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Voluntary Oral Morphine Self-Administration in Rats: Effect of Haloperidol or Ondansetron

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BORG, P. J. AND D. A. TAYLOR. *Voluntary oral morphine self-administration in rats: Effect of haloperidol or ondansetron.* PHARMACOL BIOCHEM BEHAV 47(3) 633–646, 1994.—Rats were exposed to increasing concentrations of morphine hydrochloride (up to 0.4 mg/ml) in 5% w/v sucrose solution as their sole source of drinking water. Physical dependence was established as determined by the precipitation of withdrawal behaviour following administration of 1 mg/kg IP naloxone hydrochloride on day 23. The choice between either a 5% w/v sucrose solution or a 5% w/v sucrose solution containing 0.4 mg/ml morphine hydrochloride 4 days following withdrawal resulted in rats being categorized into two groups based on their respective consumption of the morphine-containing solution. The amount of morphine solution voluntarily consumed by approximately half the rats was sufficiently high as to lead to a relapse into physical dependence to morphine. The high preference for morphine shown by these rats could not be attributed to the taste of the morphine solution. Naive rats or rats exposed to a 5% w/v sucrose solution for 23 days failed to consume significant quantities of the morphine-containing solution when provided with a choice. The administration of either an IM slow-release formulation of 70.5 mg/kg haloperidol decanoate (=50 mg/kg haloperidol) or 10 μg/kg IP ondansetron hydrochloride daily did not alter morphine ingestion in the high morphine-preferring rats.

Morphine Drinking behaviour Ondansetron Haloperidol Withdrawal

THE incorporation of morphine in drinking water to produce physical dependence in rats is a method that has routinely been used in the past with varying success. Early investigators (19,20,29,50) who employed high initial concentrations of morphine reported that rats consumed insufficient fluid to maintain a healthy status. Subsequently, Badawy et al. (3) reported a method whereby increasing concentrations of morphine ranging from 0.1 to 0.4 mg/ml (expressed as the sulfate salt) led to a vast improvement in fluid consumption, leading ultimately to a state of morphine dependence in experimental animals. The method described does not require the use of prior parenteral administration of morphine (28) or scheduled access to a morphine drinking solution (20,50). The latter, in any case, is not representative of morphine intake in human addicts.

A major advantage of inducing morphine dependence by the current method is that it provides the opportunity to observe whether rats will voluntary consume a morphine-containing solution when also supplied with a drug free solution.

Dai et al. (15) have reported that previously dependent rats will preferentially drink a solution containing morphine over one that does not, provided that sucrose is also present in both solutions. This behaviour is still observed at a time where rats, although having initially been made dependent to morphine, do not undertake a two-bottle choice phase until no overt signs of physical withdrawal are displayed following the discontinuation of morphine. The addition of sucrose is essential in this model as rats or mice will otherwise avoid the bitter taste of morphine (3,4,15,19,20,28,29,42,44,56).

It would be of major importance to determine whether voluntary morphine consumption is a consequence of postingestional effects rather than any taste factors that may contribute to the solution preferences exhibited by experimental rats. The use of quinine, an alkaloid possessing a similar bitter taste to morphine but lacking in any pharmacological effects (4), has successfully been used in place of morphine to distinguish between these factors (4,14,18,20,44,45,50).

A final aim of the following study is to determine the effect of haloperidol (a dopamine D_2 receptor antagonist) and ondansetron (a 5-HT_3 receptor antagonist) on voluntary morphine consumption. These drugs have commonly been investigated for their propensity to attenuate reinforcement, whether measured by self-administration experiments (17,47,48,54,55) or place preference conditioning (6,8,24,31,34).

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METHOD

Animals

Male Glaxo Wistar rats, supplied by the Victorian College of Pharmacy Animal House, were maintained in a temperature-controlled environment (22°C) and subjected to a 12 L : 12 D cycle. All rats were kept in individual cages and food was available ad lib.

Materials

Morphine hydrochloride was supplied by Glaxo-Macfarlane Smith (Edinburgh, UK). Naloxone hydrochloride was obtained from Endo Laboratories (New York, NY). Haloperidol decanoate was provided by Janssen-Cilag (Beerse, Belgium) and ondansetron hydrochloride was supplied by Glaxo (Middlesex, UK)

Statistics

The significance of the changes obtained was evaluated by means of the two-tailed Student's *t*-test or Mann–Whitney *U*-test where appropriate.

General Procedure

All experiments were carried out with rats being placed in individual cages. The procedure of Badawy et al. (3) was used whereby rats were introduced to increasing concentrations of morphine in their drinking water, commencing at 0.1 mg/ml (expressed as the hydrochloride salt), increasing every 48 hours to 0.15, 0.2, 0.3, and finally 0.4 mg/ml, a concentration that provided suitable doses necessary to produce significant withdrawal behaviour at the end of a 3-week period.

Withdrawal behaviour was observed for 30 min at the end of the 3-week period, in a clear perspex box (dimensions 19.5 × 20 × 50 cm) with absorbent paper being placed at the bottom of the box to measure faecal mass and to give an indication of fluid loss or urine output (no attempt was made to account for evaporation).

Other common withdrawal signs recorded were wet dog shakes, jumps, paw tremor, teeth chatter, and head shakes. Any other general behaviours such as salivation, ejaculation, and posture were also noted.

Pilot experiments showed that the severity of withdrawal did not differ between groups of rats exposed to morphine for 3 weeks and those who were exposed to morphine for 6 weeks (data not shown). This observation reinforces the proposal that dependence had been well established by the end of the 3-week period. Similarly, various doses of naloxone hydrochloride administered IP (1, 3, and 10 mg/kg) failed to produce any significant differences in any of the characteristics of the withdrawal syndrome in rats exposed to morphine for 3 weeks (unpublished observations). Thus, for all subsequent studies, a dose of 1 mg/kg IP naloxone hydrochloride was used to reduce the possibility of nonspecific effects influencing any of the general behaviours observed during the withdrawal period.

The introduction of 5% w/v sucrose into the morphine solution was considered mandatory to mask the bitter alkaloid taste of morphine during the choice phase. Sucrose is not required for the production of dependence because rats will drink morphine solution if it is the only solution presented (3); however, the amount of morphine consumed in a two-bottle choice situation by previously dependent rats is only negligible without the addition of sucrose. This finding is in agreement with other workers (3,4,19,20,28,29,42,44,56) and was found to occur in our laboratory (unpublished observations). As a result, 5% w/v sucrose was introduced at the commencement of each experiment, and during the two-bottle choice phase, the solutions offered consisted of 5% w/v sucrose in tap water and 5% sucrose in tap water with 0.4 mg/ml morphine hydrochloride.

Experiment 1

Thirty-six rats weighing 300–400 g were housed in individual cages. Twenty randomly chosen rats were exposed to increasing concentrations of morphine hydrochloride (0.1–0.4 mg/ml) in 5% w/v sucrose solution, and 16 control rats were exposed to a 5% sucrose only solution. On day 23, all morphine-treated rats and eight of the control animals were administered 1 mg/kg IP naloxone hydrochloride and withdrawal behaviour was observed. The naloxone-induced behaviour was observed for 30 min as described previously (see General Procedure; note that one morphine-treated rat was sacrificed due to unrelated illness on day 14).

At the completion of the observation period, all rats were returned to their home cages and given access to tap water ad lib for 4 days. This time frame was chosen from the work performed by Dai et al. (15), who found that by the 4th day after the cessation of chronic morphine treatment, the administration of 1 mg/kg naloxone hydrochloride failed to produce withdrawal behaviour significantly different from control animals. The previously dependent rats could thus be regarded as essentially drug free.

On day 28, a choice between 0.4 mg/ml morphine hydrochloride and 5% w/v sucrose solution or 5% w/v sucrose solution alone was offered to all subjects. Fluid intake and body weight were measured on a daily basis for the next 15 days.

At the completion of this phase, rats that were previously made morphine dependent could be categorized into one of two distinct groups based on their respective degrees of morphine preference as reflected by their drinking behaviour. Rats that consumed negligible quantities of the morphine-containing solution were classified as "low morphine-preferring" rats and those who consistently drank significantly higher volumes of the morphine-containing solutions were classified as "high morphine-preferring" rats. None of the eight control rats chose to consume significant quantities of the morphine-containing solution and were removed from the study. On day 43, the high morphine-preferring rats and the eight remaining control subjects were injected with naloxone and their behaviour observed for 30 min as described above.

The high morphine-preferring rats were returned to their home cages and continued to be given a choice as before. Some of the low morphine-preferring rats ($n = 5$) were removed from the study and the remainder ($n = 6$) were maintained to determine whether their preferences remained consistent over a longer period of time. The remaining eight control animals were removed from the study following naloxone administration on day 43.

On day 64, the eight highest morphine-preferring rats were given an intramuscular injection of 70.5 mg/kg slow-release haloperidol decanoate (= 50 mg/kg haloperidol) with the femoral muscle being the site of injection. The remaining low morphine-preferring animals ($n = 6$) were given an IM injection of an equivalent volume of sesame oil as vehicle. Fluid intake and body weight of all rats continued to be measured on a daily basis.

On day 84, the two-bottle choice phase was temporarily

discontinued and all rats were given tap water until fluid intake was stabilized.

On day 92, the two-bottle choice phase recommenced in all rats; however, morphine hydrochloride was replaced with 0.4 mg/ml quinine sulphate. This exercise was performed in an attempt to establish whether any apparent drug preferences were not attributable to a simple taste preference. Morphine hydrochloride was then reintroduced on day 119 in place of quinine sulphate up until the completion of the experiment on day 134.

Experiment 2

Twenty rats weighing 300–400 g were housed in individual cages. Fourteen randomly chosen rats were exposed to increasing concentrations of morphine hydrochloride (0.1–0.4 mg/ml) in 5% w/v sucrose solution for 23 days. Six rats were exposed to 5% sucrose solution for 23 days.

Naloxone hydrochloride (1 mg/kg, IP) was administered to all animals on day 23 and behaviour was observed for 30 min as described in General Procedure. Note that one morphine-treated rat was removed from the study on day 8 due to its abnormally low fluid intake and resultant significant weight loss. Replacement of the morphine and sucrose solution with tap water led to the consumption of normal quantities of fluid and a recovery in body weight of this rat.

After 30 min of observation, all other rats were returned to their home cages and given free access to tap water for 4 days, as described in Experiment 1. The two bottle choice phase was introduced on day 28 with the solutions offered being 0.4 mg/ml morphine hydrochloride in 5% w/v sucrose solution or 5% w/v sucrose solution alone. The choice phase spanned 27 days, and once again it become possible to categorize the rats into two distinct groups based on morphine preference as reflected by their drinking behaviour.

Commencing on day 55, the seven highest morphine-preferring rats were given a daily injection of 10 μg/kg ondansetron hydrochloride IP for 14 days, and a comparison of the volume of fluid consumed in both bottles was made in each rat for the 21 days preceding ondansetron administration and for the 14 days during treatment. The remaining six previously dependent rats were given a daily injection of normal saline (1 ml/kg) and fluid consumption was similarly compared. None of the sucrose-treated rats consumed significant volumes of morphine solution and were removed from the study.

To establish a pattern of morphine preference in subjects never before exposed to morphine, 10 naive rats were placed in individual cages and given a choice between a 5% w/v sucrose solution containing 0.4 mg/ml morphine hydrochloride or 5% w/v sucrose alone for 14 days. Fluid intake was measured and the naive rats' preference for morphine was compared to that determined in rats made previously dependent in the two-bottle choice experiments described above.

RESULTS

Experiment 1

The average daily fluid intake of morphine hydrochloride solution consumed by all rats on the last 6 days of the involuntary phase was 33.7 ± 1.5 ml (±SEM) per day, corresponding to an average estimated daily dose of morphine hydrochloride of 54.0 ± 2.3 mg/kg per rat.

The administration of 1 mg/kg IP naloxone hydrochloride produced a significant withdrawal syndrome, indicating that a reasonable state of dependence had been established in all subjects when compared to rats who received sucrose only solution (see Table 1).

After 30 min of observation, rats were returned to individual cages and given free access to tap water. The average volume of tap water consumed per rat over the 4-day period following withdrawal was 39.0 ± 1.0 ml, which was lower ($p < 0.05$) compared to the final intake by the same rats when given access to the morphine and sucrose solution (no choice). By the commencement of the two-bottle choice phase, all rats had returned to body weights similar to those achieved before the administration of naloxone hydrochloride 4 days prior to the beginning of the choice phase.

Figure 1 gives an account of the daily consumption of both the 0.4 mg/ml morphine hydrochloride in 5% w/v sucrose solution and the 5% w/v sucrose solution for each previously dependent rat during the first period of the choice phase (the data from the five rats removed from the study on day 43 have been omitted, although it should be stressed that these were all considered to be low morphine-preferring rats at the time of sacrifice). None of the control rats consumed significant quantities of morphine solution during the choice phase and therefore the data from these animals have not been included. The voluntary daily intake of morphine solution by previously dependent rats ranged from 1 to 200 ml. Overall however, the mean volume of morphine solution consumed was generally lower compared with intake by the same rats during the no-choice phase (see Fig. 1).

The eight rats who consistently showed the highest preference for the morphine solution to day 43 (see Fig. 1; rats 1, 9, 10, 11, 18, 19, 21, and 22) were administered 1 mg/kg IP naloxone hydrochloride and their behaviour was observed and recorded (Table 2).

As shown in Table 2, the high morphine-preferring rats produced significantly higher faecal mass when compared to controls ($p < 0.001$); however, they produced significantly lower faecal mass when compared to rats given no-choice morphine ($p < 0.001$) (Table 1). Similarly, the high morphine-preferring rats lost significantly more weight compared with controls ($p < 0.001$) but lost significantly less weight compared with rats given no-choice morphine ($p < 0.001$) (Table 1).

Wet dog shakes were significantly higher ($p < 0.05$) in treated rats compared with control subjects; however, they were significantly lower compared to rats who were administered naloxone and exposed to no-choice morphine solution ($p < 0.05$).

Morphine-treated rats also displayed significantly more teeth chatter ($p < 0.05$) compared with control animals. There were, however, no other significant differences between these two groups. Similarly, there were no other significant differences between treated rats in this group (i.e., rats taking morphine by choice) and treated rats administered naloxone hydrochloride on day 23 (i.e., no-choice morphine).

After the second administration of naloxone hydrochloride, rats were returned to their home cages and continued to be given a choice between morphine hydrochloride 0.4 mg/ml in 5% w/v sucrose solution and 5% w/v sucrose alone for 21 days. Figure 1 shows that for the period between days 43–64, the preference for the morphine-containing solution increased substantially in both rat 13 and rat 24 (from 7.4 ± 1.6 ml to 33.6 ± 2.1, and 3.4 ± 0.7 to 18.3 ± 2.4, respectively). Although these rats were not initially considered to be high morphine-preferring and were not administered naloxone on day 43, it was considered that their consumption had become

TABLE 1

NUMBER OF RATS DISPLAYING NALOXONE-PRECIPITATED WITHDRAWAL SYMPTOMS IN RATS CHRONICALLY TREATED WITH MORPHINE IN DRINKING WATER (EXPERIMENT 1)

	Control	Morphine-Treated
Jumps (>0)	0/8	3/19
Wet dog shakes (>0)	0/8	18/19*
Head shakes (>5)	6/8†	4/19
Paw tremor (>5)	2/8	5/19†
Teeth chatter (>5)	2/8	9/19†
Ejaculation	0/8	11/19
Yawning	0/8	5/19
Salivation	0/8	1/19
Av % weight loss (g)	0.92 ± 0.16	3.86 ± 0.15‡
Av faecal mass (g)	0.87 ± 0.24	11.52 ± 0.63‡

Morphine-treated rats (n = 19) were exposed to increasing concentrations of morphine hydrochloride up to 0.4 mg/ml in a 5% w/v sucrose solution for 23 days. Control animals (n = 8) received a 5% w/v sucrose solution for 23 days. All rats received an IP injection of naloxone hydrochloride 1 mg/kg and were observed for 30 min. The differences between control and morphine-treated rats after naloxone administration were analysed by the Mann-Whitney U-test; percentage weight loss and faecal mass (mean ± SEM) were analysed by Student's two-tailed t-test and are indicated as follows: *$p < 0.002$, †$p < 0.05$, ‡$p < 0.001$.

Values given for each behavioural characteristic refer to the number of animals responding over the total number used.

sufficiently high to induce moderate but significant dependence as reflected by withdrawal behaviour, and as a result, they were chosen amongst the group of eight rats who were to be administered haloperidol. The drinking profile of rat 9 and rat 21 did not change substantially after the second administration of naloxone (from 11.0 ± 0.8 to 11.2 ± 0.6, and 9.1 ± 1.0 to 11.5 ± 0.8, respectively) (Fig. 1); however, their mean daily consumption was no longer in the highest eight and thus these rats were administered sesame oil as vehicle. Therefore, the haloperidol-treated subjects were rats 1, 10, 11, 13, 18, 19, 22, and 24, and the control or sesame oil-treated subjects were rats 5, 9, 17, 20, 21, and 23.

The effect of haloperidol on voluntary morphine solution consumption is summarized in Table 3. Only rats 10 and 11 of the haloperidol-treated group consumed significantly less morphine solution ($p < 0.05$) over the 3 weeks after haloperidol administration when compared with the 21 days preceding drug treatment. The most profound effect of haloperidol administration was a decrease in sucrose solution consumption, an effect that was displayed in six out of eight rats ($p < 0.001$). The effect of sesame oil on the various parameters measured in the low morphine-preferring rats was variable (see Table 3).

After the 22nd day following haloperidol administration (day 86), all rats were placed on tap water until fluid intake had become relatively stable. This occurred by day 91, at which time the previously haloperidol-treated rats consumed 31.6 ± 2.1 ml and the sesame oil treated rats drank 36.7 ± 3.7 ml of tap water. Thus, on day 91, all rats were provided with a choice between 0.4 mg/ml quinine sulphate in 5% w/v sucrose solution and 5% w/v sucrose only.

Figure 2 illustrates the effect of replacing morphine with quinine in rats who had been categorized as high morphine-preferring rats (note that rat 18 has been omitted from this data due to its immediate refusal to drink any of the quinine-containing solution and due to the fact that it consistently consumed four to five times more sucrose solution than any other rat in this group).

As shown in Fig. 2, the substitution of morphine with quinine led to a progressive decrease in the consumption of the bitter-tasting solution with a concomitant increase in sucrose solution consumption. The difference in consumption between the two solutions reached significance on day 106 (day 14 of quinine substitution, $p < 0.05$) and occurred without a change in average total fluid intake.

The replacement of quinine with morphine on day 119 led to an immediate increase in consumption of the bitter-tasting solution and from 9 days following the reintroduction of morphine (day 127) to the end of the experiment both solutions were consumed in essentially similar volumes.

Experiment 2

The average daily fluid intake of morphine hydrochloride solution consumed over the last 6 days of the involuntary phase was 48.2 ± 2.5 ml, corresponding to an average estimated daily dose of morphine hydrochloride of 52.2 ± 8.8 mg/kg per rat.

The administration of 1 mg/kg IP naloxone hydrochloride on day 23 produced a significant withdrawal syndrome, suggesting that all subjects could be categorized as having developed physical dependence towards morphine by the completion of the involuntary phase (see Table 4).

After 30 min of observation following the administration of naloxone, all rats were returned to their individual cages and given free access to tap water for 4 days. The average daily volume of tap water consumed per rat over this time was 37.4 ± 1.1 ml, which was significantly lower ($p < 0.01$) than the volume of the 0.4 mg/ml morphine hydrochloride and 5% w/v sucrose solution being consumed by the same

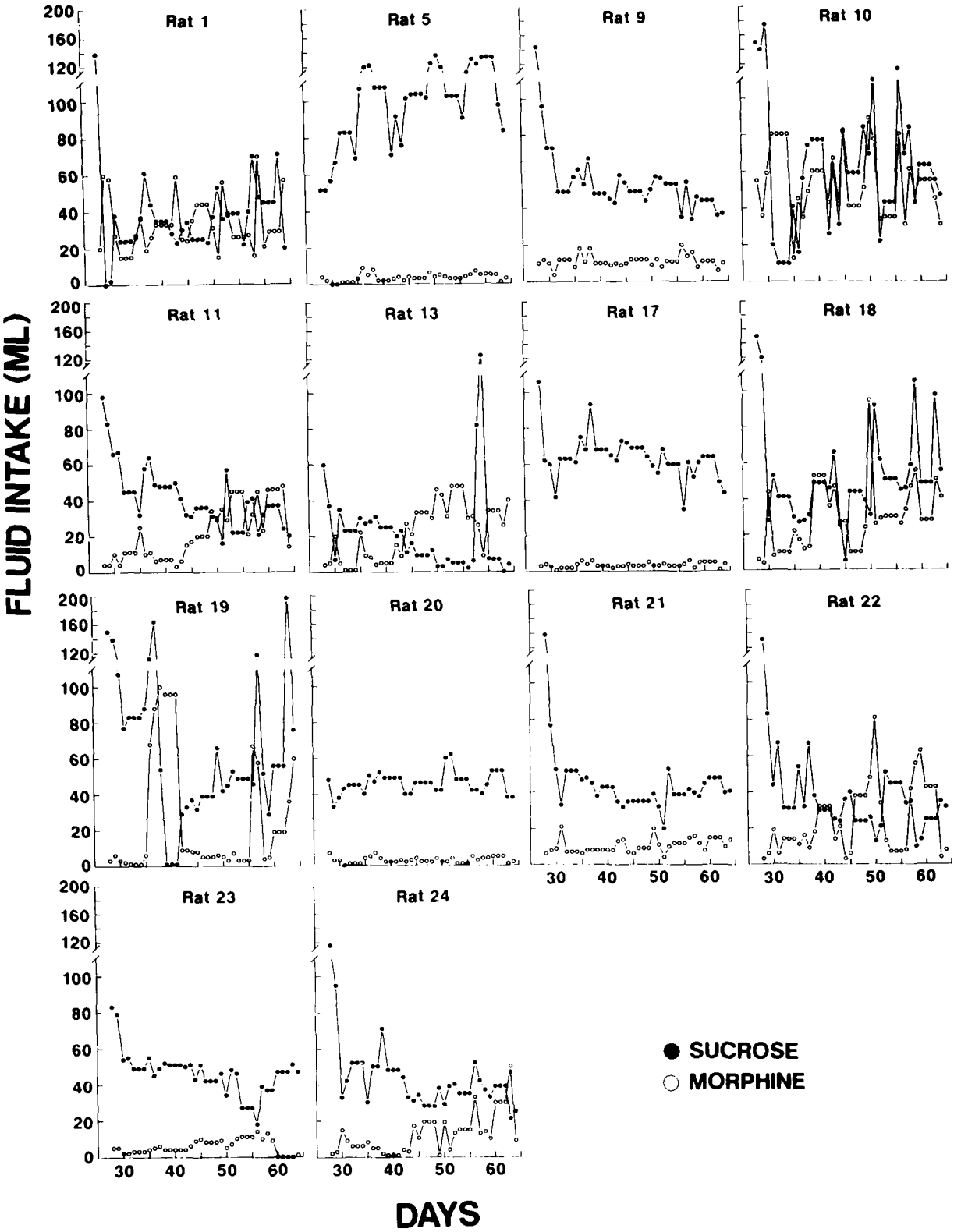

FIG. 1. Individual volume consumption of morphine/sucrose and sucrose solution of previously dependent rats following morphine withdrawal (Experiment 1). All previously dependent rats were given a choice between 0.4 mg/ml morphine hydrochloride in 5% w/v sucrose or 5% w/v sucrose alone, commencing 4 days after the precipitation of withdrawal by the administration of naloxone (day 23). On day 43, the eight highest morphine-preferring rats were readministered naloxone to observe withdrawal behaviour following the voluntary consumption of the morphine solution. Those rats (1, 9, 10, 11, 18, 19, 21, and 22) were immediately returned to their home cage and continued to be given a choice as before. The data obtained between days 43 and 64 served as control values when comparing the effect of haloperidol or sesame oil administration (day 64) on voluntary morphine/sucrose and sucrose solution consumption.

TABLE 2

NALOXONE-PRECIPITATED WITHDRAWAL SYMPTOMS IN HIGH MORPHINE-PREFERRING RATS 15 DAYS AFTER THE COMMENCEMENT OF VOLUNTARY MORPHINE SOLUTION CONSUMPTION (DAY 43; EXPERIMENT 1)

	Control	Morphine-Treated
Jumps (>0)	0/8	0/8
Wet dog shakes (>0)	0/8	5/8*
Head shakes (>5)	8/8	4/8
Paw tremor (>5)	1/8	2/8
Teeth chatter (>5)	2/8	7/8*
Ejaculation	0/8	4/8
Yawning	0/8	5/8
Salivation	0/8	2/8
Av % weight loss (g)	0.49 ± 0.11	2.48 ± 0.16†
Av faecal mass (g)	1.11 ± 0.27	8.43 ± 0.74†

Morphine-treated rats (n = 8) were given a choice between 0.4 mg/ml morphine hydrochloride in 5% w/v sucrose or 5% w/v sucrose alone for 15 days. Control animals (n = 8) received a 5% w/v sucrose solution. All rats received an IP injection of 1 mg/kg naloxone hydrochloride. The differences between control and morphine-treated rats after naloxone administration were analysed by the Mann-Whitney U-test; average percentage weight loss and average faecal mass (mean ± SEM) were analysed by Student's two-tailed t-test and are indicated as follows: *$p < 0.05$, †$p < 0.001$.

Values given for each behavioural characteristic refer to the number of animals responding over the total number used.

TABLE 3

EFFECT OF IM HALOPERIDOL (50 mg/kg) AND SESAME OIL ON THE VOLUNTARY CONSUMPTION OF MORPHINE (0.4 mg/ml IN 5% w/v SUCROSE) AND 5% w/v SUCROSE SOLUTIONS

Rat	Morphine Solution	Estimated Daily Dose	Sucrose Solution	Total Fluid
	Haloperidol-Treated (high morphine-preferring, n = 8)			
1	–	–	↓*	↓*
10	↓†	↓‡	↓*	↓*
11	↓†	↓‡	↓*	↓*
13	–	–	↓*	↓‡
18	↑‡	↑‡	↑*	↑*
19	↑‡	↑‡	↓*	–
22	–	–	↓*	↓‡
24	↑‡	↑‡	–	–
Grouped results	3/8↑ 3/8– 2/8↓	3/8↑ 3/8– 2/8↓	1/8↑ 1/8– 6/8↓	1/8↑ 2/8– 5/8↓
	Sesame Oil (vehicle) (low morphine-preferring, n = 6)			
5	–	–	–	–
9	↑†	↑†	↓*	–
17	↓†	–	↓†	↓‡
20	–	–	↓†	–
21	↑‡	↑‡	–	↑*
23	↑‡	↓‡	–	↓*
Grouped results	2/6↑ 2/6– 2/6↓	2/6↑ 3/6– 1/6↓	3/6– 3/6↓	1/6↑ 3/6– 2/6↓

A comparison was made between consumption of the solutions offered on the 21 days preceding treatment and the 21 days following treatment. Differences in consumption over this time were analysed by Student's t-test and are indicated as follows: *$p < 0.001$, †$p < 0.05$, ‡$p < 0.01$ (↑ = increase, ↓ = decrease, – = no change).

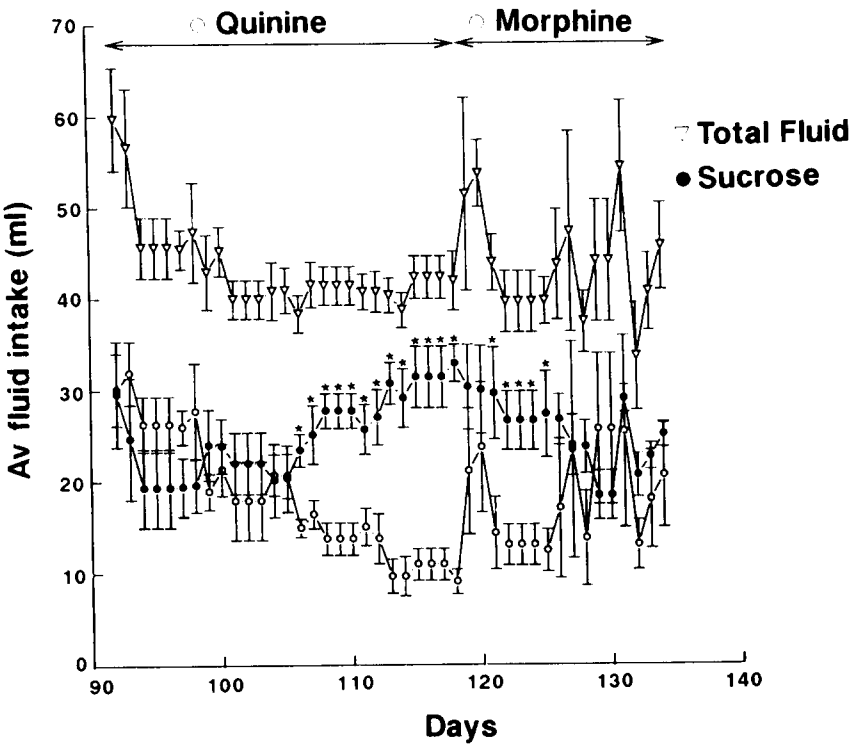

FIG. 2. Effect of replacing morphine hydrochloride with quinine sulphate on the preference for a bitter tasting solution. On day 92, rats who were previously considered to show a high preference for 0.4 mg/ml morphine hydrochloride in 5% w/v sucrose were provided with both 0.4 mg/ml quinine sulphate in 5% w/v sucrose and 5% w/v sucrose alone. On day 119, the quinine sulphate was removed and 0.4 mg/ml morphine hydrochloride was reintroduced. Note that the values for 1 rat (rat 18) who was previously considered to be high morphine preferring was not included due to its refusal to consume any of the quinine-containing solution and also because it consistently consumed four to five times more of the 5% w/v sucrose solution compared with any other rat in this group. $N = 7$ per point (mean ± SE). The average volume of 5% w/v sucrose solution consumed was significantly higher compared with the quinine-containing solution in these rats on days 106–118 and the morphine-containing solution on days 121–125 (*$p < 0.05$, two-tailed t-test).

rats during the involuntary phase. By the commencement of the two-bottle choice phase, all rats had approached body weights similar to those achieved before the administration of naloxone hydrochloride 4 days prior to the beginning of the choice phase.

Figure 3 gives an account of the voluntary consumption of both the 0.4 mg/ml morphine hydrochloride in 5% w/v sucrose solution and the 5% w/v sucrose solution during the first period of the choice phase in all rats who had previously been made dependent on morphine, as determined by the precipitation of withdrawal behaviour following the administration of naloxone. None of the control rats consumed significant quantities of morphine solution during the choice phase, and therefore the data from these animals has not been included. The voluntary daily intake of morphine solution by the previously dependent rats ranged from 1 to 200 ml. Overall, however, the mean volume of morphine solution consumed was generally lower compared with intake by the same rats during the no-choice phase.

The seven rats who consistently showed the highest preference for the morphine solution to day 55 (see Fig. 3; rats 6, 9, 11, 12, 17, 18, and 19) were administered 10 μg/kg IP ondansetron hydrochloride daily for 14 days and a comparison of voluntary consumption during treatment and for the 21 days prior to injection was made. The remaining rats received a daily injection of 1 ml/kg saline. Although rats 15 and 20 displayed a relatively high average morphine intake over the 21 days preceding injection (Fig. 3), their consumption was sporadic and highly variable. It was thus considered inappropriate to attempt to evaluate the effect of ondansetron on the behaviour shown by these rats.

As the volume of morphine solution consumption by the high morphine-preferring rats is comparable to those during the same period in Experiment 1 (see Fig. 1), it was considered unnecessary to precipitate withdrawal by the administration of naloxone in these rats. It was assumed that these subjects had attained a significant degree of physical dependence to morphine.

The effect of ondansetron on voluntary consumption of morphine or sucrose only solution is summarized in Table 5.

None of the ondansetron-treated rats significantly reduced voluntary morphine intake. Rats 11 and 12 actually increased morphine consumption ($p < 0.001$ and $p < 0.05$, respectively) with a concomitant increase in total fluid intake (both

TABLE 4

NUMBER OF RATS DISPLAYING NALOXONE-PRECIPITATED WITHDRAWAL SYMPTOMS IN RATS CHRONICALLY TREATED WITH MORPHINE IN DRINKING WATER (EXPERIMENT 2)

	Control	Morphine-Treated
Jumps (>0)	0/6	8/13*
Wet dog shakes (>0)	1/6	6/13
Head shakes (>5)	6/6*	3/13
Paw tremor (>5)	0/6	2/13
Teeth chatter (>5)	1/6	4/13
Ejaculation	0/6	9/13
Yawning	0/6	6/13
Salivation	0/6	3/13
Av % weight loss (g)	0.39 ± 0.11	3.72 ± 0.18†
Av faecal mass (g)	0.35 ± 0.11	10.65 ± 0.61†

Morphine-treated rats (n = 13) were exposed to increasing concentrations of morphine hydrochloride up to 0.4 mg/ml in a 5% w/v sucrose solution for 23 days. Control animals (n = 6) received a 5% w/v sucrose solution for 23 days. All rats received an IP injection of 1 mg/kg naloxone hydrochloride and were observed for 30 min. The differences between control and morphine-treated rats after naloxone administration were analysed by the Mann-Whitney U-test; percentage weight loss and faecal mass (mean ± SEM) were analysed by Student's two-tailed t-test and are indicated as follows: *$p < 0.05$, †$p < 0.001$.

$p < 0.001$). One of the control rats also significantly increased morphine intake ($p < 0.01$) over the time frame studied. Generally, however, the consumption of rats remained consistent regardless of which treatment regime was employed.

The 10 individually caged rats who had never been exposed to either morphine or sucrose consumed negligible amounts of morphine solution when given a choice between 0.4 mg/ml morphine hydrochloride in 5% w/v sucrose solution or 5% w/v sucrose alone for 14 days. The average daily consumption of the morphine-containing solution per rat over the 14 days was 2.0 ± 0.1 ml, with the highest volume consumed on any given day by any single rat being 6 ml (this volume only being consumed once). There was no tendency amongst any of the rats to increase their consumption of the morphine-containing solution over the 14 days. In comparison, the average daily volume of sucrose solution consumed per rat over the 14-day period was 54.4 ± 1.8 ml.

DISCUSSION

The establishment of morphine dependence under experimental conditions has been achieved successfully with the use of varying procedures, including repeated parenteral administration, implantation of a pellet(s), and the incorporation of morphine in drinking water. The latter appears to be of greatest value when it is necessary to maintain animals in a steady state of moderate morphine dependence for long experiments such as those undertaken in this study.

The procedure described by Badawy et al. (3) was used, whereby increasing concentrations of morphine hydrochloride, commencing at 0.1 mg/ml, were provided ad lib to experimental rats. This method overcomes the problem encountered by earlier investigators (19,20,29,50) who reported unsatisfactory consumption of the morphine solution when commencing at higher concentrations (0.5–1 mg/ml), resulting in severe weight loss and the death of some animals. In addition, it has been reported that increasing concentrations of morphine result in higher morphine intake over a 2-week period when compared to a single, fixed, higher morphine concentration, even when the taste of morphine is masked by the addition of saccharin (4).

The pattern of morphine drinking during the involuntary phase is comparable with other workers. The normal consumption of 0.4 mg/ml morphine hydrochloride solution provided daily doses in the range of 40–60 mg/kg. Although only one rat in this study refused to consume the morphine solution, even at low concentrations, we have observed several other rats neglecting the morphine in a similar manner in our preliminary work. This observation has also been noted by other investigators (19,20,42); however, at this stage, such behaviour cannot be explained and will require further investigation.

The incorporation of sucrose into the morphine solution, although not preferable, was considered mandatory as animals will otherwise avoid the bitter taste of the morphine solution in a choice situation. This behaviour will occur even if the animals have previously been made physically dependent to morphine. This observation has been reported extensively in the literature (3,4,15,19,20,28,29,42,44,56), and illustrates the necessity of masking the bitter alkaloid taste of morphine.

It has been suggested that the addition of sucrose may interfere with the behavioural processes of morphine dependence in several ways. Firstly, chronic sucrose or glucose administration enhances 5-HT synthesis in rat brain, possibly by inhibition of the activity of liver tryptophan pyrrolase. This, in turn, leads to increased circulating levels of tryptophan (2). As 5-HT is implicated in many of the pharmacological actions of morphine, it follows that an activation of the tryptaminergic system may interfere with neurochemical changes that occur following morphine administration, ultimately leading to a modification in the normal behaviour one would observe following morphine consumption. For example, it has been suggested that an increased 5-HT level in several synaptic areas of the brain may result in a diminished tolerance to morphine (44).

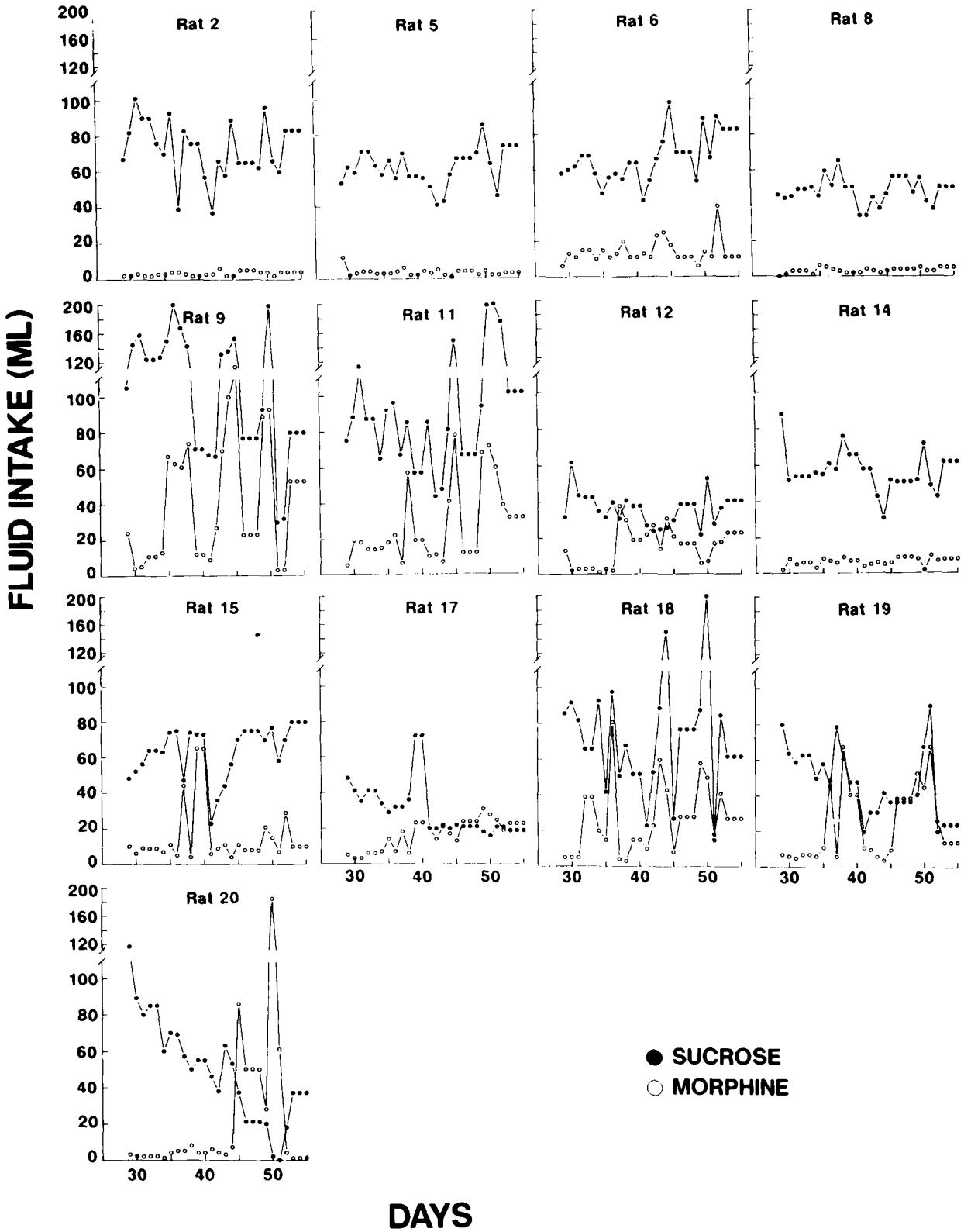

FIG. 3. Individual volume consumption of morphine/sucrose and sucrose solution of previously dependent rats following morphine withdrawal (Experiment 2). All previously dependent rats were given a choice between 0.4 mg/ml morphine hydrochloride in 5% w/v sucrose or 5% w/v sucrose alone, commencing 4 days after the precipitation of withdrawal by the administration of naloxone (day 23). The rats who consistently consumed a relatively large quantity of the morphine-containing solution were considered to be rats 6, 9, 11, 12, 17, 18, and 19. The data obtained between days 34–55 served as control values when comparing the effect of ondansetron or saline administration (day 55) on voluntary morphine/sucrose and sucrose solution consumption.

TABLE 5

EFFECT OF IP ONDANSETRON 10 μg/kg OR SALINE ON THE VOLUNTARY CONSUMPTION OF MORPHINE (0.4 mg/ml IN 5% w/v SUCROSE) AND 5% w/v SUCROSE SOLUTIONS

Rat	Morphine Solution	Estimated Daily Dose	Sucrose Solution	Total Fluid
	Ondansetron-Treated (high morphine-preferring, n = 7)			
6	–	–	–	–
9	–	–	–	–
11	↑*	↑*	↑↑	↑*
12	↑‡	↑‡	↑↑	↑*
17	–	–	↓‡	–
18	–	–	–	–
19	–	–	–	–
Grouped results	2/7↑ 5/7–	2/7↑ 5/7–	2/7↑ 4/7– 1/7↓	2/7↑ 5/7–
	Saline (vehicle) (low morphine-preferring, n = 6)			
2	–	–	–	↑↑
5	–	–	–	–
8	↑↑	↑‡	–	–
14	–	–	–	–
15	–	–	–	–
20	–	–	–	–
Grouped results	1/6↑ 5/6–	1/6↑ 5/6–	6/6–	1/6↑ 5/6–

On Day 55, rats were categorized into two groups based on morphine preference as reflected by drinking behaviour. High morphine preferring rats (n = 7) received an intraperitoneal injection of 10 μg/kg ondansetron hydrochloride at 16:00 h on a daily basis for 14 days. Low morphine-preferring rats (n = 6) received a corresponding daily injection of 1 ml/kg normal saline. All rats continued to be offered both 0.4 mg/ml morphine hydrochloride in 5% w/v sucrose or 5% w/v sucrose alone throughout the treatment period and a comparison was made between consumption of the solutions offered on the 21 days preceding treatment and the 14 days during treatment. The differences in consumption over this period were analysed by Student's two-tailed t-test and are indicated as follows: *$p < 0.01$, †$p < 0.001$, ‡$p < 0.05$ (↑ = increase, ↓ = decrease, – = no change).

While this possibility cannot be ignored, it should be noted that the amount of morphine and sucrose solution consumed by all rats did not differ greatly during the involuntary phase, yet each rat had an individual susceptibility to consuming the morphine solution during the choice phase (see below). This behaviour occurred even though, presumably, all rats had an increased circulating level of tryptophan. Furthermore, none of the rats exposed to the 5% sucrose-only solution for 3 weeks chose to consume significant quantities of the morphine solution during the choice phase.

The second argument against the use of sucrose in this model is the possibility that a caloric imbalance may influence any changes observed in biochemical parameters such as brain protein metabolism and neurotransmitter metabolism during long-term morphine treatment (56). Rats, however, have been reported to be excellent caloric meters and achieve the same caloric intake regardless of whether or not sucrose is present in the diet (29).

To our knowledge, the susceptibility of individual rats to relapse into consuming morphine by choice has not been discussed elsewhere in any great detail. It is acknowledged that the C57BL/6J strain of mice will preferentially consume a morphine/saccharin solution over tap water (4,25,26) or a similarly tasting quinine/saccharin solution (18), and the DBA/2J strain will either avoid morphine-containing solutions in the presence of tap water or consume relatively equal amounts of morphine/saccharin and quinine/saccharin when provided with a choice. However, because of fewer genetically well-defined inbred strains of rats compared with mice, similar data with respect to rats has not been reported as frequently. More recently, however, Rönnback et al. (45) have shown that within the Sprague–Dawley strain of rat, there is a percentage of individual animals with the ability to acquire a high morphine preference in a two-bottle choice situation. Similarly, Sudakov et al. (51) reported that within the Wistar strain of rat, animals could be subdivided with respect to their sensitivity to the development of morphine dependence induced either by drinking morphine or by the IP injection of increasing doses of morphine.

Many workers have provided morphine in a two-bottle choice situation to naive rats at the commencement of the experiment, usually in very low concentrations (0.01–0.05 mg/

ml) in the presence of sucrose or saccharin, and selected rats or mice according to their preference ratio for morphine vs. tap water (4,20,42). Rönnback et al. (44,45) have also used this method, but have designed a balanced fluid diet (56) that partially masks the bitter taste of morphine. Alternatively, the procedure of cyclical reinforcement has been used (40,50) without the addition of a sweetening agent to increase rats' preference for morphine solution. Using this procedure, Nichols et al. (40) reported a selective breeding of two strains of Sprague–Dawley rats that differ in their susceptibility to morphine addiction. Stolerman et al. (50), however, reported that all rats preferred morphine solution over tap water by the completion of the experiment. Similarly, Khavari et al. (29), whose technique more closely resembles that of our own, and Dai et al. (15), whose procedure we have duplicated, found that on average, all previously dependent rats ultimately preferred a morphine-containing solution over a drug-free solution by the completion of the choice phase. It should be mentioned, however, that rats were grouped three or four to a cage in the latter experiment and thus data on individual animals is lacking. It is suggested that experiments using the current model should therefore be carried out with rats being housed individually.

Furthermore, the expression of morphine preference as percentage of total fluid consumed rather than total morphine solution ingested is not appropriate with respect to this model. There is no correlation between the consumption of the two solutions offered, meaning that rats that drink considerable amounts of the morphine solution will vary greatly in the volume of sucrose solution consumed. Some rats drink excessive quantities of both solutions, and others drink relatively low volumes of sucrose solution. The main criteria for categorizing rats as high or low morphine-preferring is the consistency in which they consume the morphine-containing solution. We propose that previously dependent rats who consistently consume at least 15–20 ml of the morphine solution daily, regardless of sucrose solution consumption, are regarded as high morphine-preferring rats.

The use of quinine has been routinely used in place of morphine to determine whether individual preferences are attributable to the bitter taste of morphine. Our results clearly show that rats who previously consumed significant volumes of morphine-containing solution in a two-bottle choice situation selectively reduce their intake of quinine-containing solution while maintaining a steady intake in total fluid. One would expect that this reduction would not occur immediately, as the bitter taste of quinine would have acquired secondary reinforcing properties. If, in fact, the preference for morphine was not due to its bitter taste, rats would eventually discriminate between the primary reinforcer (i.e., rewarding properties of morphine) and the secondary reinforcer (i.e., bitter taste of quinine and morphine) and consequently reduce their intake of quinine solution. The time taken for this process to occur is a reflection of the animals' ability to discriminate, which would further be dependent on the initial volume of morphine solution consumed. Thus, rats with a high voluntary morphine consumption would be expected to reduce their voluntary quinine consumption more quickly compared with the lower morphine-preferring rats.

The time taken for a significant reduction in voluntary quinine solution intake in Experiment 1 was 14 days, which is comparable to that observed by Stolerman et al. (50) who used concentrations of 0.5 mg/ml morphine and 0.25 mg/ml quinine. The reintroduction of morphine in place of quinine led to an immediate increase in voluntary morphine consumption. This observation would be expected as the rewarding properties of morphine would be immediately reinforcing.

Other studies have illustrated that if naive rats are given a choice between increasing concentrations of quinine sulphate in vehicle or vehicle alone, all experimental animals will avoid the quinine-containing fluid (4,44). By contrast, the presentation of initially low concentrations of morphine to naive rats is reported to select out different strains of rats based on solution preferences (4,14,20,44). These observations, along with our own results, strongly suggest that the preferences for the morphine solution are a reflection of the drug's rewarding properties rather than its bitter taste. However, this conclusion could not be confirmed by Jurna et al. (28), who reported that animals treated with high doses of morphine preferred solutions of quinidine sulfate (1%) to tap water.

Finally, Rönnback et al. (45) have demonstrated that when the concentrations of morphine presented to rats is randomly changed, animals will adjust their consumption such that their morphine intake is essentially similar each day.

These observations, taken together with the fact that naive rats or rats previously exposed to a sucrose-only solution will consume negligible quantities of a 0.4 mg/ml morphine solution in a two-bottle choice situation, strongly validate the current model used and suggest that there may be a predisposition amongst rats to self-administer morphine.

The differences obtained in withdrawal severity when comparing involuntary and voluntary morphine intake has been described in the Results section. These differences are not surprising considering the lower intake of morphine during the voluntary phase. This is in keeping with the belief that the severity of withdrawal is proportional to both the degree of dependence and morphine intake. While it is considered that diarrhoea and weight loss are good indicators of morphine withdrawal, there is evidence within our laboratory and from others (20) to suggest that these effects can occur at very low doses of oral morphine, even if given acutely. That rats displayed some of the somatic signs of withdrawal, namely wet dog shakes, paw tremor, and teeth chatter, as well as significant weight loss, provides clear evidence that these rats had voluntarily relapsed into a state of morphine dependence.

The use of haloperidol as a possible agent in attenuating voluntary morphine consumption stems from the well-documented role of dopamine in reward and reinforcement [see Koob and Bloom for review (30)]. Specifically, the indirect stimulant actions of morphine on the mesolimbic dopamine system and on its afferents in the nucleus accumbens are thought to account for its rewarding properties (5,7,9,16,30, 32,46,53). Furthermore, the place preference induced by the administration of heroin or morphine has successfully been reversed by pimozide (6), haloperidol, and SCH23390 (31), although negative results were obtained with the use of flupenthixol (34).

The effect of administration of neuroleptics on morphine or heroin self-administration, however, is unclear. Although Smith and Davis (48) reported that high doses of haloperidol reduced morphine response rates and Ettenberg et al. (17) reported a reduction in heroin self-administration following treatment with high doses of flupenthixol, the influence of the effects of neuroleptics on motor function during these studies cannot be ignored. The extent to which DA receptor antagonists impair motor function appears to be dependent on the task being performed, therefore making it difficult to extrapolate or predict such effects.

Haloperidol (a dopamine D_2 receptor antagonist) was administered IM as the decanoate at a dose of 70.5 mg/kg,

corresponding to 50 mg/kg haloperidol. Pharmacologically effective levels of haloperidol are achieved almost immediately, with peak brain levels occurring at approximately 16 days following administration (36). After this time, the level of haloperidol falls away markedly, particularly after day 25. Similarly, the rate of excretion is decreased within several days following administration, remains rather sustained, and finally decreases gradually after 16 days (35).

The effect of haloperidol on voluntary morphine consumption, if anything, further confirmed the validity of the model described. Neuroleptics have been reported to drastically reduce water intake (27,43,49). The most likely explanation for this occurrence stems from the ability of this class of compound to produce excessive secretion of antidiuretic hormone (1,52) as a result of stimulation of the supraoptic and paraventricular nuclei (27). An alternative possibility is that DA receptor blockade attenuates drinking by an impairment of central motor systems (43), although this claim has been refuted (39,54). The reduction in fluid intake observed in the majority of rats administered haloperidol in Experiment 1 was almost exclusive for the sucrose-only solution. Of the two rats who consumed significantly less morphine solution following haloperidol treatment, there was an even greater reduction in voluntary sucrose-only solution consumption. It is most likely that the comparatively minor decrease in morphine solution intake in these rats is therefore a result of the profound effect of haloperidol in reducing total fluid intake. Thus, rats selectively reduced their total fluid intake while maintaining a consistent intake of morphine during exposure to haloperidol. No satisfactory explanation can be provided as to why this effect of haloperidol was completely reversed in one rat (rat 18; Table 3).

It should be noted that the effect on fluid intake produced by haloperidol progressively decreases with time (33), ultimately leading to an adaptation by treated animals (27) and a return to normal fluid intake. These observations, along with the pharmacokinetic data discussed above, ensure that the experiments conducted with quinine were not influenced by prior treatment with haloperidol (Experiment 1).

The facilitatory relationship between 5-HT_3 receptors and the mesolimbic dopaminergic system has recently been well documented [for review see Costall et al. (16)].

5-HT_3 receptor antagonists also block the place preference induced by morphine (8,24), as well as the place aversion produced by low doses of naloxone in morphine-dependent rats (23). Pretreatment with ondansetron, however, failed to affect the incidence of wet dog shakes, paw shakes, and salivation observed during naloxone-precipitated withdrawal in these same animals (23). This lack of correlation between place aversions and observable abstinence has also been reported by others (22,37,38), suggesting that the motivational impact of opiate abstinence may not always be addressed in conventional models of withdrawal.

The daily administration of 10 μg/kg ondansetron produced no significant changes in voluntary morphine consumption in the current method (Experiment 2). There were also no significant differences in the sucrose solution consumption or total fluid intake when compared to saline-treated controls, suggesting that ondansetron does not influence the behaviour of rats within this model, at least with respect to drinking. The dose of ondansetron is comparable to those used in place preference experiments (23,24) and other behavioural studies (10,11,12,21), and an attempt to increase the dose within the range 0.3–1 mg/kg IP daily did not produce any significant changes in any of the parameters measured (unpublished observations).

The results presented here are in contrast to those reported by Sevilla et al. (47) in several ways. Firstly, these workers reported that all previously dependent rats consumed significant quantities of morphine solution in a two-bottle choice situation, and secondly, that ondansetron significantly attenuated this preference. Oakley et al. (41) reported similar findings with ethanol in the marmoset. The reason for these discrepancies presently remains unclear, although it should be noted that in both of these studies, ondansetron was administered either before withdrawal of the drug (47) or on the first day of withdrawal of the drug (41). It remains to be seen whether the administration of ondansetron produces a different effect on voluntary morphine consumption whether administered during the development of dependence or after dependence has been established. The timing of ondansetron administration with respect to physical withdrawal may also have important implications. The fact that rats appear to have an inherent disposition to relapse voluntarily into a state of morphine dependence, however, signifies that the administration of ondansetron before the commencement of the choice phase may not be a feasible option.

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