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Production of Physical Dependence in Rats by Drinking a Morphine Solution

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LEUNG, C. M. K., C. W. OGLE AND S. DAI. *Production of physical dependence in rats by drinking a morphine solution.* PHARMACOL BIOCHEM BEHAV **25**(5) 1001–1006, 1986.—The plasma concentrations of morphine and glucose, the body weight, and the severity of the naloxone-precipitated withdrawal syndrome were studied in female rats in which morphine dependence was induced by administration of the opiate, with or without sucrose, in their drinking water. It was found that sucrose encouraged the animals to consume more morphine and that the initial plasma concentrations of the opiate, as well as the rate of development of physical dependence, were higher than the group not given sucrose. Plasma glucose concentrations, maximum plasma morphine levels and the maximum severity of the naloxone-precipitated withdrawal syndrome were, however, not significantly different between the two groups. The findings suggest that both regimens of administering the opiate in drinking fluid are effective in inducing morphine dependence in rats; the addition of sucrose tends to speed up the development of physical dependence, probably by increasing intake of the opiate through consuming more sucrose solution.

Morphine Sucrose Physical dependence Rats

PHYSICAL dependence on morphine has been successfully induced in experimental animals by various techniques [2, 5–9, 11, 12]. However, it has been suggested that administration of the opiate in drinking water might be the more efficient method [1, 2, 6, 12]. Previous studies have shown that a regimen of starting with a low concentration of morphine and gradually increasing the narcotic content in a 5% sucrose drinking solution can produce morphine dependence in rats within one week [12]. However, it is currently unknown whether the addition of sucrose in the morphine solution leads to a significantly higher plasma morphine concentration which could hasten the development of physical dependence in contrast to its administration in tap water only.

In the present study, the method of self-administration of morphine by the oral route in rats, with or without sucrose, was examined. The daily consumption of morphine solution and solid food, and the body weight gain of the animals, were recorded. The rates of development of physical dependence, in relation to the plasma concentrations of morphine and glucose, were also investigated.

METHOD

General

Female Sprague-Dawley rats, weighing 140–160 g, were used. They were housed, 3 or 4 per cage, in an air-conditioned room in which the temperature was maintained at 23±1°C and relative humidity at 60–70%, and were exposed to a 12:12 hr light-dark cycle. The animals were given standard laboratory chow (Ralston Purina Co., 4.17 kcal/g) and drinking fluid ad lib.

Administration of Morphine

The rats were randomly divided into controls and morphine-treated groups. The control animals received either plain tap water or 5% sucrose (w/v) in tap water as drinking fluid. Those given morphine treatment received either morphine-containing tap water (morphine/tap water), or morphine-containing 5% sucrose (w/v) in tap water (morphine/sucrose) to drink. The amount of morphine sulphate in drinking solutions was increased (48 hr apart) from 0.1 to 0.2, 0.3 and finally 0.4 mg/ml (expressed as the salt). The last concentration was maintained until the end of the 3-week observation period.

During the 3-week experimental period, the body weight of each rat was measured every 2 days. Daily fluid and food intakes were estimated for each animal by averaging the consumption per day of the 3 or 4 rats in each cage.

Evaluation of Physical Dependence

The development of physical dependence was estimated by the naloxone-precipitated withdrawal syndrome [4,5] on days 1, 2, 4, 7, 14, and 21 in separate groups of rats. After

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various durations of morphine administration, naloxone HCl 1 mg/kg (expressed as the salt) was given intraperitoneally to the animals. Immediately after naloxone injection, the following behavioural parameters were observed for 20 min: wet-dog shakes, head shakes, diarrhoea, ptosis, chattering teeth, writhing, chewing, paw tremor and irritability to touch and handling. The number of parameters observed in each animal was recorded as the number of withdrawal signs. The amount of faeces excreted during the 20-min observation period, and the percentage body weight loss at 4 hr after naloxone injection, were also recorded. No fluids or food were allowed before these measurements were completed.

Plasma Morphine Assay

The fluorometric method developed by Kupferberg, Burkhalter and Way [10] was adopted for assaying morphine in plasma. Separate groups of morphine/sucrose- and morphine/tap water-treated rats were used for this study. Plasma (1–2 ml) was obtained from blood, collected into heparinised tubes, from the descending aorta of ether-anaesthetised animals. After mixing each plasma sample with 0.5 g of sodium bicarbonate in a stoppered glass tube, 8 ml of 10% n-butanol in chloroform was added. The mixture was then agitated for 15 min in a shaker at 2 cycles per sec, following which it was transferred into a teflon centrifuge tube and centrifuged at 2800 g for 8 min. A 5-ml aliquot of the separated organic phase was then transferred to a glass centrifuge tube containing 1.6 ml of 0.01 M HCl. After gentle shaking, the solution was spun at 1500 g for 30 min. Following this final centrifugation, 1 ml of the acid phase was removed and added to 1 ml of 0.5 M Tris-HCl buffer (pH 8.5), and 0.1 ml of a 1/10 dilution of potassium ferri-ferrocyanide reagent (57.7 mg potassium ferricyanide + 5 mg potassium ferrocyanide dissolved in 100 ml distilled water). The solution was mixed thoroughly and allowed to stand for 10-min. Its fluoresence at 440 mμ, resulting from excitation at 250 mμ, was measured in an Aminco-Bowman spectrophotofluorometer. Recovery rate of the procedure was determined by a duplicate test using another plasma sample from the same animal, to which was added a known amount of morphine sulphate (1 μg, expressed as the salt).

Determination of Plasma Glucose Concentration

The plasma glucose concentration was determined by the glucose oxidase/peroxidase method of Lott and Turner [13], in separate groups of rats. Twenty-five μl of the plasma sample, collected as described in the morphine assay method, was added to 2.5 ml of a glucose oxidase reagent (GOD/POD colour reagent; DCL, Canada), and kept at 37°C for 10 min. Absorbance measurements were then carried out at 505 nm using a spectrophotometer (Varian, Cary 219).

Drugs

Morphine sulphate was purchased from Macfarlan Smith Ltd (Middlesex). Naloxone HCl was a gift from Endo Lab. (NY). Naloxone was dissolved in 0.9% NaCl (w/v) (saline), and was injected intraperitoneally in a volume of 1 ml/kg. Solutions of the drugs were prepared immediately before use.

Statistics

The data were analysed for significance of differences by means of Student's two-tailed *t*-test.

RESULTS

Daily Fluid Intake

Figure 1 shows the daily fluid intake throughout the 3-week experimental period. The daily fluid consumption of the tap-water controls was consistently and significantly lower than that of the sucrose- or morphine/sucrose-treated rats. Intake of the morphine/tap water group tended to be higher than those of the plain tap water controls, and statistical significance was observed on days 1, 5, 6, 7, 9, 14, 17, 19 and 20. In contrast, fluid consumption of the sucrose- and the morphine/sucrose-treated animals was essentially similar. Significant differences were reached only on days 15, 16, 19 and 20, when the sucrose-drinking controls consumed more than the morphine/sucrose-treated group.

Daily Food Intake

Figure 2 illustrates the daily food intakes in rats subjected to various drinking regimens. The daily food intake of animals drinking tap water was significantly higher than that of the sucrose-drinking groups throughout the whole experimental period, irrespective of the presence or absence of morphine in the sucrose solution.

Body Weight

The body weight of the rats in each group increased steadily, from 140–150 g to 225–237 g, during the 3-week observation period. There were generally no significant differences among them.

Naloxone-Precipitated Withdrawal Syndrome

Tables 1, 2 and 3 show the quantitative assessment of the naloxone-precipitated withdrawal syndrome, including the number of withdrawal signs, faecal weight at 20 min and % body weight loss at 4 hr after injection, exhibited by animals receiving different drinking regimens for various durations. In each of these parameters, there were no significant differences between the tap water- and the sucrose-treated controls throughout the experimental periods. Chronic morphine treatment, however, markedly elevated the magnitude of the withdrawal syndrome in all three parameters. The morphine/sucrose-treated animals had already exhibited significantly greater 20-min faecal weight and 4-hr body weight loss only 24 hr after drinking the opiate solution, withdrawal signs were greater on day 2. Morphine/tap water-drinking rats, on the other hand, did not show any significant changes when compared to their controls until day 2 (20-min faecal weight and 4-hr body weight loss) and day 7 (number of withdrawal signs). The magnitude of the withdrawal parameters in the morphine/tap water-treated animals was initially significantly smaller than that of the morphine/sucrose-drinking rats, but gradually increased until no significant differences were observed on days 2 (4-hr body weight loss), 4 (20-min faecal weight) and 7 (number of withdrawal signs).

Plasma Level of Morphine

Table 4 shows the plasma concentrations of morphine in rats drinking morphine solution with or without sucrose. In the morphine/sucrose-treated animals, a noticeable level of morphine was detected after 24 hr. This gradually increased and plateaued off by day 14. The morphine/tap water-

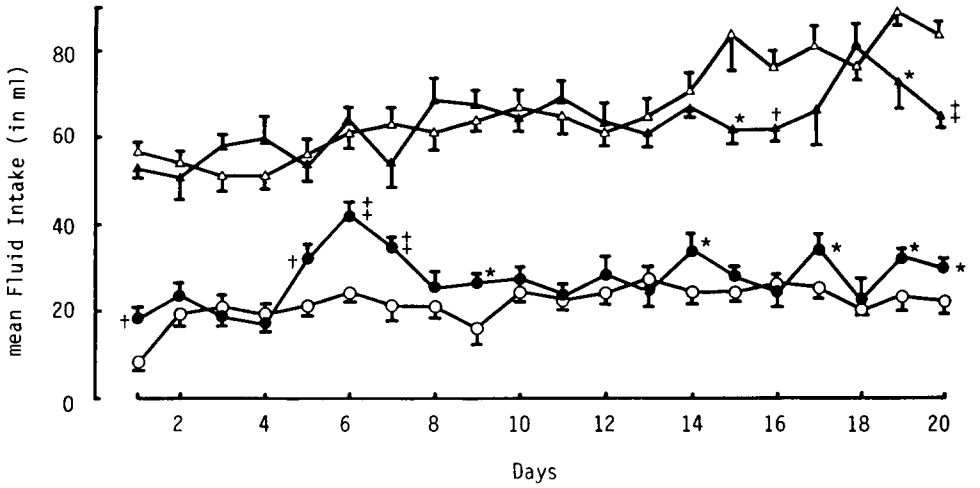

FIG. 1. Daily fluid intakes of rats drinking (○) tap water (n=15), (△) 5% sucrose (n=16), (●) morphine/tap water (n=16), or (▲) morphine/sucrose (n=16). The values plotted are the means±S.E.M. *$p<0.05$, †$p<0.01$, ‡$p<0.001$ when compared with the corresponding values in their own plain tap water- or sucrose-drinking controls.

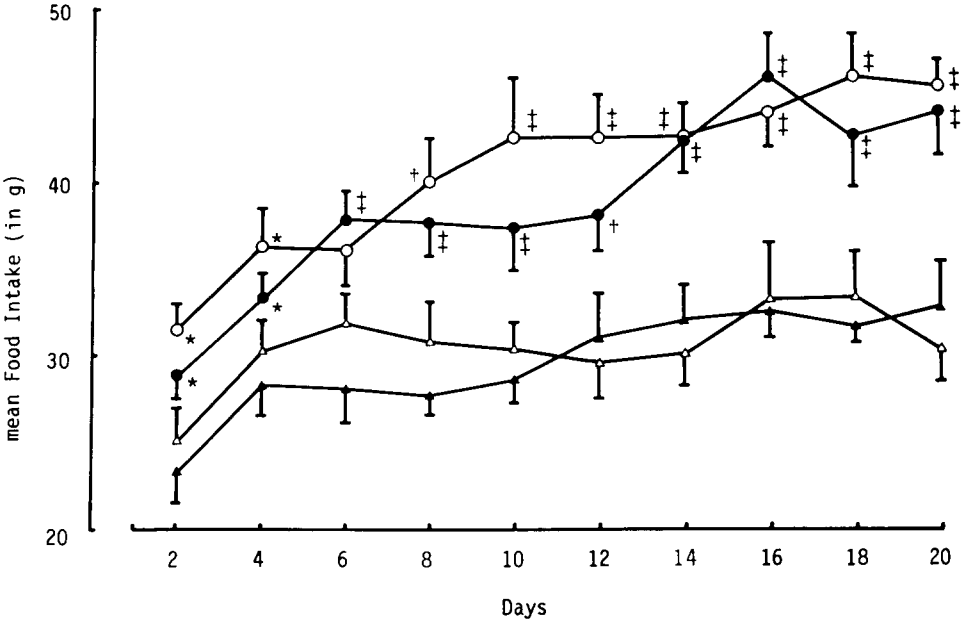

FIG. 2. Daily food intakes of rats drinking (○) tap water (n=15), (△) 5% sucrose (n=16), (●) morphine/tap water (n=16), or (▲) morphine/sucrose (n=16). The values plotted are the means±S.E.M. *$p<0.05$, †$p<0.01$, ‡$p<0.001$ when compared with the corresponding sucrose-drinking groups.

drinking groups, however, did not show a detectable amount of plasma morphine on days 1, 2 and 4. On day 7, a noticeable plasma level of morphine was revealed, but was significantly lower than that of the morphine/sucrose-treated rats ($p<0.01$). The level rapidly increased and was maximum by day 14; this final level was statistically similar to that of the morphine/sucrose-consuming group.

Plasma Glucose Concentrations

The plasma glucose concentrations in rats after 21 days of drinking tap water (n=8), 5% sucrose (n=8), morphine/tap water (n=19), or morphine/sucrose (n=14) were 124.00±6.44, 154.56±6.18, 156.04±8.26, or 143.41±5.81 μg/dl, respectively. They were not significantly different from each other.

DISCUSSION

Rats often refuse to consume significant amounts of morphine, presumably due to its bitter taste. Successful attempts have been made to overcome this problem by initially providing a low concentration of morphine in the drinking water [2] or by masking the bitter taste with sucrose [6]. A

TABLE 1

NUMBER OF WITHDRAWAL SIGNS EXHIBITED BY RATS DURING THE 20-MIN OBSERVATION PERIOD AFTER NALOXONE INJECTION (1 mg/kg, IP)

		Chronic treatment		
Number of days after drinking treatment	Tap water (8)	Morphine/tap water (16)	5% Sucrose (10)	Morphine/Sucrose (10)
1	0.77 ± 0.27	0.63 ± 0.18	0.86 ± 0.44	2.01 ± 0.50¶
2	0.98 ± 0.33	0.77 ± 0.32	0.38 ± 0.19	3.21 ± 0.40‡#
4	0.48 ± 0.35	1.63 ± 0.37	0.84 ± 0.72	2.85 ± 0.31*§
7	0.50 ± 0.49	2.75 ± 0.56*	0.57 ± 0.33	2.63 ± 0.42†
14	0.67 ± 0.48	2.75 ± 0.37†	0.63 ± 0.29	3.33 ± 0.26‡
21	0.54 ± 0.29	3.50 ± 0.19‡	0.71 ± 0.57	3.61 ± 0.30‡

The values are the means ± S.E.M. The number of animals in each group is shown in parentheses.
*$p<0.05$, †$p<0.01$, ‡$p<0.001$ when compared with the corresponding values in the plain tap water- or sucrose-treated controls.
§$p<0.05$, ¶$p<0.01$, #$p<0.001$ when compared with the corresponding values in the morphine/tap water-treated group.

TABLE 2

FAECAL WEIGHT (g) AT 20 MIN AFTER NALOXONE INJECTION (1 mg/kg, IP)

		Chronic treatment		
Number of days after drinking treatment	Tap water (8)	Morphine/tap water (16)	5% Sucrose (10)	Morphine/sucrose (10)
1	0.25 ± 0.09	0.61 ± 0.16	0.36 ± 0.10	1.66 ± 0.59*‡
2	0.76 ± 0.12	2.06 ± 0.30*	1.07 ± 0.69	2.97 ± 0.03†‡
4	0.27 ± 0.03	2.11 ± 0.21†	0.10 ± 0.09	2.55 ± 0.22†
7	0.16 ± 0.06	5.57 ± 1.13†	0.27 ± 0.06	4.98 ± 0.97†
14	0.34 ± 0.12	5.97 ± 0.99†	0.21 ± 0.12	7.20 ± 1.38†
21	0.42 ± 0.10	7.55 ± 0.71†	0.21 ± 0.11	6.95 ± 1.01†

The values are the means ± S.E.M.
The number of animals in each group is shown in parentheses.
*$p<0.01$, †$p<0.001$ when compared with the corresponding values in the plain tap water- or sucrose-treated controls.
‡$p<0.05$ when compared with the corresponding values in the morphine/tap water-treated group.

TABLE 3

BODY WEIGHT LOSS (%) AT 4 HR AFTER NALOXONE INJECTION (1 mg/kg, IP)

		Chronic treatment		
Number of days after drinking treatment	Tap water (8)	Morphine/tap water (16)	5% Sucrose (10)	Morphine/sucrose (10)
1	2.41 ± 0.60	2.33 ± 0.36	2.41 ± 0.25	4.45 ± 0.20‡§
2	1.97 ± 0.53	3.46 ± 0.17†	1.84 ± 0.72	4.20 ± 0.74*
4	2.01 ± 0.70	5.01 ± 0.42‡	0.91 ± 0.07	4.97 ± 0.61‡
7	1.08 ± 0.26	5.50 ± 0.88‡	1.06 ± 0.12	5.88 ± 0.77‡
14	0.95 ± 0.70	6.15 ± 0.38‡	1.55 ± 0.62	6.60 ± 0.52‡
21	1.86 ± 0.22	5.87 ± 0.39‡	0.96 ± 0.93	5.95 ± 0.83‡

The values are the means ± S.E.M. The number of animals in each group is shown in parentheses.
*$p<0.05$, †$p<0.01$, ‡$p<0.001$ when compared with the corresponding values in the plain tap water- or sucrose-treated controls.
§$p<0.001$ when compared with the corresponding value in the morphine/tap water-treated group.

TABLE 4

PLASMA CONCENTRATIONS OF MORPHINE (μg/ml) IN MORPHINE-DRINKING RATS

Drinking regimens	Number of days after drinking treatment					
	1	2	4	7	14	21
Morphine/ sucrose	0.12 ± 0.06 (8)	0.13 ± 0.014 (8)	0.70 ± 0.14 (10)	0.61 ± 0.07 (10)	1.09 ± 0.43 (8)	1.04 ± 0.13 (10)
Morphine/ tap water	— (10)	— (8)	— (10)	0.15 ± 0.11* (10)	0.89 ± 0.09 (10)	1.01 ± 0.12 (10)

The values are the mean ± S.E.M. The number of animals in each group is shown in parentheses.
*$p<0.01$ when compared with the corresponding value in the morphine/sucrose-treated group.

combination of the two methods has been reported to be effective in producing morphine dependence in rats [5]. Previous work has shown that this combined method can induce morphine dependence in rats in a very short period of time [12]. The present study shows that morphine/sucrose-treated rats have an earlier onset of marked naloxone-precipitated withdrawal effects than morphine/tap water-drinking animals. This confirms past work and suggests that the addition of sucrose enhances the development of morphine dependence, because the animals tend to drink more, and, consequently, to achieve a higher initial plasma level of morphine within 24 hr. However, the differences in the severities of naloxone-precipitated withdrawal syndromes between morphine/sucrose- and morphine/tap water-treated rats ceased to be statistically significant by days 4–7, during which the former groups still showed significantly higher plasma morphine concentrations. These findings suggest that the severity of naloxone-precipitated withdrawal syndrome in rats, which reflects the degree of opiate dependence, may not be directly proportional to plasma morphine concentrations. It is possible that measurement of brain morphine levels might be more relevant when considering withdrawal syndromes that involve central functions. It has been reported that the proportion between morphine levels in plasma and central compartments are dependent on the route, methods and schedule of administration as well as the measuring time after treatment with morphine [3,14]. However, this lack of direct proportionality may also be due to differences in the sensitivities of the measuring methods for morphine concentrations and the withdrawal syndrome. In view of these uncertainties, a firm conclusion on the relationship between plasma morphine level and the severity of the withdrawal syndrome cannot be drawn at the moment. The current investigation also reveals that the amount of the opiate consumed by the morphine/sucrose-drinking group during the last 2 weeks of the experimental period is still consistently higher than that of the morphine/tap water-treated animals. However, the plasma morphine concentrations in these two groups were not statistically different. The findings suggest that with these chronic methods of morphine administration, there seems to be a limiting effect on the plasma levels of morphine. Since the amount of morphine consumed between the two groups was different, although their plasma morphine levels were the same, it is likely that factors affecting narcotic absorption, elimination, volume of distribution as well as the half-life might contribute to this phenomenon.

The present investigation also reveals that consistent drinking of 5% sucrose solution for up to 21 days does not lead to significant changes in plasma glucose concentration. The rats appear capable of mobilising the sugar to maintain a similar plasma glucose level to that of the tap water-drinking controls. These findings, therefore, suggest that the plasma glucose concentration is not an essential factor which determines the rate of development of morphine dependence or the severity of the naloxone-precipitated syndrome. The results also suggest that chronic morphine administration and the development of opiate dependence do not significantly interfere with carbohydrate metabolism. Animals with an increased consumption of sucrose tended to eat less (Fig. 2), but the overall caloric intake among the groups, taking into account both sucrose and chow consumption, was about the same (170 kcal/rat/day). This could be another reason for the lack of differences between the plasma glucose levels in these groups. The absence of differences in total caloric intake is also supported by the lack of statistically significant differences in animal body weights.

This study confirms that morphine dependence in rats can be induced by oral administration of the opiate. Sucrose addition speeds up the development of morphine dependence but does not increase the severity of the naloxone-precipitated withdrawal syndrome. Intake of sucrose, in the quantities used, appears to have no significant effect on animal body weight, appearance and plasma glucose level and, therefore, might not be a limiting variable in the interpretation of morphine action. The reason why an increased consumption of morphine does not lead to a higher level of plasma morphine is not clear. The possible effect of sucrose on the pharmacokinetics of morphine cannot be excluded and further investigations are needed.

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