Pharmacology Biochemistry & Behavior, Vol. 17, pp. 495–501, 1982. Printed in the U.S.A.

A New Ingestion Method for Long-Term Morphine Intoxication in Rat

JENNY ZEUCHNER, LARS ROSENGREN, ANDRZEJ WRONSKI AND LARS RÖNNBÄCK[1]

Institute of Neurobiology, University of Göteborg, Göteborg, Sweden

Received 9 December 1981

ZEUCHNER, J., L. ROSENGREN, A. WRONSKI AND L. RÖNNBÄCK. *A new ingestion method for long-term morphine intoxication in rat.* PHARMAC. BIOCHEM. BEHAV. 17(3) 495–501, 1982.—A new method for long-term morphine intoxication in rat was developed. It was designed to deal with the nutritional imbalance and body weight loss that generally occurs using conventional techniques for morphine treatment. The morphine is administered in a nutritionally complete diet. Also pair-feeding is used to deal with intoxicated rats that do not eat the same amount of food as controls. The technique was validated during the study of different intoxication conditions, using different initial doses, dose increments and final doses. An initial dose of 25 mg morphine/kg b.w., raised exponentially up to 340 mg/kg b.w. in 8 days, made the rats dependent, as tested by withdrawal signs, precipitated by excluding morphine from the diet, or by administration of antagonists. A final dose of up to 715 mg morphine/kg b.w. was reached in 13 days without decreased food intake. However, initial doses of 340 or 715 mg/kg led to impaired weight gain and diet consumption. Plasma morphine levels of 3 μg/ml serum were reached on a dose of 340 mg/kg b.w. Also, preference for morphine diet over control diet was evaluated by choice tests. The technique is simple, time-saving and inexpensive, allowing the treatment of numerous animals for long periods under standardized intoxication conditions. No animals get ill or die.

Morphine intoxication Ingestion method Rat

MANY methods have been developed in order to make animals physically dependent upon morphine. Morphine can be injected intraperitoneally, intramuscularly or subcutaneously. These routes of administration are characterized by good absorption of the drug. The method is often also used as premedication in "combined methods" (see below). A disadvantage is the unfavourable weight gain in morphinized animals compared to that of controls (*cf.* 15,22]). Furthermore, due to the low solubility of morphine in water, large volumes have to be injected when higher doses are administered. Another disadvantage with the injection technique is the uneven plasma levels of morphine with high concentrations following the injections [3]. It has been shown that uniform serum levels of morphine are to be preferred as a means of making animals dependent [3,9].

Morphine intoxication is also produced by implantation of morphine-containing pellets in the subcutis or the muscles [1, 4, 12, 19, 29, 30]. The pellets tend to become encapsulated, thus creating low serum levels. Repeated anesthesia and surgery often lead to a high frequency of infections. Therefore, the method is suitable for intoxication periods not longer than 4–6 days [12,29].

A number of methods have been developed where morphine is administered orally (see [16, 25, 26]. One method is to offer the test animal a solution of morphine in tap water as the sole drinking source [2, 6, 11, 15, 21, 25, 26]. This procedure is easy to handle without manipulation of the animal. However, the animal avoids drinking the morphine solution, unless premedicated or forced to drink. It has been suggested that this is caused by the bitter taste of morphine even in low concentrations. Thus, morphine consumption will be directed by the animal's need for water [11]. Therefore it may become necessary to control the consumption of morphine by having the animal drink morphine solution. Also, it has been observed that by dissolving morphine in a sucrose solution, rats increased their consumption, and dependence could be demonstrated. However, the different caloric intake makes weight gain hard to control.

In addition, morphine can be administered via catheters or probes, either in the stomach or in blood vessels. By using these methods the bitter taste is avoided. However, complicated equipment, together with repeated anaesthesia and/or surgery, is necessary. If self-administration is used, then some form of learning procedure or premedication with morphine injections is required (see [2,16]).

Another method for treating is to administer morphine in solid food together with morphinized drinking water. By use of this method, rats have been made dependent in such a short period as a week [15]. However, it is very laborious to avoid a caloric imbalance. Furthermore, if an automatic food intake measuring-apparatus is not used, morphine consumption is difficult to measure (see [32]).

In summary, in most chronic morphine intoxication models weight loss or poor weight gain is a prominent fea-

[1]Send reprint requests to Dr. Lars Rönnbäck, Institute of Neurobiology, University of Göteborg, P.O.B. 33 031, S-400 33 Göteborg, Sweden.

ture, and the caloric imbalance between intoxicated rats and control rats is hard to avoid. When determining changes in biochemical parameters (i.e., brain protein metabolism, neurotransmitter metabolism) during long-term morphine treatment caloric imbalance between intoxicated and control rats could not be allowed to influence the results.

Our aim was to develop a model for long-term morphine intoxication of rats where the nutrient intake in intoxicated and control animals should be complete, and caloric imbalance avoided. We chose a method using oral intake of morphine dissolved in a nutritionally complete fluid diet.

METHOD

Animals

A total of 209 male rats of the Sprague-Dawley strain were used in this study. The weights of the animals at the beginning of the experiments were around 150 g. No mortality was seen during the studies. No animals got ill or were withdrawn from the experiments.

Drugs

Morphine-chloride, naloxone and nalorphine-chloride were bought from Apoteksbolaget, Sweden.

Cages

The rats were housed individually in cages ($41 \times 25 \times 15$ cm^3) in a room with constant temperature. Lights were turned on between 7 a.m. and 5 p.m. The fluid diet was administered in graded Richter tubes. Consumption could easily be determined every day.

Diet Composition

The ingredients of the diet (Table 1) were mixed to a stabilized solution in a Warren Blender Mixer with vitamins according to Table 2. Routinely 90 ml in graded Richter tubes were given to each rat every day.

Procedure

Upon arrival from the supplier, the rats were weighed and placed individually in cages. Prefeeding for 2–3 days on control fluid diet was performed to adapt the animal to the new diet. Morphine was added in a dose of 25 mg/kg b.w. on the first day of treatment, increasing up to 55, 90, 130, 175, 225, 280 and 340 mg/kg b.w./day, or in some experiments continued increase up to 405, 475, 550, 630 and 715 mg/kg b.w./day. The daily dose of morphine was achieved when the animal had consumed the 90 ml diet offered.

The rats were fed and the consumption determined daily at 6–7 p.m. and weighed every 2nd–4th day. During choice tests or withdrawal, food consumption and body weight were determined up to four times daily.

Development of Physical Dependence

Physical dependence was evaluated by observing withdrawal signs following administration of nalorphine (20–30 mg/kg b.w.) or naloxone (6 mg/kg b.w.) injected intraperitoneally, or by excluding morphine from the diet. The following abstinence symptoms were observed: (1) decrease in food intake, (2) weight loss, (3) diarrhea, (4) irritability when the animal was handled. The method made it possible to elucidate the preference for mophine diet vs control diet by choice test. The morphine tube was randomly kept in a fixed position in every cage, either to the right or to the left in the different cages.

TABLE 1

COMPOSITION OF THE FLUID DIET

Casein hydrolysate (Sigma Chem. Co. St. Lous, MO)	41.4 g
L-cyst inium chloride monohydrate (Merck)	0.5 g
DL-methionine (Merck)	0.3 g
Vitamin mixture (see Table 2)	3.0 g
Salt mixture Rogers-Harper (United States Biochem. Corp., Cleveland OH)	10.0 g
Corn Oil (DAGAB, Sweden)	8.5 g
Olive oil (DAGAB, Sweden)	28.5 g
Sucrose (DAGAB, Sweden)	150.9 g
Morphine-chloride (Apoteksbolaget, Sweden)	x mg
Lecitin (Kebo, Sweden)	2.0 g
Distilled water	to 1.0 l

Morphine-chloride added to diet of experimental animals:

$$x = \frac{\text{dose morphine (mg/kg b.w.)} \times \text{mean body weight (g)}}{\text{mean volume diet consumed per rat (ml)}}$$

Determination of Morphine Plasma Levels

Free morphine in the plasma of the morphine-drinking rats was determined by gas chromatography after acetylation to heroin [23,27] (Department of Clinical Chemistry, Central Hospital of Uddevalla).

Histological Examination of Small Intestine and Kidney

Small intestine and kidney were dissected from morphine-intoxicated, abstinent and control rats, after 50 days diet consumption, and usual pellet eating rats of similar weight. They were fixed in 4% formaldehyde, embedded in paraffin and prepared for routine light microscopy examination, 10 μm sections, stained with HTX-Eosin.

Injection Technique

As a reference 12 rats with a beginning weight of 150 g were injected intraperitoneally twice daily with a morphine solution. The initial dose was 5 mg morphine per kg b.w. and rat. The dose increase was 5 mg morphine per kg b.w. per day. Six controls were injected with similar volumes of saline as the intoxicated rats. The animals were kept for 20 days and reached a daily morphine dose of 100 mg/kg b.w.

Statistical Evaluations

Statistical analyses were done according to Student's *t*-test. S.E.M. values are given.

RESULTS

Morphine Intoxication with the Peroral Method

Free consumption of morphine diets, containing 340 mg/kg b.w. (Fig. 1) or 715 mg/kg b.w. (Fig. 2, curve 2) was

TABLE 2

COMPOSITION OF THE VITAMIN DIET FORTIFICATION MIXTURE*

	Grams
Vitamin A acetate (200,000 units per gram)	4.5
Vitamin D_2 (400,000 units per gram)	0.25
Alpha tocopherol	5.0
Ascorbic acid	45.0
Inositol	5.0
Choline chloride	75.0
Menadione	2.25
Paminobenzoic acid	5.0
Niacin	4.5
Riboflavin	1.0
Pyridoxine hydrochloride	1.0
Thiamine hydrochloride	1.0
Calcium pantothenate	3.0
	Mg
Biotin	20
Folic acid	90
Vitamin B 12	1.35

*A mixture of the above vitamins triturated in dextrose to a total amount of 1 kg (bought from the United States Biochemical Corporation, Cleveland, OH).

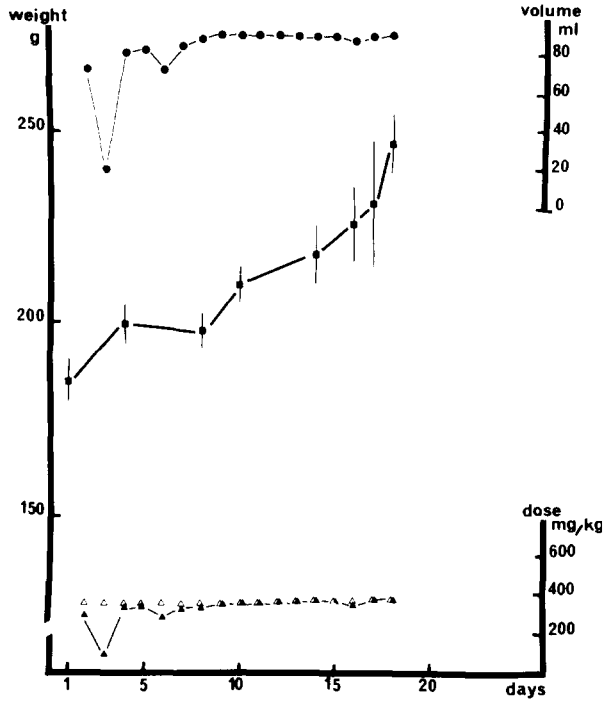

FIG. 1. Free fluid diet consumption of 6 male rats on a morphine dose of 340 mg/kg b.w. (providing 90 ml consumed). The daily morphine diet ingestion was 72.8±10.6 ml/day during the first 6 days of the experiment (until day 8 in the figure). The mean weight gain of this period is 2.1 g/day. Rats receiving control diet or morphine in an escalating fashion show a daily weight gain of about 6 g. From day 9 the rats consumed 90 ml fluid diet, giving a mean weight increase of 4 g/day. △=Morphine dose (mg/kg b.w./day) offered the animals. ▲=Morphine dose (mg/kg b.w./day) consumed. ●=Volume diet consumed (ml). ■=Body weight (g).

studied. When offered 715 mg morphine/kg b.w. in 90 ml diet, the mean weight gain during the initial 7 days was 0.4 g/day, with a mean fluid diet intake of 53.9±7.9 ml. Offering the rats 340 mg morphine/kg b.w. in 90 ml diet the weight gain during the first 6 days was 2.1 g/day. The mean daily diet intake was 72.8±10.6 ml. At the end of the experiment, nalorphine (20 mg/kg b.w. (IP) precipitated an abstinence reaction.

Comments on Diet Consumption

Rats drinking 90 ml/day of the control diet showed a mean weight increase of around 6 g/day (in the weight class 150–250 g). (Fig. 2, curve 3). Eighty-five to ninety percent of the fluid diet was consumed from 6 p.m. to 6 a.m., if the diet was served at 6 p.m.

To establish optimal conditions for long-term morphine intoxication concerning b.w. gain and food intake, experiments were performed where the initial dose of morphine and the daily dose increase were varied. One group of rats were started on 60 mg/kg b.w., increasing by 60 mg/kg/day. Two other groups were started on 45 and 25 mg/kg b.w., respectively, increasing daily with the same amount. The body weight and food intake of the intoxicated animals were compared with the body weight of the controls during the experiment. Rats intoxicated with an initial dose of 25 mg morphine per kg b.w. and a daily increase of 25 mg per kg showed a b.w. gain which parallelled with that of the controls for the first 8 days. Daily doses of 700 mg morphine/kg were reached prior to a decreased food intake.

Starting with 25 mg morphine/kg b.w./day, and increasing exponentially by adding 25 mg morphine/kg b.w. and another 5 mg/kg b.w./day up to 715 mg/kg/day during 13 days, the mean b.w. gain was 4 g/day during the first 8 days, i.e., up to a dose of 340 mg/kg b.w. (Fig. 2, curve 1). Rats offered and consuming 90 ml control diet/day showed a daily weight gain of 5.9 g (Fig. 2, curve 3).

Plasma Levels of Morphine

When consuming 340 mg morphine/kg b.w. from 6 p.m. one day, the animals had plasma morphine levels of 3.3±0.8 μg/ml at 00.00 a.m., 3.1±0.6 μg/ml at 9:00 a.m., 2.6±0.5 μg/ml at 3 p.m.

Abstinence Reaction

Withdrawal signs were precipitated by exclusion of morphine from the diet or by administration of antagonists (nalorphine or naloxone). Weight loss and decreased food intake were consistent and easily measurable signs of abstinence. Within 5 min. after IP injection of naloxone (6 mg/kg b.w.) animals intoxicated on 340 mg morphine/kg for 20–30 days showed tremor and irritability, crying during handling. During the next hour, a weight loss and decreased food intake (compared to controls) were seen together with diarrhea. Maximal intensity of the withdrawal reaction was

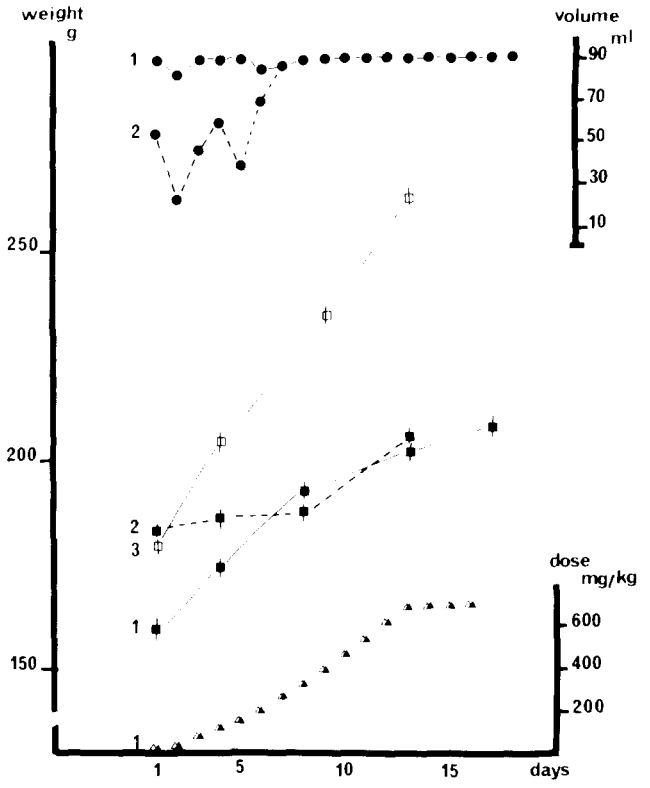

FIG. 2. Curve 1: 18 animals were started on a morphine dose of 25 mg/kg b.w. on day 1, reaching 715 mg/kg on day 13. The weight gain curve is parallel to curve 3 (controls) during the first 8 days, i.e., up to a dose of 340 mg/kg b.w. □=Body weight controls. ■—■=Body weight (g). △=Morphine dose (mg/kg b.w./day) offered the animals. ▲=Morphine dose (mg/kg b.w./day) consumed. ●=Volume morphine diet consumed (ml). Curve 2: Free diet consumption of 6 male rats on a morphine dose of 715 mg/kg b.w. During the first 7 days the daily diet consumption was 53.9±7.9 ml, thereafter stabilizing on 90 ml/day. Weight gain during the initial period is 0.4 g/day. ■---■=Body weight (g). ●---●=Volume morphine diet consumed (ml). Curve 3: Shows weight gain (□—□) of 12 rats offered and consuming 90 ml control fluid diet daily. In the weight class 150–250 g there is a mean daily weight gain of 5.9 g. Rats getting pellets and water ad lib have a mean daily weight gain of 6.3 g.

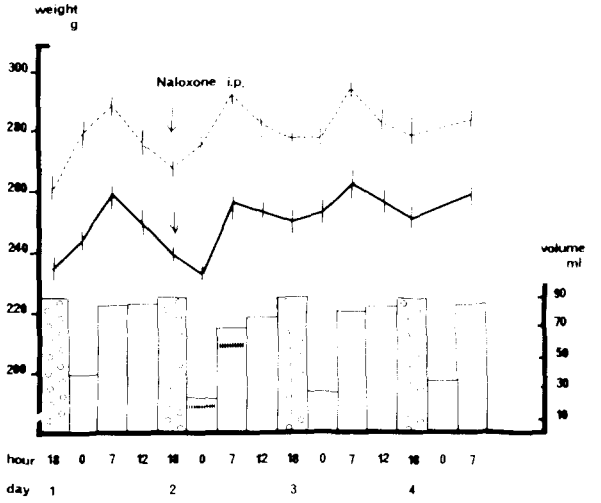

FIG. 3. Six mg naloxone per kg b.w. were injected IP on 10 rats intoxicated for 20 days (block line) on a morphine dose of 340 mg/kg, as described in the text. Within 5 min the animals showed tremor and irritability, including crying during handling. A decrease in food intake (III in volume columns) compared to control, b.w. loss and diarrhea, were seen within the first hour following the naloxone injection, reaching maximal intensity in 6 hrs (0 hr day 2). Similarly, 6 ng naloxone per kg were injected on 11 control rats (dotted line). At 0 hr on day 2 the intoxicated animals given naloxone showed a mean weight decrease of 7 g compared to the b.w. 18 hr day 2, while the control animals increased with 7 g during the same period. Controls showed no signs of abstinence following naloxone injection. Food intake and body weight were returned to normal 24 hrs after the naloxone injection. Body wt. and accumulated food intake (columns with circles indicate accumulated daily food intake in ml), starting at 18 hrs each day, is shown for 6 hr periods. Similar results were seen when nalorphine (30 mg/kg b.w.) was injected IP.

seen 4–6 hrs after injection of the antagonist. The abstinent rats decreased by 7.0±2.0 g in weight compared to the control rats, increasing in b.w. with 7.0±1.8 g during the same period. The body weight and food intake returned to normal during the next 18 hrs (Fig. 3). Similar results were obtained after nalorphine (30 mg/kg b.w.) IP injection.

If withdrawal was induced by exclusion of morphine from the diet when the animals consumed 340 mg morphine/kg b.w., the rate at which the animals lost weight was greatest over the first 13 hrs (mean weight loss 1.9 g/hr, Fig. 4). The maximal weight loss was seen in 48 hrs. The shortest intoxication period to produce withdrawal symptoms was investigated. Intoxications from one day (25 mg/kg) up to 4 days (end dose 130 mg/kg b.w., 5 animals in each group) were followed by excluding morphine from the diet. After one day (25 mg/kg b.w.) and two days (25, 55 mg/kg b.w.) of intoxication, a stagnation of weight gain followed morphine withdrawal. Morphine withdrawal from rats intoxicated for 3 days (end dose 90 mg/kg b.w.) or 4 days (end dose 130 mg/kg b.w.) gave a more complete abstinence reaction registered as a decrease in b.w. by 3 g (nonsignificant) and 8 g (significant) one day after withdrawal. (see Fig. 5). The decrease in food intake was significant compared to controls, with a nonsignificant difference between the groups of intoxicated animals.

Figure 6 shows an experiment for 46 days with 3 choice tests. The dose 340 mg morphine per kg was reached on the 8th day of the intoxication period. Prior to the 3rd choice test the morphine dose was increased to 715 mg/kg. It could be seen from the choice tests that the degree of dependence on morphine diet increased during the intoxication period.

Some rats (around 10–15%), preferred extremely high morphine amounts (in the range of 250 mg/kg b.w./day), while another 10–15% consumed extremely little morphine (50 mg/kg/day) even in the very last choice test. Most animals preferred 150–250 mg/kg b.w./day) in the choice situation described.

Light Microscopy of Kidney and Small Intestine

Macroscopically, visceral and thoracal organs looked normal after long-term fluid diet intake. Light microscopy of

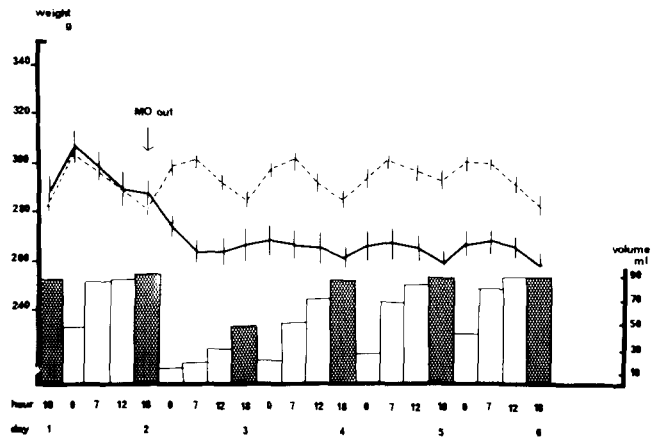

FIG. 4. Twelve rats were intoxicated with morphine for 26 days on a dose of 340 mg/kg b.w. Morphine withdrawal (six rats, black line) resulted in a decreased b.w. of 35 g within 48 hrs. A decreased food intake, diarrhea and irritability was observed within 6–12 hrs. Body weight and accumulated food intake (cross-hatched columns indicated accumulated daily fluid diet intake, ml), starting at 18 hrs each day, is shown for 6 hr periods. The six morphinized control rats were run in parallel (dotted line).

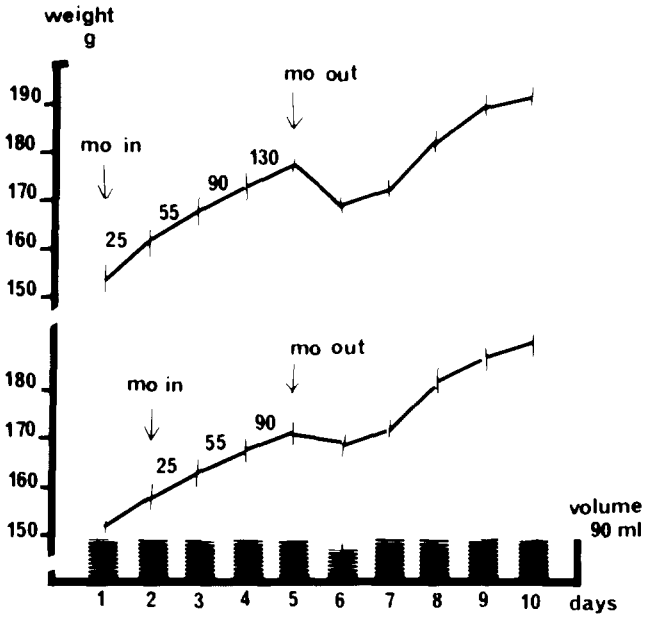

FIG. 5. Five male rats were given morphine in an escalating fashion for 3 and 4 days, respectively. Rats receiving morphine for 4 days (upper curve) in an escalating fashion showed a mean weight decrease of 8.2 g (significant, $p<0.001$), and rats receiving morphine for 3 days (lower curve) showed a mean weight decrease of 2 g (nonsignificant) one day after withdrawal. A decrease in food intake was registered: there was a significant ($p<0.05$) decrease of the two groups, compared to controls, a nonsignificant difference between the groups. S.E.M. values are given. Column=Volume diet consumed (ml).

kidney from morphine intoxicated (for 50 days on a dose of 340 mg/kg b.w.), abstinent and control rats, was normal. The epithelium of proximal and distal tubules and loops of Henle were indistinguishable from pellet feeding litter-mates with consumption of water ad lib. The same was found at different levels of the small intestinal tract.

Morphine Intoxication with the Intra-peritoneal Injection Technique

The intoxication period was 20 days with an initial morphine dose of 5 mg/kg b.w. and a final dose of 100 mg/kg b.w. At the end of the intoxication period the intoxicated animals had mean b.w. slightly below the weight at the beginning of the experiment. They weighed around 100 g less than the saline controls.

DISCUSSION

One disadvantage with previous methods of long-term morphine intoxication is poor weight gain in the intoxicated animals. One reason for this is that morphinized rats eat less than controls, thus causing a caloric imbalance. A relative undernutrition of morphinised animals could interfere with brain protein synthesis and neurotransmitter metabolism during tolerance development (see [5]), as it is well known that protein/calorie malnutrition affects specific nervous tissue proteins (see [13,31]). Therefore, it is extremely important to eliminate the biochemical effects of an undernutrition when neurochemical correlates to tolerance development are to be studied. Some authors (see [15]) have been aware of this problem, although it has been very laborious to assure a full caloric balance for the animals.

In order to develop a method for long-term morphine treatment which avoids caloric imbalance, oral intake was chosen. This type of procedure has received some attention in recent years (e.g., [16, 25, 26]). It is advantageous, because it is relatively inexpensive, since no special equipment is involved, and large numbers of animals can be used simultaneously. We developed a system where morphine was diluted in a complete fluid diet, modified after De Carli and Lieber [7]. The diet, with a content of sucrose, made it possible to overcome the bitter taste of opium alkaloid which is aversive to animals. Some authors (see [20]) have described direct effects of sucrose on hepatic lipid metabolism. However, Lees [17] could not confirm such effects. In our studies the animals showed a weight gain on the diet offered, with no significant difference from rats offered pellets and water ad lib.

The diet was served in graded Richter tubes. The amount of food (and thereby morphine consumption) could easily be recorded. If the animals did not consume the diet offered, pair-feeding could be adopted to ensure caloric balance between intoxicated and control animals. During intoxication of rats in the weight class and with the morphine-dose increment suggested in this study, this was never necessary. However, if the present method will be used to test different drug combinations, pair-feeding might optimize the experimental conditions. As rats are nocturnal, they eat most of their food at night. However, fluid intake is more uniform over day and night [10]. According to our observations 85–90% of the total fluid diet, and thereby morphine intake, was consumed at night.

Iwamato and Klaassen [14] reported that an orally administered dose of morphine achieved plasma levels over a 6 hr period which was only $^1/_5$th of the plasma levels observed at

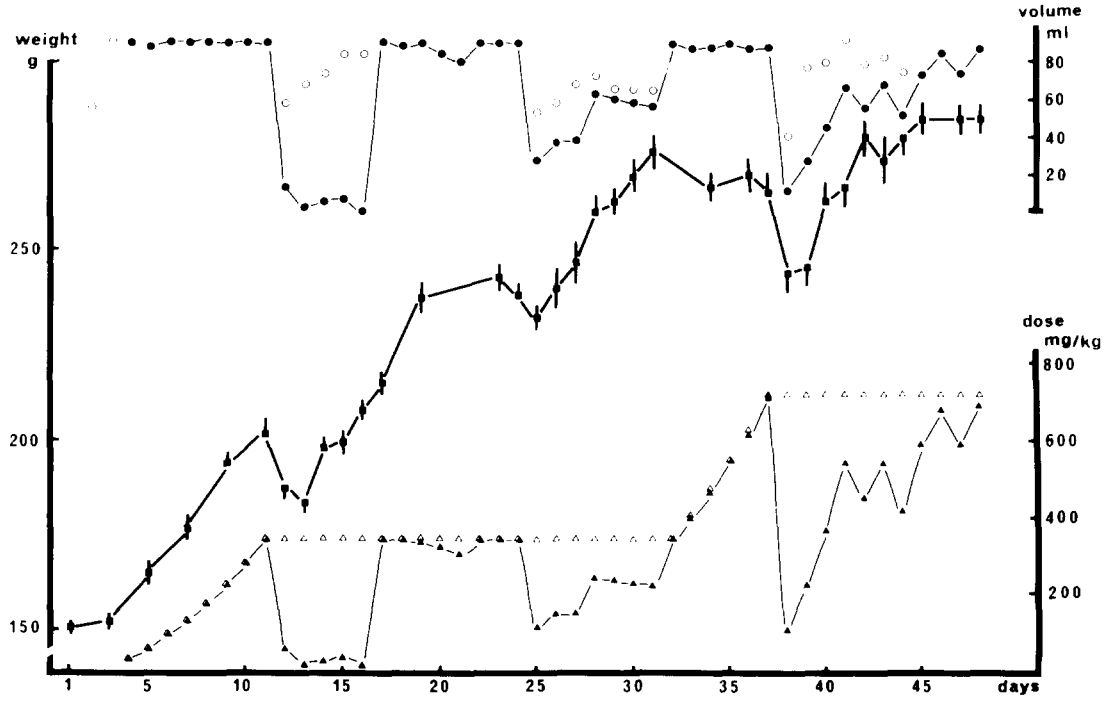

FIG. 6. Morphine intoxication schedule (18 rats), starting with a dose of 25 mg/ b.w., increasing exponentially up to 340 mg/kg. During days 31–38 the dose was increased to 715 mg/kg. Three choice-tests revealed an increase in morphine preference over control during the period studied, i.e., more morphine was consumed with longer intoxication period. ●=Volume (ml) morphine diet consumed. ○=Volume (ml) control diet consumed. ■=b.w. (g). △=Morphine dose (mg/kg b.w./day) offered the animals. ▲=Morphine dose (mg/kg b.w./day) consumed.

the same dose given intravenously. In our studies we reached plasma morphine levels of 3 μg/ml 6 hrs after 85–90% of the daily morphine dose of 340 mg/kg b.w. was consumed, indicating that the animals were exposed to significant morphine doses even in daytime. Similarly, Gellert and Holtzman [11] reported rather steady plasma levels of morphine between doses in an oral self-administration procedure.

The explanation for low but steady plasma levels of morphine following oral administration has been discussed by Del Villar *et al.* [8], Sanchez and Tephly [24] and by Walsh and Levine [28] who have suggested that the intestinal tract to act as a depot from which morphine is continuously reabsorbed. It should be noted that Deneau and Seevers [9] in 1964 pointed out that maintaining continuous exposure of an organism to morphine is the most important factor in establishing dependence. This fact was further supported by Cerletti *et al.* [3].

It was found that starting with morphine doses of 25 mg/kg b.w. and increasing exponentially to final doses of up to 340 mg/kg b.w., or increasing linearly with 25 mg/kg/day up to 715 mg/kg permits full nutrient intake of the experimental animals (i.e., 90 ml fluid diet in our method). These are extremely high doses as compared to those reported in the literature. Withdrawal signs were precipitated within 5 min with naloxone or nalorphine, or within 6–10 hrs after excluding morphine from the diet in animals intoxicated for 20 days or more, with a final dose of 340 mg/kg. Abstinence signs could be precipitated after just 3–4 days after oral intoxication, indicating that our model is effective in producing morphine dependence. This is satisfactory compared to the findings of Way *et al.* [29] who demonstrated withdrawal signs precipitable after removal of morphine pellets implanted for 3 days. Gellert and Holtzman [11] were able to precipitate withdrawal syndrome by naloxone in rats which had been drinking 16 mg morphine per kg b.w./day for 6 days.

It was also found that in choice situations individual rats consumed different amounts of morphine even after long intoxication periods. Some intoxicated rats (10–15% of total) consumed very small amounts of morphine in the choice-test. Similarly, Gellert and Holtzman [11] reported that on an average 1 rat of 10 refused to drink morphine diluted in tap water which could, of course, be explained by the bitter taste of the morphine solution. It should be noted that all rats drank high concentrations of morphine when the morphine was given in the diet. Ten to fifteen percent of the rats in our study preferred extremely high amounts of morphine diet in choice situations. In future studies we intend to investigate these groups of rats with high morphine and low morphine intake during choice situations with respect to tolerance and dependence development.

In summary, the intoxication model presented ensures both experimental and control animals a nutritionally complete fluid diet. The only difference between the animals is the morphine itself. The method might thus serve as a new and important experimental basis of studying neurochemical

responses to opiates with no interference of the biochemical effects caused by a relative undernutrition due to the food-depriving effect of morphine. Routinely the tolerant rats consume 340 mg morphine per kg b.w. with plasma morphine levels of 3 μg/ml over the day. It is simple, time-saving and inexpensive to intoxicate numerous animals in parallel for long periods, as frequent injections of morphine or surgical pellet implantation/removal are eliminated.

ACKNOWLEDGEMENTS

This study was supported by grants from the Swedish Medical Research Council, from Svenska Läkaresällskapets Foundation for Medical Research, from Torsten and Ragnar Söderberg's Foundation, from Tore Nilsson's Foundation for Medical Research, Svenska Sällskapet for Medical Research, Lars Hierta's Foundation, from the Medical Faculty, University of Göteborg, and from the Royal Society of Art and Science.

REFERENCES

1. Bläsig, J., A. Herz, K. Reinhold and S. Zieglgänsberger. Development of physical dependence on morphine in respect to time and dosage, and quantification of the precipitated withdrawal syndrome in rats. *Psychopharmacologia* **33:** 19–38, 1973.
2. Cappell, H. and A. E. le Blanc. Some factors controlling oral morphine intake in rats. *Psychopharmacologia* **21:** 192–201, 1971.
3. Cerletti, C., S. H. Keinath, M. M. Reidenberg and M. W. Adler. Chronic morphine administration. Plasma levels and withdrawal syndrome in rats. *Pharmac. Biochem. Behav.* **4:** 323–327, 1976.
4. Cicero, T. J. and E. R. Meyer. Morphine pellet implantation in rats: Quantitative assessment of tolerance and dependence. *J. Pharmac. exp. Ther.* **181:** 404–408, 1973.
5. Craves, F. B., H. H. Loh and J. L. Meyerhoff. The effect of morphine tolerance and dependence on cell-free protein synthesis. *J. Neurochem.* **31:** 1309–1316, 1978.
6. Davis, W. and J. R. Nichols. Physical dependence and sustained opiate-directed behavior in the rat. A preliminary report. *Psychopharmacologia* **3:** 139–145, 1962.
7. De Carli, L. M. and C. S. Lieber. Fatty liver in the rat after prolonged intake of ethanol with a nutritionally adequate new liquid diet. *J. Nutr.* **91:** 331–336, 1967.
8. Del Villar, E., E. Sanchez and T. R. Tephly. Morphine metabolism II Studies on morphine glucuronyl transferase activity in intestinal chromosomes of rats. *Drug Metab. Dispos.* **2:** 370–374, 1974.
9. Deneau, G. A. and M. H. Seevers. Pharmacological aspects of drug dependence. *Adv. Pharmac. Chemother.* **3:** 267–283, 1964.
10. Freund, G. Alcohol consumption and its circadian distribution in mice. *J. Nutr.* **100:** 30–36, 1970.
11. Gellert, V. F. and S. G. Holtzman. Development and maintenance of morphine tolerance and dependence in the rat by scheduled access to morphine drinking solutions. *J. Pharmac. exp. Ther.* **205:** 536–546, 1978.
12. Gellert, V. F. and S. B. Sparber. A comparison of the effects of naloxone upon body weight loss and suppression of fixed-ratio operant behavior in morphine-dependent rats. *J. Pharmac. exp. Ther.* **201:** 44–54, 1977.
13. Haglid, K. G., L. Rosengren, L. Rönnbäck, P. Sourander and A. Wroński. The influence of early protein-calorie malnutrition on levels of a glial brain-specific protein S 100 in discrete brain areas. In: *Proc. 2nd Meeting Eur. Soc. Neurochem.*, edited by V. Neuhoff. Gottingen: Verlag Chemie, 1978, p. 593.
14. Iwamoto, K. and C. D. Klaasen. First pass effect of morphine in rats. *J. Pharmac. exp. Ther.* **200:** 236–244, 1977.
15. Khavari, K. A. and M. E. Risner. Opiate dependence produced by *ad libitum* drinking of morphine in water, saline and sucrose vehicles. *Psychopharmacologia* **30:** 291–302, 1973.
16. Kumar, R. and I. P. Stolerman. Resumption of morphine self-administration by ex addit rats: An attempt to modify tendencies to relapse. *J. comp. physiol. Psychol.* **78:** 457–465, 1972.
17. Lees, R. S. The plasma lipid response to two types of dietary carbohydrate. *Clin. Res.* **13:** 549, 1965.
18. Lieber, C. S. and L. M. De Carli. Ethanol dependence and tolerance: A nutritionally controlled experimental model in the rat. *Res. Commums chem. Path. Pharmac.* **6:** 983–991, 1973.
19. Maggiolo, C. and F. Huidobro. Administration of pellets of morphine to mice; abstinence syndrome. *Acta physiol. latinoam.* **11:** 70–78, 1961.
20. McDonald, I. Some influences of dietary carbohydrate on liver and depot lipids. *J. Physiol.* **162:** 334, 1962.
21. McMillan, D. E., J. D. Leander, T. W. Wilson, S. C. Wallace, T. Fix, S. Redding and R. T. Turk. Oral ingestions of narcotic analgesics by rats. *J. Pharmac. exp. Ther.* **196:** 269–279, 1976.
22. Mucha, R. F. and H. Kalant. Increased weight gain as a morphine withdrawal response in rats. *Pharmac. Biochem. Behav.* **11:** 197–201, 1979.
23. Rasmussen, K. E. Quantitative morphine assay by means of gas-liquid chromatography and on-column silylation. *J. Chromat.* **120:** 491–495, 1976.
24. Sanchez, E. and T. R. Tephly. Morphine metabolism 1. Evidence for separate enzymes in the glucuronidation of morphine and p-nitrophenol by rat hepatic microsomes. *Drug Metab. Dispos.* **2:** 247–253, 1974.
25. Stolerman, I. P. and R. Kumar. Preference for morphine in rats: Validation of an experimental model of dependence. *Psychopharmacologia* **17:** 137–150, 1970.
26. Thompson, T. and W. Ostlund. Susceptibility to readdiction as a function of the addiction and withdrawal environments. *J. comp. physiol. Psychol.* **60:** 388–392, 1965.
27. Wallace, J. E., J. D. Biggs and K. Blum. Gas-liquid and thin-layer chromatographic determination of morphine in biologic specimens. *Clinical chim. Acta* **36:** 85–91, 1972.
28. Walsh, C. T. and R. H. Levine. Studies of the enterohepatic circulation of morphine in the rat. *J. Pharmac. exp. Ther.* **195:** 303–310, 1975.
29. Way, E. L., H. H. Loh and F. H. Shen. Simultaneous quantitative assessment of morphine tolerance and physical dependence. *J. Pharmac. exp. Ther.* **167:** 1–8, 1969.
30. Wei, E. and E. L. Way. Application of the pellet implantation technique for the assessment of tolerance and physical dependence in the roden. In: *Methods in Narcotic Research*, edited by S. Ehrenpreis and A. Niedle. New York: Marcel Dekker, 1975, pp. 243–259.
31. Wroński, A. Nutritional effects on rat brain protein metabolism, with special reference to the S-100 protein. Thesis, 1977, Göteborg.
32. Yanaura, S. and T. Suzuki. Eating pattern of morphine dependent rats. *Jap. J. Pharmac.* **29:** 753–762, 1979.