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THE ADDICTION CYCLE TO NARCOTICS IN THE RAT AND ITS RELATION TO CATECHOLAMINES

TAI AKERA* and THEODORE M. BRODY*

Departments of Pharmacology, University of Michigan, Ann Arbor, Mich. and Michigan State University, East Lansing, Mich., U.S.A.

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Abstract—Tolerance to and physical dependence on several narcotic analgesics were demonstrated in the rat. Daily doses of morphine at 30 and 120 mg/kg and levorphanol at 6 and 15 mg/kg given in divided doses three times daily were capable of producing tolerance to the analgesic actions. With these treatment regimens withdrawal, by substituting saline for the drug or by using nalorphine, induced severe withdrawal signs characterized by weight loss, marked diarrhea, piloerection, irritability, muscle rigidity and anorexia. In our opinion, the weight loss was the best index of withdrawal; it was dose-dependent and could be quantified. Weight loss was not the result of anorexia. With the above treatments, no deviation from the normal growth curve of the rats was observed. Adrenal hypertrophy with chronic administration of morphine was not demonstrated. Tolerance to methadone was difficult to achieve and none was observed with chronic thebaine treatment. The severity of withdrawal from levorphanol was as great as or greater than that seen after withdrawal from chronic morphine treatment.

While an increase in whole brain norepinephrine (NE) was observed during chronic morphine treatment, no such increase was detected with the chronic administration of levorphanol or methadone, nor was a dose-dependent relationship established. The higher chronic levels of morphine and levorphanol resulted in no higher brain NE concentration. Withdrawal of the animal from morphine reduced brain NE levels to control values. No evidence was obtained indicating that the increased NE levels observed during chronic morphine treatment resulted from an increase in NE synthesis. Morphine enhanced the urinary excretion of catecholamines even during chronic treatment. During withdrawal after chronic drug treatment, larger amounts of epinephrine and norepinephrine were excreted, epinephrine being the primary free amine excreted. The excretion after withdrawal was dose-dependent. No relationship exists between any phase of the addiction cycle and the levels of brain NE. The increase in brain NE after morphine is peculiar to this drug and is not a characteristic of the drug class.

IN 1954 VOGT[1] demonstrated that a single injection of morphine was capable of depleting the catecholamines of the cat brain. Since that time, a series of reports[1–9] have appeared indicating that morphine can alter brain catecholamine content in various species. Since profound disturbances of the functions of the central nervous-system have long been known to occur upon withdrawal from the chronic administration of morphine, it was not unexpected that studies attempting to relate the depletion of brain catecholamines to either tolerance development or the abstinence syndrome were pursued.

* Present address: Dept. of Pharmacology, Michigan State University, East Lansing, Mich., U.S.A.

Generally, it has been observed that single doses of morphine reduce the brain norepinephrine (NE) level,[1-6] although in a few studies increases in amine content have been reported.[2, 7] In the rat, the chronic administration of morphine resulted in supranormal levels of brain NE.[3, 5, 6, 8] This rise was observed irrespective of the frequency of treatment. In other species, no increase in brain NE content has been reported.[5, 6, 9, 10] When rats were abruptly withdrawn from long-term morphine treatment there appeared to be no decrease in brain NE.[5, 6, 8] In contrast, a marked fall in brain catecholamine levels was observed in the dog after morphine abstinence.[5, 6, 9]

The purpose of the present investigation was to reexamine the effects of morphine upon tissue and urinary catecholamines in the rat by using a dosage schedule comparable to that used in studies with higher animals to produce tolerance and physical dependence in the rat. The present work was also extended to include narcotic analgesics other than morphine to determine whether the changes in catecholamines observed with morphine were unique to this drug or also occurred with related narcotic drugs.

EXPERIMENTAL PROCEDURE

Drug treatment. Female white rats of the Holtzmann strain were used in all experiments. At the beginning of the study these animals weighed between 120 and 150 g. Animals were weighed daily and the mean body weight of control and drug-treated groups was recorded. At the termination of chronic drug treatment, prior to sacrifice, the rats weighed between 180 and 240 g. All animals were maintained on a diet of standard laboratory chow and tap water. During urine collection periods, the food pellets were powdered and placed in a separate container in order to minimize contamination of the urine samples.

Morphine sulfate, levorphanol tartrate, methadone hydrochloride and thebaine hydrochloride were administered s.c. at 8-hr intervals 7 days a week. All doses were calculated as the salt. With morphine and levorphanol the dose was increased twice a week. The initial dose of morphine sulfate was 5·0 mg/kg/day (1·67 mg/kg t.i.d.) and was gradually increased to 30 mg/kg/day at the end of 3 weeks. The animals in this group were maintained at this dose prior to sacrifice. In another group of rats receiving morphine sulfate, a level of 120 mg/kg/day was attained in 7 weeks. Two groups of animals received levorphanol tartrate chronically, one group starting at 1·0 and the other at 2·5 mg/kg/day and gradually increasing the dose to 6·0 and 15·0 mg/kg/day at the end of 3 weeks. The initial dose of methadone hydrochloride was 5·0 mg/kg/day; it was elevated twice weekly for 3 weeks and then weekly to 60 mg/kg at the end of 12 weeks. Thebaine hydrochloride was administered in a dose of 10 mg/kg/day throughout the period of 8 weeks and served as an additional control. In all groups the final dosage was maintained for at least 8 days prior to the determination of catecholamines. Control rats received a comparable volume of isotonic saline solution. A separate control series was run with each drug-treated group.

Saline-treated rats, chronic drug-treated rats and rats receiving a single drug injection were sacrificed 1½ hr after the final injection of the test drug or saline, and tissue catecholamines were determined. When animals were withdrawn from chronic drug administration, they were sacrificed 32 hr after the last drug injection. Saline solution was injected s.c. in lieu of drug according to the particular drug regimen.

When tranylcypromine sulfate (Parnate, SKF 385B) was used, it was administered subcutaneously in a dose of 10 mg/kg 10 hr prior to the sacrifice of the animal.

Catecholamines. All animals were sacrificed under anesthesia after the i.p. injection of 0·4 ml/100 g body wt. of a 4% hexobarbital solution. The tissues were rapidly removed, washed carefully to remove excess blood, and weighed. The whole brain was homogenized in 7·0 ml of 0·4 N perchloric acid in a Dounce ball-type homogenizer.

The homogenates were allowed to stand for 30 min at 5° and were then centrifuged at 755 *g* for 5 min in a refrigerated centrifuge (0–3°). The supernatants were poured off into individual beakers and the tissue residues were rehomogenized with 3·0 ml of 0·4 N perchloric acid, allowed to stand for 30 min and centrifuged as above. The two extracts were combined and brought to pH 4·0 with 5·0 M and 0·25 M K_2CO_3. The resulting precipitate of potassium perchlorate was removed by centrifugation at 755 *g* for 2 min. The supernatant was poured into a beaker containing 1·0 ml of 1% disodium EDTA and 1·0 ml of 1% sodium thiosulfate. Purification on alumina columns and spectrofluorometric estimates of catecholamine levels were performed as described by Stern and Brody.[11]

Urinary catecholamines were determined in samples collected from individual animals placed in suitable metabolic cages for a period of 24 hr beginning immediately after the morning drug injection. Two subsequent injections (either drug or saline) were made during the collection period. When animals were withdrawn from chronic drug treatment, saline was substituted for the drug and the collection of urine begun just after the first saline injection. To study the acute effects of morphine on urinary catecholamines, three equally spaced doses of morphine were administered to control rats over the 24-hr collection period. Urine was collected in 6-ounce brown bottles containing 2 ml of 1 N HCl. At the end of the collection period the cage and collecting funnel were thoroughly rinsed with 0·05 N HCl and the total volume of the specimen brought to approximately 25 ml. The urine was then filtered through a rapid filter paper (Reeve Angel No. 202). The filtrate was purified on alumina columns and catecholamines were estimated according to procedures previously described.[11] With this method, 98 per cent of epinephrine (E) and 85–92 per cent of NE added to urine samples could be recovered. No corrections for recovery were made in the data reported here. Tissue catecholamines are expressed as μg/g wet wt. of tissue and urinary catecholamines as μg/kg body wt./24 hr urine sample. Data were analyzed using the *t* test for significance of differences.[12]

RESULTS

Effect of morphine, levorphanol, methadone and thebaine on rats during chronic treatment and withdrawal

With the dosage schedule employed, in groups of animals maintained at either 30 or 120 mg/kg morphine sulfate per day, there was no reduction in the growth curve during chronic administration. This is shown in Fig. 1 where the mean body wt. of 35 saline-treated animals is compared to the mean body wt. of 35 animals receiving morphine sulfate in a final dose of 120 mg/kg/day, which was the highest dose used. The dosage schedule is also seen in this figure. Since the initial dose of morphine sulfate (1·67 mg/kg) was not analgesic to rats,[13] the only observable change in these animals was a slight reduction in spontaneous activity during the first few days of morphine treatment. After 2 weeks of chronic treatment, rats receiving doses of

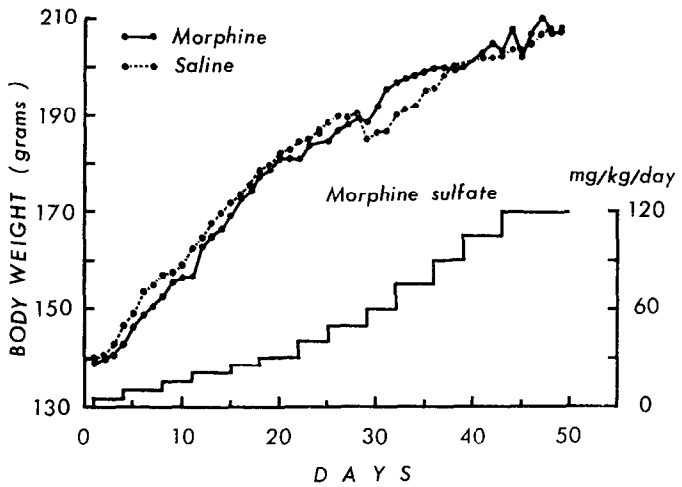

FIG. 1. The body weight of rats during chronic treatment with increasing doses of morphine. Morphine sulfate was injected at 8-hr intervals, 7 days a week, following the dosage schedule shown in the lower part of the figure. Body weight was estimated every day at 9.00 a.m. before the morning injections. Each point represents the mean body wt. of 35 rats in each group of morphine-treated rats (solid line) and saline-treated controls (dotted line). S.E. for each point is approximately 1·0 per cent of the observed values.

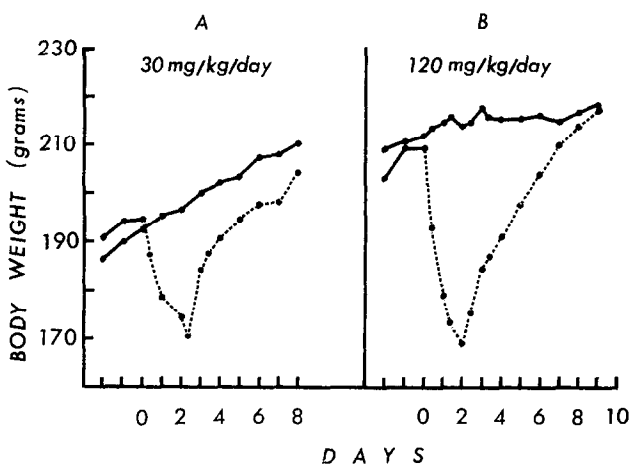

FIG. 2. The effect of morphine withdrawal upon the body wt. of rats after chronic treatment. The rats have been receiving (A) 30 mg/kg morphine sulfate/day after 4 weeks of chronic treatment with increasing doses of morphine, or (B) 120 mg/kg morphine sulfate/day after 8 weeks of the chronic treatment. Morphine was injected at 8-hr intervals. At zero, saline was substituted for morphine injection in one group while morphine injections were continued in the other group at each dose level. Dotted lines show the mean body weight of morphine-withdrawn groups (5 rats in the 30 mg/kg/day group and 4 rats in the 120 mg/kg/day group) and solid lines show the mean body wt. of 5 rats in each group receiving morphine continuously. S.E. for each point is approximately 2·3 per cent of the observed values.

20 mg/kg or more per day showed a marked increase in activity after each injection, which was noticeable 15 min after morphine administration and lasted for approximately 1 hr. After 5 weeks of chronic treatment, this stimulation was much less obvious. No analgesia (response to tail-pinch) was observed after the first 2 weeks of chronic treatment with morphine indicating an extremely rapid development of tolerance to the analgesic effects.

Upon substitution of saline for morphine in chronically treated animals, a prominent abstinence syndrome was precipitated. The signs of withdrawal included a profound weight loss, marked diarrhea, piloerection, irritability and resistance to handling, muscle rigidity and anorexia. A "depressant" phase was not observed,[14] nor were "wet dogs",[15] a consistent sign of withdrawal with the morphine schedule described here. The loss in body weight seemed to be the best index of the severity of the physical dependence. Fig. 2A illustrates the loss in body weight upon abrupt withdrawal. These animals had been receiving 30 mg/kg/day in three divided doses. At zero, saline was substituted for the morphine injections. The fall in body weight was rapid and the maximum loss occurred between 48 and 72 hr, at which time other withdrawal signs were also most prominent. The values shown represent the mean body weight of 5 animals in each group. Recovery was slow and at 9 days the animals had not completely regained the weight lost after withdrawal. The severity of the abstinence syndrome, including weight loss, is dose-dependent. Animals tolerant to higher levels of morphine, *i.e.* the group maintained at 120 mg/kg/day, show a greater weight loss and more intense withdrawal signs. Fig. 2B shows a plot of the loss in body weight in animals withdrawn from this level of morphine addiction; this group of animals lost almost 25 per cent of their normal body weight upon withdrawal. If morphine is readministered to these animals during withdrawal, the increase in body weight is extremely rapid and normal levels are attained about 36 hr after chronic drug treatment is reinstituted. Fig. 3A illustrates a typical experiment where 2 animals were withdrawn and morphine was readministered 32 hr after withdrawal; a very rapid reversal in body weight is seen. These animals had been maintained on 40 mg/kg morphine sulfate/day for 8 days prior to withdrawal.

That the weight loss observed on abrupt withdrawal was not entirely the result of anorexia was indicated by a study shown in Fig. 3B. This group of 4 animals had been maintained on 30 mg/kg morphine sulfate per day and were continued on that dose for the entire experiment. At zero, food was withdrawn. The fall in mean body weight is modest when compared to the loss of weight seen after abrupt withdrawal of drug in Figs. 2A and 3A. Moreover, the recovery of weight when food is made available is considerably slower than that observed after abrupt withdrawal and subsequent reinstitution of the normal drug schedule.

When 25 mg/kg of *n*-allyl normorphine (nalorphine) was administered to chronically morphinized rats, the same general pattern of withdrawal signs was observed. The signs, however, were more rapid in onset and terminated more rapidly. Although a marked weight loss was also seen after *n*-allyl normorphine, the loss was not as severe as that seen on abrupt withdrawal of morphine. *n*-Allyl normorphine alone had no effect on the body weight of normal rats.

Addiction to levorphanol was similar to that observed after morphine. Animals made tolerant to levorphanol showed no decrease in body weight compared to saline-treated controls (Fig. 4). At the time of sacrifice these animals were receiving 15 mg/kg

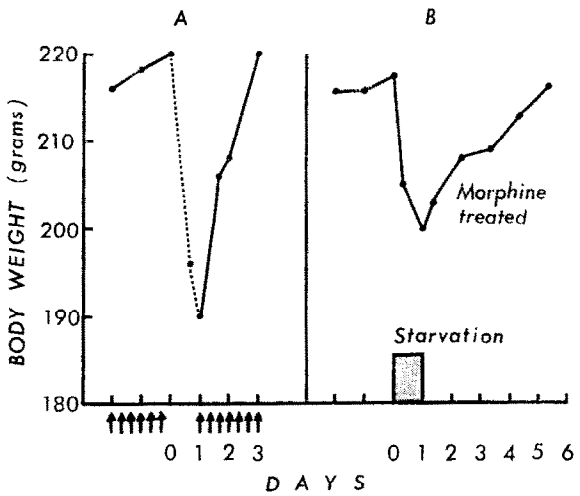

FIG. 3. Comparison of the effect of 24-hr withdrawal from morphine and from food upon the body wt. of rats after chronic treatment with morphine.

(A) Two rats were injected with 40 mg/kg morphine sulfate/day in three divided doses as indicated by arrows after chronic treatment with increasing dose of morphine for 4 weeks. Each arrow indicates the s.c. injection of 13·3 mg/kg morphine sulfate. Saline was substituted for 3 morphine injections where the arrows are not shown.

(B) These rats were receiving 30 mg/kg morphine sulfate/day after chronic treatment with an increasing dose of morphine for 4 weeks. At zero, food, but not drinking water, was withdrawn. Free access to food was allowed after 24 hr. Injections of morphine were continued. Each point represents the mean body weight of 4 rats. S.E. for each point is approximately 2·0 per cent of the observed values.

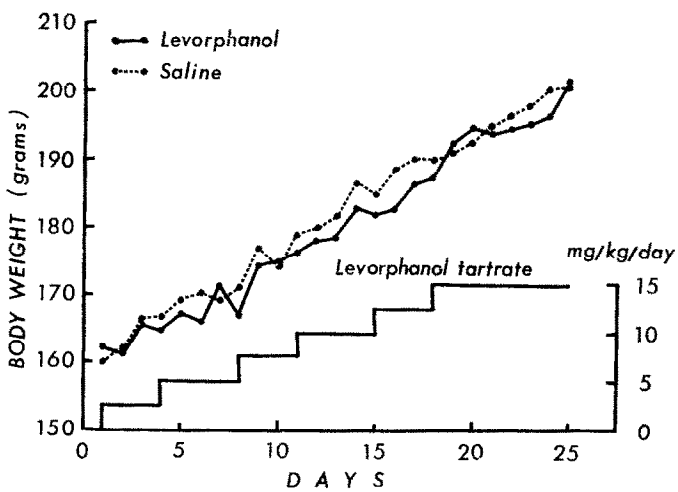

FIG. 4. The body wt. of rats during chronic treatment with an increasing dose of levorphanol. Levorphanol tartrate was injected at 8-hr intervals, 7 days a week, following the dosage schedule shown in the lower part of the figure. Body wt. was estimated every day at 9.00 a.m. before the morning injections. Each point represents the mean body wt. of 14 rats in the levorphanol-treated group (solid line) and of 10 rats in the saline-treated control group (dotted line). S.E. for each point is approximately 1·5 per cent of the observed values.

levorphanol tartrate per day. Tolerance development was extremely rapid, but the increase in spontaneous activity seen early during morphine treatment was not prominent when levorphanol was used. The abstinence signs on abrupt withdrawal from levorphanol were similar to those seen after morphine withdrawal. Peak reduction in body weight was observed 32 hr after the last dose of levorphanol tartrate (Fig. 5) and the recovery of body weight in this group of animals took approximately 6 days. If the rats were withdrawn from the lower maintenance dose of levorphanol, 6 mg/kg/day, a smaller fall in body weight was observed and the other abstinence signs appeared to be less severe. Again, the intensity of abstinence correlated well with the maintenance dose of the drug.

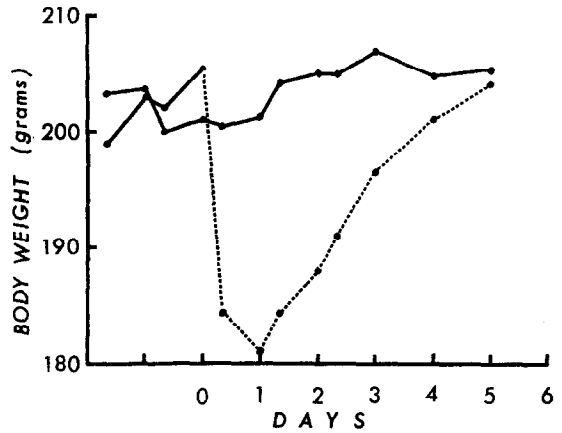

FIG. 5. The effect of levorphanol withdrawal upon the body wt. of rats after chronic treatment. The rats were receiving 15 mg/kg levorphanol tartrate/day after 4 weeks of chronic treatment with an increasing dose of levorphanol at 8-hr intervals. At zero, saline was substituted for levorphanol injections in one group (dotted line) while levorphanol injections were continued in the other group (solid line). Each point represents the mean body wt. of 5 rats in each group. S.E. for each point is approximately 2·5 per cent of the observed values.

Rats chronically treated with thebaine showed no change in body weight either during chronic drug treatment (Fig. 6) or upon abrupt withdrawal of the drug. There was no development of physical dependence.

With methadone, it was not possible to maintain the body weight of rats at control levels even with the dosage schedule used. When animals were started at 5 mg/kg/day, as were the morphine animals, and the dosage was elevated in the same manner as in the morphine group, the growth curve fell precipitously as compared to saline- or thebaine-treated animals. When this occurred, the dose of methadone was reduced and thereafter increased weekly instead of twice a week. With this regimen the methadone group gained weight but never attained the control level (Fig. 6). At the end of a 12-week treatment with methadone, the mean body weight of rats was approximately 10 per cent lower than that of controls. Furthermore, upon withdrawal from this dosage schedule rats failed to show any loss in body weight, but rather exhibited a gain in weight upon abrupt withdrawal from as much as 60 mg/kg methadone/day. After 3 weeks, when the dose had been increased to 15 mg/kg/day, the analgesic effect of methadone was still marked and the rats were severely depressed for approximately $1\frac{1}{2}$ hr after each injection. Thus, the development of tolerance was much slower with

methadone than with either morphine or levorphanol in the rat. Analgesia and sedation were observed with methadone until the experiments were terminated. There were also local edema, induration and hair loss at the site of injection.

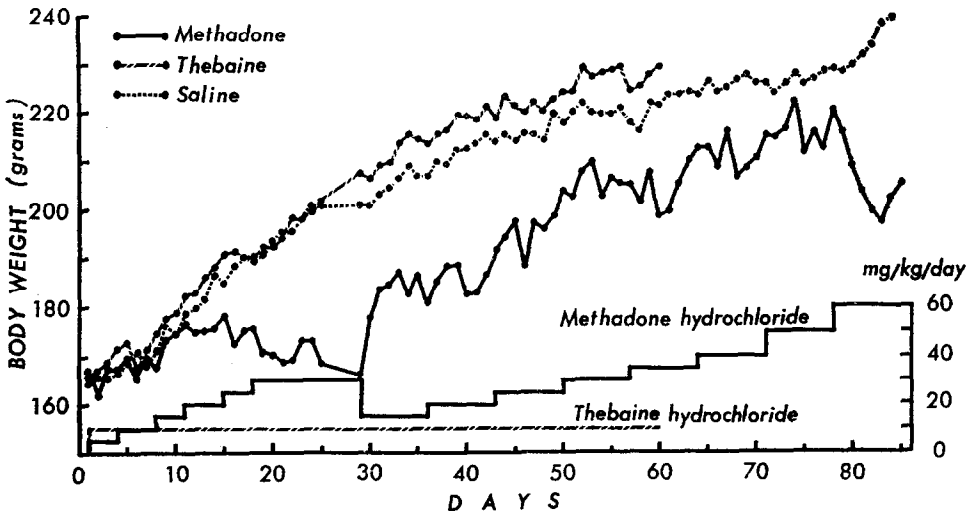

FIG. 6. The body wt. of rats during chronic treatment with methadone and thebaine. Methadone or thebaine was injected at 8-hr intervals, 7 days a week, following the dosage schedule shown in the lower part of the figure. Body wt. was estimated every day at 9.00 a.m. before the morning injections. Each point represents the mean body wt. of 14 methadone-treated rats (solid line), 14 thebaine-treated rats (broken line), and 10 saline-treated controls (dotted line). S.E. for each point is approximately 2·0 per cent of the observed values.

Effect of chronic treatment on rat adrenals

Adrenal hypertrophy has been observed frequently during chronic treatment with morphine.[15–19] In the present study adrenal weights were determined in saline-treated controls, in normal rats receiving no treatment, in animals receiving 120 mg/kg morphine sulfate per day chronically, and in a group dosed with levorphanol tartrate, 6 mg/kg/day. The treated animals were sacrificed 4–7 weeks after chronic treatment had begun and 1½ hr after the last injection. The data indicate (Table 1) that with the dosage schedule described above there is no increase in adrenal weight during chronic treatment with these narcotic analgesics. Nor do repeated injections of saline cause adrenal hypertrophy.

TABLE 1. EFFECT OF CHRONIC TREATMENT WITH SALINE, MORPHINE AND LEVORPHANOL ON ADRENOL WEIGHTS OF RATS

Treatment	Dose (mg/kg/day)	No. of animals	Adrenal glands (wet wt., mg/pair)*
Normal		12	47·5 ± 1·2
Saline controls		6	48·4 ± 2·7
Chronic morphine sulfate	120	7	41·9 ± 2·7
Chronic levorphanol tartrate	6	6	47·7 ± 2·5

* Mean ±S.E.

Effect of morphine on tissue NE levels

As shown in Table 2, after 4 weeks of chronic treatment with increasing doses of morphine (final, 30 mg/kg/day), the brain NE levels of rats were elevated. They returned to control levels upon abrupt withdrawal from the drug 32 hr after the last dose. A single dose of 10 mg/kg had no effect on brain NE levels when injected into nonaddicted rats. Tranylcypromine sulfate, a monoamine oxidase inhibitor,[20] increased brain NE levels of chronically morphinized, withdrawn or control animals in proportion to the original content of catecholamine found in the brains of these animals without inhibitor (Table 2); the levels increased in these groups 63, 59 and 61 per cent respectively.

TABLE 2. EFFECT OF MORPHINE ON BRAIN NE LEVELS AND THE INFLUENCE OF MONOAMINE OXIDASE INHIBITOR IN RATS

Treatment	Dose	No. of animals	Norepinephrine (μg/g wet wt. tissue $\pm$S.E.)
Saline controls		25	0·44 ± 0·01
Tranylcypromine sulfate	10 mg/kg	7	0·72 ± 0·02
Morphine sulfate, single dose	10 mg/kg	6	0·44 ± 0·04
Chronic morphine sulfate	30 mg/kg/day	13	0·51 ± 0·01*
Morphine abstinent (32 hr)	30 mg/kg/day	14	0·43 ± 0·02†
Chronic morphine sulfate + Tranylcypromine sulfate	30 mg/kg/day + 10 mg/kg	4	0·81 ± 0·06
Morphine abstinent (32 hr) + Tranylcypromine sulfate	30 mg/kg/day + 10 mg/kg	4	0·73 ± 0·02
Chronic morphine sulfate	120 mg/kg/day	12	0·50 ± 0·01*
Morphine abstinent (32 hr)	120 mg/kg/day	4	0·45 ± 0·01‡

* Different from control, $P < 0·001$.
† Different from chronic morphine sulfate (30 mg/kg/day), $P < 0·001$.
‡ Different from chronic morphine sulfate (120 mg/kg/day), $P < 0·01$.

After 7 weeks of treatment with morphine, at which time the rats were receiving 120 mg/kg/day, the same increase in brain NE was observed as with the lower morphine dosage used above. Again, 32 hr after abrupt withdrawal the brain levels were essentially normal. Rats addicted to the higher level of morphine exhibited a greater loss in body weight and a more marked withdrawal syndrome than those animals withdrawn from the lower level.

Effect of levorphanol on brain NE levels

Brain NE levels of rats chronically treated with increasing doses of levorphanol tartrate for 4 weeks were within the normal range (Table 3). These animals had been receiving 6 mg/kg of the drug per day. The concentration of brain NE was unaltered when a higher dosage schedule was employed or when the rats were withdrawn abruptly from the higher drug level. Since in a limited number of rats chronically treated with levorphanol there appeared to be a large increase in the urinary excretion of catecholamines, it was possible that this increased output was obscuring the rise in brain levels. Tranylcypromine sulfate was therefore used to inhibit monoamine oxidase and to attempt to unmask the amine change. When these studies were performed, analysis of brain NE in levorphanol-tolerant rats 10 hr after the s.c. injection of 10 mg/kg of the monoamine oxidase inhibitor showed no significant difference in brain NE compared to the saline-treated group (Table 3).

Effect of methadone on brain NE levels

A single dose of 5 mg/kg methadone hydrochloride lowered the brain NE levels markedly when injected into normal rats. Even 20 mg/kg methadone, lethal if given to normal rats, failed to alter brain NE levels when injected into rats in the course of chronic treatment (Table 3), nor were levels changed upon abrupt withdrawal from methadone.

TABLE 3. EFFECT OF LEVORPHANOL, METHADONE AND THEBAINE ON BRAIN NE LEVELS AND THE INFLUENCE OF A MONOAMINE OXIDASE INHIBITOR

Treatment	Dose	No. of animals	Norepinephrine (μg/g wet wt. tissue $\pm$S.E.)
Saline controls		25	0·43 ± 0·01
Tranylcypromine sulfate	10 mg/kg	7	0·72 ± 0·02*
Levorphanol tartrate single dose	2 mg/kg	4	0·43 ± 0·01
Chronic levorphanol tartrate	6 mg/kg/day	5	0·43 ± 0·02
Levorphanol abstinent (32 hr)	6 mg/kg/day	5	0·47 ± 0·02
Chronic levorphanol tartrate	15 mg/kg/day	5	0·45 ± 0·02
Chronic levorphanol tartrate }	6 mg/kg/day	7	0·76 ± 0·03*
Tranylcypromine sulfate }	10 mg/kg		
Methadone HCl, single dose	5 mg/kg	5	0·33 ± 0·02*
Chronic methadone HCl	60 mg/kg/day	6	0·46 ± 0·03
Methadone withdrawn (32 hr)	60 mg/kg/day	5	0·46 ± 0·01
Thebaine HCl, single dose	3·3 mg/kg	7	0·41 ± 0·02
Chronic thebaine HCl	10 mg/kg/day	5	0·45 ± 0·04
Thebaine withdrawn (32 hr)	10 mg/kg/day	4	0·44 ± 0·02

* Different from controls, $P < 0·001$.

Effect of thebaine on brain NE levels

A single dose of 3·3 mg/kg thebaine did not alter brain NE levels in normal rats. No change was observed during chronic treatment or upon abrupt withdrawal (Table 3).

Urinary excretion of catecholamines

The urinary excretion of free catecholamines was markedly increased when 10 mg/kg morphine sulfate was administered to nonaddicted rats three times at 8-hr intervals (Table 4). After 4 weeks of chronic treatment with increasing doses of morphine, the urinary output of free catecholamines was still enhanced. These animals were receiving 10 mg/kg morphine sulfate three times a day. However, the increase observed was somewhat less in these animals than in nonaddicted rats receiving the same dose. On the other hand, when morphine was abruptly withdrawn from the chronically morphinized rats, the urine collected from 8 to 32 hr after the final injection of morphine showed a very high concentration of free E and NE.

When the higher dose of morphine sulfate was administered (120 mg/kg/day), a greater excretion was observed, although the same basic pattern seen with the smaller dose held for this dosage schedule. In all experiments, although changes in E and NE were observed, the predominant amine excreted was E, particularly in rats withdrawn from morphine indicating perhaps that the major source of enhanced excretion was the adrenal glands.

When chronically morphinized rats were withdrawn from 120 mg/kg/day for a period of 2 weeks, they regained their normal body weight and all overt signs of abstinence disappeared. At this time the urinary excretion of E and NE was at control levels.

TABLE 4. EFFECT OF MORPHINE ON URINARY EXCRETION OF CATECHOLAMINES

Treatment	Dose	No. of animals	Urinary epinephrine*	Urinary norepinephrine*
Saline controls		8	1·36 ± 0·29	2·28 ± 0·24
Acute morphine sulfate	30 mg/kg	8	3·61 ± 0·65†‡	4·85 ± 0·65†
Chronic morphine sulfate	30 mg/kg/day	8	2·47 ± 0·26†	3·22 ± 0·34†
Morphine abstinent	30 mg/kg/day	7	6·17 ± 0·83†‡	3·35 ± 0·41†
Acute morphine sulfate	120 mg/kg	8	14·05 ± 1·57†§	6·88 ± 0·83†
Chronic morphine sulfate	120 mg/kg/day	8	5·28 ± 0·65†	5·04 ± 0·55†
Morphine abstinent	120 mg/kg/day	8	21·36 ± 3·37†§	8·63 ± 1·26†
Day 13 of morphine abstinence		8	0·67 ± 0·10†	1·92 ± 0·19

* Expressed as μg/kg/24 hr, mean ±S.E.

† Different from controls, $P < 0·01$.

‡ Different from chronic morphine sulfate 30 mg/kg/day, $P < 0·01$.

§ Different from chronic morphine sulfate 120 mg/kg/day, $P < 0·001$.

DISCUSSION

In the present investigation it has been demonstrated that tolerance and physical dependence in rats can be achieved with relatively low doses of narcotic analgesic drugs. It has been clearly shown that this state may be achieved without any significant reduction in the growth curve of the animal. Most previous studies[15, 19, 21] indicated a reduction in the growth curve during chronic morphine treatment. Sollman[22] reported an increase in growth in the rat receiving small doses of morphine mixed in the diet. No description of the development of tolerance or physical dependence was mentioned, however. The present study confirms the observation of Weeks[23] who reported in chronically cannulated rats the rapid development of tolerance and physical dependence and no weight loss with small doses of morphine while the drug was self-administered at frequent intervals. In our study, with doses of morphine or levorphanol comparable to those used in larger mammals and administered three times daily, the growth rate of rats was not inhibited. Under these conditions it was still possible to demonstrate tolerance to the analgesic effects and to observe the typical abstinence signs when the drugs were withdrawn. Reduction in the growth curve observed by others may result from infrequent injections, since as early as 8 hr after the final dose of morphine the body weight of addicted rats begins to decrease.

The abstinence syndrome in rats could be demonstrated with doses of morphine sulfate as low as 10 mg/kg/dose or with 2 mg/kg/dose of levorphanol tartrate, doses commonly used in other mammalian species. Abstinence symptoms (profound weight loss, marked diarrhea, piloerection, irritability, resistance to handling, muscular rigidity and anorexia) could be demonstrated either by the substitution of saline for the narcotic drug or upon nalorphine injection.

In our opinion, the loss of body weight upon withdrawal is the best index of addiction in rats. It is objective, easy to recognize, and dose-dependent with both morphine and levorphanol. It is not merely the result of anorexia. In addition to loss of body weight, the other symptoms described above were also observed when the animals were withdrawn from either morphine or levorphanol. The "wet dog" phenomenon, designated as an important abstinence sign by Martin *et al.*,[15] was not observed in this study.

Undoubtedly the dose and the frequency of administration must be determined for each individual narcotic drug. Methadone, for example, seems to require more frequent administration. Elimination of methadone is rapid in the rat according to Way *et al.*[24] and Rickards *et al.*[25] Wikler and Frank[26] were able to demonstrate abstinence symptoms qualitatively similar to those produced by morphine after the four times daily administration of methadone to dogs. In the present study we were able to demonstrate tolerance to methadone in the rat, but it was difficult to demonstrate physical dependence in the species. With thebaine, no tolerance, physical dependence or loss of body weight upon withdrawal was observed.

Observations on the state of the animal during chronic drug administration will vary with the size and the frequency of the dose. Morphine should be given at least three times daily, since the duration of action of morphine is less than 8 hr. Any change resulting from the single or twice daily injection may result from repeated withdrawal or stress rather than from the chronic effect of morphine *per se*. Martin *et al.*[15] reported that rats were apparently withdrawn before each injection when morphine was given twice daily. Kaymakcalan and Woods,[14] administering 100 mg/kg morphine to rats twice daily, observed "sedation" during withdrawal. This was not observed in the present study. During withdrawal from morphine or levorphanol the rats were extremely irritable, which is probably comparable to the excitation generally seen in other mammalian species.

In the present study no increase in adrenal weight was seen during chronic treatment with morphine or levorphanol, nor did the repeated injection of saline increase adrenal weight. The previously observed increases in adrenal weight reported during chronic treatment with morphine[15–19] were explained by hypothalamic or pituitary stimulation.[19, 27, 28] The above authors employed single or twice daily drug injections, and the rats were undoubtedly withdrawn part of each day after having been addicted to morphine. Since withdrawal is likely to be a stress, these changes in adrenal weight probably result from the repeated stress rather than from the primary effect of morphine. Our results may also indicate that no continuous stress was imposed upon our animals during the course of chronic morphine or levorphanol administration. An alternate explanation would be that our dosage was too small to induce changes in adrenal weight. The initial dose of morphine sulfate used in the present study, 1·67 mg/kg/dose, is well below that needed to induce changes in the hypophyseal–adrenal system.[28] As the dose is increased it is conceivable that the rats would become tolerant and therefore the adrenal effect was not observed.

The increase observed in brain NE during the course of narcotic addiction seems to be a specific response to morphine and is not related to tolerance development. In the present study we could not demonstrate a dose-dependent effect. Rats receiving 120 mg/kg morphine sulfate per day had no higher brain NE levels than those animals receiving the lower dose of 30 mg/kg/day. The NE content in both these groups was significantly higher than control levels. Maynert and Klingman[5] observed an increase in brain NE only at a dose of 200 mg/kg/day but not at 150 mg/kg/day. Undoubtedly this difference is largely the result of the frequency of drug administration.

The above authors speculated that the increase in brain NE during chronic morphine treatment might involve increased synthesis. From their data and from ours with the monoamine oxidase inhibitor, tranylcypromine, there is a significantly greater amount of norepinephrine present in the brains of the chronically morphinized groups. How-

ever, the relative increase is not different from that observed in controls after the monoamine oxidase inhibitor. Maynert and Klingman observed a 49 per cent increase in brain NE of controls after monoamine oxidase inhibition and 56 per cent after the same treatment in chronically morphinized animals. Our data are remarkably similar; NE increased 63 and 58 per cent respectively in the two groups after tranylcypromine. While the absolute increases in brain NE may be significantly different, the percentage increases are not. Under the experimental conditions used, it is difficult to ascertain whether there is an enhanced NE synthesis in the brain. A more meaningful approach would be to measure the rate of decrement of NE levels after an agent that blocks the synthesis of NE.

In the present study there was a decrease in brain NE during abstinence, the amine concentration returning to essentially control levels. Although Maynert and Klingman observed this in the dog and rabbit, they did not obtain this result in the rat. There was no significantly greater reduction in the levels during abstinence in the presence of a monoamine oxidase inhibitor in our study.

A greater reduction of brain NE found in other species during abstinence by Maynert and Klingman led them to the postulation that elevated levels of free NE within the brain could play a role in antagonizing the depressant effects of morphine during chronic treatment and also induce an excitation during abstinence where depressant effects of morphine were suddenly removed. It is more logical to assume that the decrease in the brain NE level during withdrawal is the result of stimulation rather than the cause.

If the catecholamines play an important role in the addiction cycle, other potent addictive drugs should produce like increases in brain NE during chronic drug administration. Studies at two dose levels of levorphanol failed to demonstrate a similar type of response. Neither levorphanol treatment (at final drug levels of 6 or 15 mg/kg/day) produced any change in brain NE levels, nor was there a decrease in NE concentration during withdrawal. With both treatments severe abstinence symptoms including weight loss were observed during withdrawal. Experiments with monoamine oxidase inhibitor revealed no significant differences in brain NE levels between levorphanol-treated and control animals.

Methadone treatment did not alter brain NE levels during chronic treatment, nor could the loss of weight be demonstrated upon drug withdrawal. Development of tolerance to the lethal effect of methadone was apparent since 5 of 17 rats (29 per cent) died when 10 mg/kg methadone HCl was injected into previously unexposed rats, whereas a 10 or even 20 mg/kg dose had no lethal effect during the course of chronic treatment with an increasing dosage schedule. The development of tolerance, particularly to the analgesic action is slow. Similar conclusions regarding development of tolerance to methadone have been reported by Bianchi and Franceschini.[29] Tolerance to the effect of methadone on brain NE can be observed in Table 4. Here it is noted that while 5 mg/kg of methadone will significantly reduce the brain NE levels in control rats, during chronic drug treatment, 20 mg/kg has no effect, and there is no reduction in brain NE when the drug is withdrawn.

Thebaine, which does not produce addiction, did not produce any change in the brain level of NE during chronic treatment or after withdrawal. From the present study in the rat we must conclude that there is no relationship between the addiction cycle and the levels of brain catecholamines. This conclusion is based upon the demon

stration that: 1) the increase in brain NE levels during chronic morphine treatment is not dose-dependent, whereas the intensity of the abstinence syndrome increases with the larger dose; 2) levorphanol, a potent narcotic analgesic which produces tolerance and physical dependence, does not influence brain NE during chronic treatment nor does the brain concentration of NE fall during drug withdrawal; 3) methadone does not increase NE levels, although some degree of tolerance is achieved. Similar conclusions have been drawn from studies of addiction and brain catecholamines in the monkey.[10]

Partial tolerance was observed to the action of morphine in increasing the urinary excretion of free catecholamines particularly at high dose treatment. Gunne[6] also reported the initial rise and the subsequent reduction in excretion during chronic morphine treatment. It is also apparent that this response is dose-dependent. The control values are probably higher than those of "untreated" animals and represent the response to the injection of saline. The control values after the thirteenth day of abstinence more closely resemble "untreated controls", since the animals are now probably tolerant to the saline injection. The urinary excretion of free catecholamines after a course of addiction to levorphanol followed the same general pattern observed with morphine treatment.

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