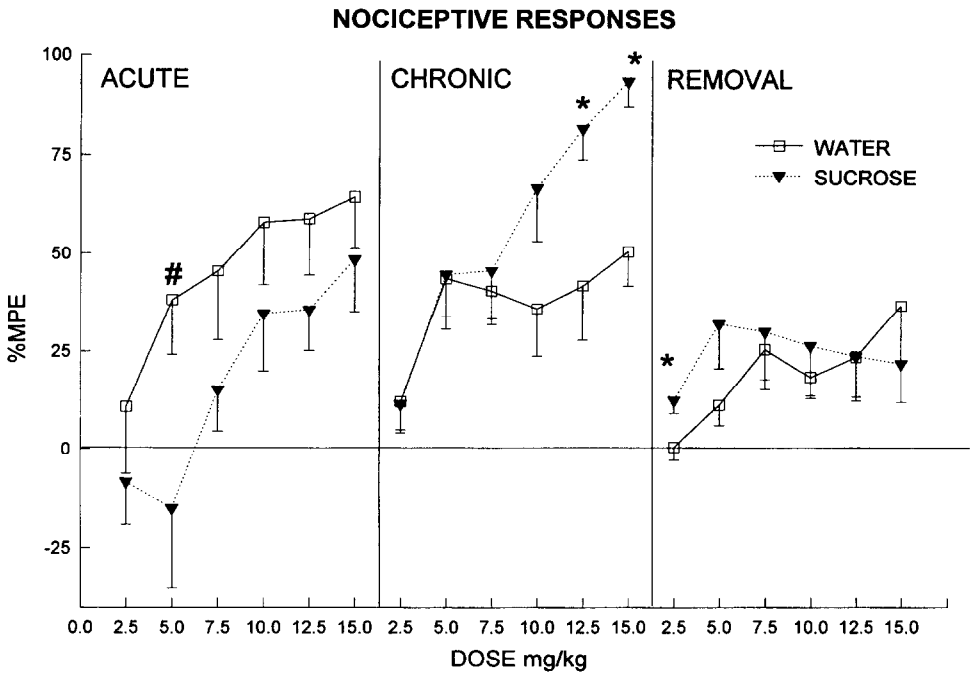

FIG. 1. Nociceptive responses were measured as %MPEs:

$$\%\text{MPE} = \left(\frac{\text{test latency} - \text{baseline latency}}{\text{maximal latency} - \text{baseline latency}}\right) \times 100.$$

In the acute phase, a 32% sucrose solution (dotted line) or water (solid line) was available for a 5-h drinking period. During the chronic phase, the sucrose solution and water, or water alone were available ad lib for 3 weeks. In the sucrose removal phase, all rats were given ad lib access to water for 3 weeks. #Indicates % MPEs of rats drinking sucrose were significantly ($p < 0.05$) less than those of rats drinking water. *Indicates % MPEs of rats drinking sucrose significantly ($p < 0.05$) greater than those of rats drinking water.

Three weeks after the sucrose solution was removed, %MPEs did not vary as a function of the dose of morphine. Moreover, across doses, %MPEs did not differ as a function of prior dietary conditions. However, post hoc tests indicated that at the lowest dose of morphine, rats that had drunk sucrose had significantly greater % MPEs than animals that had received only water, $t(18) = 2.53$, $p < 0.05$.

Analysis Across All Phases

Repeated measures ANOVAs were conducted on both diet groups together and each group separately to determine the changes across time. There was a significant interaction between dietary condition and time of testing, $F(2, 36) = 5.82$, $p < 0.01$ (Fig. 1). For rats drinking only water, %MPEs tended to decline with repeated testing, $F(2, 18) = 2.65$, $p = 0.098$. Nociceptive responses of animals drinking sucrose varied as a function of time, $F(2, 18) = 15.10$, $p < 0.01$. For animals drinking sucrose, %MPEs were significantly greater after chronic intake of sucrose than after acute intake ($p < 0.01$). After sucrose was removed for 3 weeks, %MPEs significantly decreased ($p < 0.01$) relative to the chronic phase. No differences in %MPEs were found when comparing the acute and removal phases.

DISCUSSION

Animals drinking a sucrose solution for a brief period of time were less responsive to morphine's analgesic actions than rats drinking water. In contrast, after consuming the sucrose solution for 3 weeks, the same animals were more responsive to the analgesic properties of morphine than rats drinking only water. Removal of the sucrose solution was associated with a reduction in the analgesic potency of morphine in rats formerly consuming the sugar. Thus, there were no differences in nociceptive responses as a function of prior dietary conditions during the final test. The results of this experiment support the proposal that the duration of intake of palatable foods or fluids is important in determining the effects of these substances on MIA.

As in previous studies (1,3,10,17) in the present experiment, short-term intake of a sweet solution was associated with a reduction in MIA. One possible explanation for this finding comes from research showing that short-term intake of palatable foods or sweet tasting solutions leads to the release of endogenous opioids (8,9). For example, Dum and colleagues (8,9) reported that intake of palatable sucrose-containing foods was associated with an increase in the amount of beta-endorphin occupying hypothalamic receptors. It has been further proposed that the release of these endogenous opioids can lead to the development of crosstolerance to morphine, thereby reducing the analgesic potency of morphine in animals given acute access to palatable foods and fluids (2,10,17).

In contrast to short-term intake, chronic intake of sucrose solutions is associated with an increase in the analgesic potency of morphine (11,15,18,20). This increase in potency may be

related to alterations in opiate receptor binding. In support of this suggestion, opiate receptor binding affinity was significantly greater in rats drinking a 32% sucrose solution in addition to water than in rats drinking only water (14). Additionally, Morley and colleagues (19) found that glucose added to an in vitro opiate assay containing membranes isolated from whole brain, significantly increased binding of tritiated naloxone. Thus, morphine may bind more tightly to receptors in rats drinking sucrose for an extended period of time.

When sucrose was removed, MIA was decreased. This decrease also may be associated with modifications in opiate receptor binding. Evidence for this comes from work demonstrating that rats that drank a sucrose solution for 3 weeks, and then had the sugar removed for three weeks displayed opiate receptor binding midway between that of rats that consumed the sucrose solution, and rats that drank only water for the entire 6 weeks (14). These findings suggest that changes in opiate receptor binding as a function of sucrose intake are not permanent.

For rats drinking only water throughout the experiment, MIA tended to decrease across testing periods. This finding indicates that animals drinking water were becoming tolerant to the analgesic actions of morphine. This agrees with previous work demonstrating that onset of tolerance to MIA occurs rapidly (4,21). In contrast, for rats consuming the sucrose solution and water, MIA was significantly greater during the second test period than during the first. This suggests that sucrose intake may prevent the development of tolerance to MIA. During the third test, after sucrose had been removed for 3 weeks, nociceptive responses for rats that had previously been consuming the sugar declined relative to the chronic sucrose feeding period.

It is possible that length of exposure alone does not account for the variations in the effects of palatable substances on MIA observed in different experiments. Bergmann and colleagues (1) found that suppression of MIA induced by a sweet solution began as soon as 24 h after introduction and persisted over a 5-week period. This finding is in contrast to that of the present experiment and of others using long periods of access to sweet solutions (11,15,20). Another difference between experiments finding suppression and enhancement of MIA by sweet fluids is the nutrient value of the solution. Experiments finding suppression of MIA with sweet liquids have used saccharin/glucose solutions that are minimally caloric (less than 0.2 kcal/ml) (1,10,12,13,16,17). In contrast, studies reporting enhancement of MIA with sweet fluids have used sucrose solutions containing substantially more calories (11,15,18,20). Added support for the relevance of the nutritive value of palatable solutions on analgesia comes from work directly comparing the effects of chronic intake of a sucrose solution or saccharin solutions [(18); D'Anci, unpublished results). When given morphine, nociceptive responses were greatest in rats drinking the sucrose solution, followed by rats drinking water, and then rats drinking saccharin. In conjunction with this finding, it is interesting to note that while chronic sucrose intake was associated with increased opiate receptor binding affinity, chronic saccharin intake was accompanied by a reduction in receptor binding affinity (14).

Another factor that could contribute to the disparities in the actions of palatable substances on MIA is whether animals are given a palatable fluid alone or a choice of water and a palatable fluid. A suppression in nociceptive responses has been observed most often in experiments in which rats' intake is restricted to the palatable fluid [e.g., (1,10,12,13)]. In comparison, nociceptive responses are enhanced when rats are given a choice of a palatable fluid and water [e.g., (11,14,15,18,20)]. In the present experiment, in the acute condition rats were given only the sucrose solution, while in the chronic condition, they received a choice of the sucrose solution and water. This difference in access conditions could have contributed to the divergent responses in MIA between the acute and chronic phases of the experiments. However, this seems unlikely because in a subsequent study there were no differences in MIA as a function of whether rats were chronically drinking only a sucrose solution, or drinking sucrose and water (D'Anci, unpublished results).

In summary, the results of this experiment help to resolve differences among prior studies on the effects of palatable substances on nociceptive responses. The present experiment demonstrates that duration of exposure to a palatable sucrose solution is important in mediating MIA. Moreover, these findings indicate that when investigating the consequences of psychoactive drugs, one should look, not only at the pharmacological actions of the drug, but also examine how environmental variables interact with these pharmacological actions to alter behavior.

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