Pharmacology Biochemistry & Behavior, Vol. 34, pp. 919–922. © Pergamon Press plc, 1989. Printed in the U.S.A. 0091-3057/89 $3.00 + .00

BRIEF COMMUNICATION

A Method for Computer Control of Morphine Ingestion by Rats

P. S. ERIKSSON*[1] AND L. RÖNNBÄCK*†

**Institute of Neurobiology and †Department of Neurology, University of Göteborg, Göteborg, Sweden*

Received 12 December 1988

ERIKSSON, P. S. AND L. RÖNNBÄCK. *A method for computer control of morphine ingestion by rats*. PHARMACOL BIOCHEM BEHAV **34**(4) 919–922, 1989.—In this paper, we describe a method for controlling the administration of liquid diet and morphine to sixteen rats using a computer. Morphine ingestion treatment was performed with 6 feeding occasions per 24 hr, all experimental animals receiving similar drug doses. The amount of drug was individual and based on body weight at each feeding occasion. Control and experimental animals were kept under isocaloric conditions. Corrections of drug doses in order to compensate for changes in body weight were made every 24 hr. Sensors registered the exact time of complete drug and diet consumption and prevented overdistribution. Rats were administered 103 mg/kg b.wt. morphine during 24 hr. In another experiment rats were administered 191 mg/kg b.wt. morphine during 48 hr, and no weight loss or decrease in fluid diet intake was registered during the time of drug administration in either of the experiments. After exclusion of morphine from the fluid diet, the body weight loss was 6.1% and 8.3%, respectively, and the liquid diet intake decreased by 12.4 ml and 13.4 ml, respectively, compared with control animal intake. This demonstrates the induction of physical drug dependence. A major advantage of using computer-aided administration of morphine-admixed, fluid diet is the stepwise, small dose increments provided several times a day, resulting in higher drug dose per unit time when compared with ingestion procedures using one feeding occasion per day. The method enables rats to rapidly ingest large morphine doses under standardized conditions.

Computer Morphine Ingestion Liquid diet Physical dependence Rat

PHYSICAL dependence on morphine can be induced in rodents by a variety of techniques, including implantation of morphine pellets (1,2) or morphine-containing reservoirs (4), infusion of morphine (12) or treatment with slow release morphine emulsion (3). Several oral route administration procedures have been described, including morphine admixed drinking water (5), saline or sucrose solutions (6) and drug admixed food (11).

We have previously reported on an ingestion model with morphine dissolved in a liquid diet (14). Morphine-treated and control rats received similar amounts of calories, nutrients and fluid. Body weight gain was similar in treated and control rats. A disadvantage with our previous liquid diet intoxication procedure was that the dose increment was slow.

In order to make rats ingest higher morphine doses in shorter times, the liquid diet and drug supply was controlled by a computer. With this procedure, the drug dose was escalated six times per day. Physical dependence was ascertained by quantifying the decreases in body weight and food intake during abstinence.

METHOD

Animals

Forty-six male Sprague-Dawley rats were used (body weight 200 g; A-lab, Stockholm, Sweden).

Drugs

Morphine-hydrochloride was obtained from Apoteksbolaget, Göteborg, Sweden.

Housing

The rats were maintained in individual cages ($41 \times 25 \times 15$ cm^3) in a room with controlled temperature and humidity, and with lights on between 7 a.m. and 5 p.m.

Liquid Diet

Ninety ml liquid diet was administered to each rat during every 24-hr period. Casein was used as a protein source in the liquid diet

[1]Requests for reprints should be addressed to Dr. Peter Eriksson, Institute of Neurobiology, University of Gothenburg, P.O. Box 33031, S-400 33 Gothenburg, Sweden.

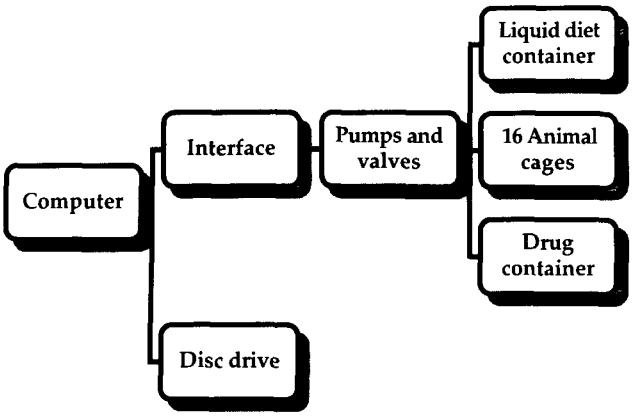

FIG. 1. Schematic presentation of the computerized liquid diet and drug distribution apparatus.

which, furthermore, included a vitamin mixture, salt plus cystine, methionine and sugar mixed to a fluid with olive and corn oil stabilizers. For details see (14).

Computer

The experimental set-up was controlled with a Commodore 64 micro-computer connected to a relay interface (Fig. 1).

Software/Computer Program

Drug treatment could be provided for a variable number of days and for a variable number of feeding occasions per 24 hr with individual amounts of the drug, i.e., similar drug doses, at each feeding, under equal caloric conditions. It was also possible to correct the drug doses to compensate for changes in body weight every 24 hr. The computer program was written in Basic.

Interface

An interface was built with outputs to control three pumps, sixteen valves and inputs to read signals from sixteen liquid diet indicators located in each cage. The interface was fed with 220 V AC and delivered 12 V DC to the pumps and valves.

Drug and Diet Administration

Control of the administered volume of drug solution (drug dose) was achieved by supplying each cage with fluid at a known flow rate for a measured time via a polypropylene capillary (∅ 0.8 mm). The current was directed to the different cages by a high-precision eight-way valve. Liquid diet was administered via a separate system of tubes (∅ 6 mm) with individual valves, using the above mentioned principle for volume control.

Diet Intake Detection

A liquid diet level indicator located in a glass cup in each cage monitored the time for complete consumption of an administered volume of drug and diet mixture. Furthermore, the indicators prevented overdistribution in the case of diet intake failure on three consecutive feeding occasions (Fig. 2).

Assessment of Physical Dependence

The abstinence signs registered were weight loss and decrease in food intake according to (8).

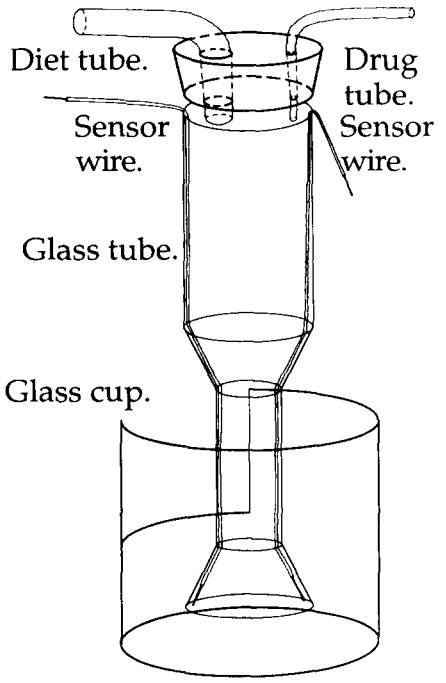

FIG. 2. Drug and liquid diet was administered via tubes to a glass cup where the diet and drug were mixed. The diet consumption indicator consists of two electrodes locted in the bottom of the afferent glass tube. The electrodes are connected to an adjustable sensor checking the resistance between the two electrodes being low when diet remains and infinite when the diet is consumed.

Documentation/Data Storage

Data concerning the time for administration of the drug and liquid diet and the time for complete drug and diet consumption were stored on floppy discs (Commodore 1541).

Statistics

Statistical analyses were made according to a two-way ANOVA model, with weight change as a response variable. The drug administration and experimental setup were used as classification variables, the first regarded as fixed, the second as random. The analyses were done with the SAS procedure GLM (General linear models).

RESULTS

Experiment I (Fig. 3)

Ten animals were used, separated into five paired trials with one treatment and one control animal. All trials started with two days of prefeeding with the liquid diet. On the first day of drug treatment morphine was added to the liquid diet according to the following schedule: 2.3 mg/kg b.wt. morphine at 2 p.m., 4.2 mg/kg b.wt. morphine at 6 p.m., 6.2 mg/kg b.wt. morphine at 10 p.m., 8.3 mg/kg b.wt. morphine at 1 a.m., 11 mg/kg b.wt. morphine at 4 a.m. and 13.3 mg/kg b.wt. morphine at 9 a.m. On the second day of drug treatment, the doses were escalated in the following manner, using the same times as on day 1: 16.3, 18.8, 22.0, 25.8, 29.3 and 33.3 mg/kg b.wt. morphine. On the

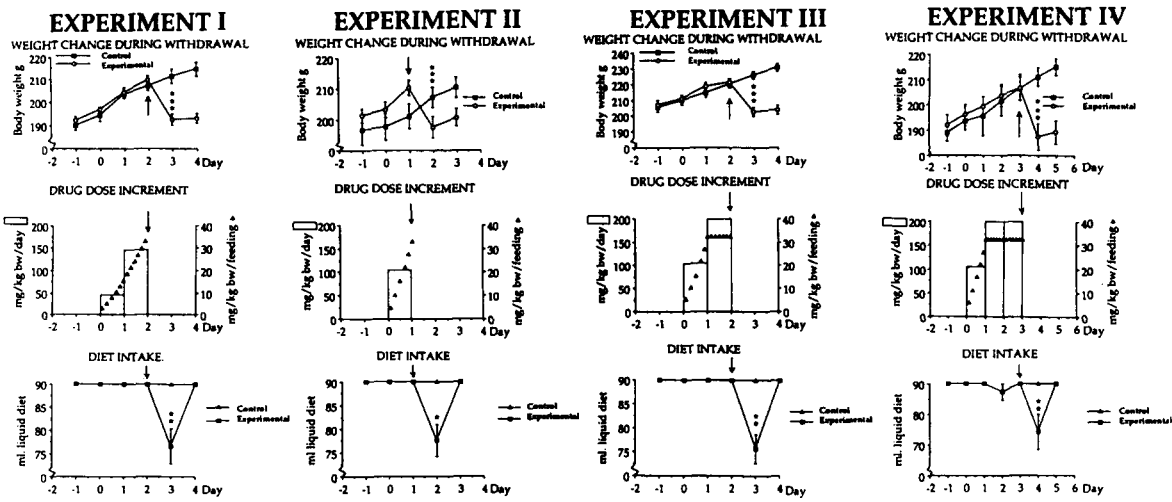

FIG. 3. Experiment I. Two days of drug dose escalation with six increments/24 hr reaching a dose at the last feeding of 33.3 mg/kg b.wt. with the total intake on day 1 being 45 mg/kg b.wt. and on day 2 146 mg/kg b.wt. Ten animals were used, separated into 5 paired trials. Experiment II. One day of drug dose escalation with six dose increments/24 hr reaching a dose at the last feeding of 33.3 mg/kg b.wt. with the total intake being 103 mg/kg b.wt./day. Ten animals were used separated into 5 paired trials. Experiment III. One day of drug dose escalation with six dose increments/24 hr reaching a dose at the last feeding of 33.3 mg/kg b.wt. with the total intake being 103 mg/kg b.wt. on the first day, followed by a one day, six dose treatment to provide 200 mg/kg b.wt./day. Sixteen animals were used in one trial. Experiment IV. One day of drug dose escalation with six increasing doses/24 hr reaching a final dose of 33.3 mg/kg b.wt., i.e., totally 103 mg/kg b.wt./day. The next two days were full-dose intoxications, i.e., six 33.3 mg/kg b.wt. doses giving a total daily intake of 200 mg/kg b.wt. Ten animals were used, separated into 5 paired trials. (A) Changes in body weight after exclusion of morphine from the diet (arrow). (B) Boxes show average daily dose (mg/kg b.wt./day), cones indicate drug dose per administration (mg/kg b.wt./feeding). (C) Changes in fluid diet intake after exclusion of morphine from the diet. *$p<0.05$, **$p<0.01$, ***$p<0.001$ SEM values are shown for whole groups.

third day, morphine was excluded from the diet. This exclusion of morphine induced a weight loss of 8.3% compared with a weight gain of 2% in the control group ($p<0.001$), and this was expressed as percent of the prewithdrawal weight. The liquid diet intake of the withdrawal rats decreased by 13.4 ml ($p<0.01$) compared with that of the control animals.

Experiment II (Fig. 3)

Ten animals were used, separated into five paired trials with 2 animals in each. After two days of prefeeding with liquid diet, morphine was distributed to one of the two animals, according to the following schedule: 4.2 mg/kg b.wt. morphine at 2 p.m., 8.3 mg/kg b.wt. morphine at 6 p.m., 13.3 mg/kg b.wt. morphine at 10 p.m., 18.3 mg/kg b.wt. morphine at 1 a.m., 25.8 mg/kg b.wt. morphine at 4 a.m., and 33.3 mg/kg b.wt. morphine at 9 a.m. On the next day, the morphine was excluded from the diet, inducing a 6.1% weight loss compared with a weight gain of 2% in the control group ($p<0.001$), expressed as percent of the prewithdrawal weight. The mean decrease in liquid diet intake was 12.4 ml ($p<0.05$) compared with the diet intake of controls.

Experiment III (Fig. 3)

Sixteen animals were used, eight controls and eight experimentals. The experiment was started with two days of prefeeding with standard liquid diet. On the first day of treatment, morphine was distributed to the experimental animals according to our standard semi-exponential, dose-increment schedule used in Experiment II. On the second day of treatment, six equivalent morphine doses were given to provide 200 mg/kg b.wt./day. On the third day, following one day of morphine dose escalation and one day on the dose 200 mg/kg b.wt./day, morphine was excluded from the diet, inducing a 8.4% decrease in body weight compared with a weight gain of 2% in the control group ($p<0.001$), these being expressed as percent of the prewithdrawal weights. The liquid diet intake was decreased by 14.5 ml ($p<0.01$) compared with the intake of controls.

Experiment IV (Fig. 3)

Ten animals were used separated into five paired trials with one treatment and one control animal. All trials started with two days of prefeeding with the liquid diet. On the first day of drug treatment, morphine was distributed to one of the two animals according to our standard semi-exponential, dose-increment schedule used in Experiment II. On the second day of treatment, a minor decrease in diet intake (2.8 ± 2.7 ml; n.s.) was registered. After three days of drug treatment, morphine was excluded from the diet. Upon withdrawal of morphine from the diet there was a 9.4% decrease in body weight compared with a weight gain of 2% in the control group ($p<0.001$), these being expressed as percent of the prewithdrawal body weights. The liquid diet intake was decreased by 15.8 ml ($p<0.01$) compared with control animal intake.

DISCUSSION

Devices designed to measure the water intake in rats have been described previously (5, 7, 10). A food intake measuring apparatus has also been described (13). In the present study we report on a computerized apparatus for the distribution of drug and liquid diet, which is also capable of detecting the liquid diet consumption. There are several advantages in using a computerized liquid diet distribution of morphine compared with manual treatment.

The sampling and processing of data are easy and fast. A total and overall control of food and drug consumption is achieved during the experimental period. Furthermore, by using the described computer controlled system, it is possible to administer liquid diet and drug at several occasions per 24 hr (6 times), including night hours without personal attendance. Several feeding occasions per 24 hr facilitates smooth increments and decrements of the drug dose. Sensors continuously monitor the time for the spontaneous consumption of food/drug. The eating pattern of dependent rats has been shown to be altered (13) and this could be either studied or considered when planning dose profiles. Rats can be treated for a variable number of days, with computer aided distribution of drug and liquid diet. Physical dependence was reached in 24 hr of morphine ingestion. An increasing degree of physical dependence was indicated by the comparisons between weight loss and cumulated drug dose (mg/kg b.wt./day) in Experiments II, III and IV. This confirms earlier findings in our laboratory (8), where a correlation was demonstrated between cumulated mean daily morphine dose and weight loss. The weight loss registered in the present study was in the same range as earlier findings in our laboratory (8). Using morphine adulterated food and morphine admixed sucrose solution, the weight loss was 4% following exclusion of morphine from the diet after five days of treatment with an average dose of 77 mg/kg b.wt./day (9). On the other hand, treatment with drug admixed food for seven days at 95 mg/kg b.wt./day resulted in a 9.2% body weight loss (13). Minor differences concerning the degree of physical dependence in terms of weight loss could be attributed to differences in experimental conditions, including variations amongst the rats. In conclusion, experiments in which the administration of high drug doses must be reached in a short time are one of the best applications for computer-aided drug and diet administration.

ACKNOWLEDGEMENTS

This study was supported by grants from the Swedish Medical Research Council (grant No. 25X-06005), from the Medical Faculty of the University of Göteborg, from Anders Otto Svärds Foundation, and from Anna Ahrenbergs Foundation. The skilful technical assistance of Kjell Örtlund, Sture Benzon and Dr. Håkan Hallberg is highly appreciated. The statistical advice of Sven Persson Department of Statistics, University of Göteborg, is also highly appreciated.

REFERENCES

1. Bläsig, I.; Herz, A.; Renhold, K.; Zieglgänsberger, S. Development of physical dependence on morphine in respect to time and dosage, and quantification of the precipitated withdrawal syndrome in rats. Psychopharmacology (Berlin) 33:19–38; 1973.
2. Cisero, T. J.; Meyer, E. R. Morphine pellet implantation in rats. Quantitative assessment of tolerance and dependence. J. Pharmacol. Exp. Ther. 184:404–408; 1973.
3. Collier, H. O. J.; Francis, C. L.; Schneider, C. Modification of morphine withdrawal by drugs interacting with humoral mechanisms: Some contradictions and their interpretation. Nature 37:220–223; 1972.
4. Goode, P. G. An implanted reservoire of morphine solution for rapid induction of physical dependence in rats. Br. J. Pharmacol. 41: 558–566; 1971.
5. Hill, J. H.; Stellar, E. An electronic drinkometer. Science 114:43–44; 1951.
6. Khavari, K. A.; Risner, M. E. Opiate dependence produced by *ad libitum* drinking of morphine in water, saline and sucrose vehicles. Psychopharmacology (Berlin) 30:291–302; 1973.
7. Kurihara, H.; Hayashi, T.; Alam, M. R.; Tadokoro, S.; Miura, T. Automatic measurement of drinking in rats; effects of hypophysectomy. Pharmacol. Biochem. Behav. 9:697–702; 1978.
8. Rönnbäck, L.; Eriksson, P. S.; Zeuchner, J.; Rosengren, L.; Wronski, A. Aspects of abstinence after morphine ingestion. Pharmacol. Biochem. Behav. 28:87–93; 1987.
9. Risner, M. E.; Khavari, K. A. Morphine dependence in rats produced after five days of ingestion. Psychopharmacologia 28:51–62; 1973.
10. Stolerman, I. P.; Kumar, R. Regulation of drug and water intake in rats dependent on morphine. Psychopharmacologia 26:19–28; 1972.
11. Suzuki, T. M.; Shimada, T.; Yoshii, T.; Uesugi, J.; Yanaura, S. Development of physical dependence on and tolerance to morphine in rats treated with morphine-admixed food. Prog. Neuropsychopharmacol. Biol. Psychiatry 7:63–71; 1983.
12. Teiger, D. G. Induction of physical dependence on morphine, codeine and meperidine in the rat by continuous infusion. J. Pharmacol. Exp. Ther. 190:408–415; 1974.
13. Yanaura, S.; Suzuki, T. Eating pattern of morphine dependent rats. Jpn. J. Pharmacol. 29:753–762; 1979.
14. Zeuchner, J.; Rosengren, L.; Wronski, A.; Rönnbäck, L. A new ingestion method for long-term morphine intoxication in rat. Pharmacol. Biochem. Behav. 17:495–501; 1982.