Pharmacology Biochemistry & Behavior, Vol. 35, pp. 15–20. © Pergamon Press plc, 1990. Printed in the U.S.A. 0091-3057/90 $3.00 + .00

Is There a Genetic Control of Morphine Preference in Rat?

LARS RÖNNBÄCK[1]

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

Received 10 February 1989

RÖNNBÄCK, L. *Is there a genetic control of morphine preference in rat?* PHARMACOL BIOCHEM BEHAV **35**(1) 15–20, 1990.—Morphine preference was tested in two-bottle, voluntary-choice situations on physically dependent Sprague-Dawley rats. The animals ingested morphine which was dissolved in a fluid diet. Choice tests were performed under similar experimental conditions as the ingestions. Approximately 10% of the physically dependent rats voluntarily preferred large amounts of morphine already after a short treatment. The preference level was found to correlate with the animals' requirement for the drug. There was a gradual increase in morphine preference in F_1 and F_2 offspring of extremely high morphine preference rats. In F_3 (of such extremely high morphine preference rats) up to 65% died shortly after birth. The surviving rats showed a low morphine preference after ingestion when adult. Our result of increasing preference over two generations and death of a large number of rats in the third generation with a low morphine preference of the surviving rats was seen also in F_4–F_8. The data suggest that high morphine preference is under genetic control.

Choice test Genetic Ingestion Morphine Physical dependence Preference Rat

CONSIDERABLE attention has been devoted to the self-administration of pharmacologically active drugs by nonhuman subjects, with the underlying hope of gaining insight into the complex etiology of human drug addiction. Separation between addiction-prone and addiction-resistant animals could facilitate the identification of causal factors in opiate addiction. Many self-administration routes have been used (3, 4, 10, 12–14). As it has been claimed that the stimuli associated with the effects of morphine administration are probably more important than the drug itself for the active drug-seeking behaviour (1,5), many authors have considered voluntary preference tests by ingestion-intoxicated rats to be preferred when studying drug addiction. One problem has been the lack of suitable choice models. Morphine has been dissolved in water or sucrose with the result being a bitter taste and/or caloric imbalance. The differences in preference level found between various strains (2), to some degree, might be related to other factors than true drug proneness.

It was earlier shown (7, 9, 15) that within one strain of rat there is a minor percentage of individual animals with the ability both to aquire high morphine preference in a two-bottle voluntary choice situation after a short ingestion period and to increase the preference further after a second treatment period. The aim of the present study was to evaluate whether or not this high morphine preference could be enriched by breeding, which has in fact been suggested earlier (6). High morphine preference rats within one strain might be a valuable tool in the search for a neurochemical basis for morphine addiction.

METHOD

Animals

Male and female inbred Sprague-Dawley rats with a body weight (b.wt.) of 150 g were bought from A-lab, Stockholm, Sweden.

Morphine Ingestion

The rats, placed in individual plastic cages ($33 \times 23 \times 12$ cm^3), received their fluid diet from plastic tubes, one for each rat. Morphine was dissolved in the fluid diet. The ingestion procedure and diet composition has been described extensively in earlier papers (8,15). The morphine dose was increased up to 340 mg/kg b.wt. during 8 days. Fluid diet and morphine consumption was determined daily. It was previously shown (8) that on this regime the rats were made physically dependent on morphine, as tested by precipitation of withdrawal signs after administration of antagonists or by excluding the drug from the diet.

Choice Tests

Two tubes were accessible to each rat, one with morphine, and the other with control diet. The amounts of calories, nutrients and fluid were similar whether or not the rats chose morphinized or control diets. Morphine diet consumption was expressed either as % preference, i.e., amount of morphine diet consumed in percent

[1]Requests for reprints should be addressed to Dr. Lars Rönnbäck, Institute of Neurobiology, University of Göteborg, P.O.B. 33 031, S-400 33 Göteborg, Sweden.

of total daily fluid diet intake, or as amount of morphine consumed in mg/kg b.wt./day. There was a statistically significant correlation between the two ways of expressing morphine intake ($r = .98$, 47 male rats, weighing 225–275 g; $r = .97$, 43 female rats, weighing 200–260 g; linear regression analysis). The placing of the diet tubes to the right or to the left in the cages did not significantly affect the preference level.

Experimental Protocol

Morphine preference (Fig. 1). Male rats ingested morphine up to a dose of 340 mg/kg b.wt./day during 8 days and were kept on that dose for another 1, 2, 3, 4, 10, 16 or 38 days in the different experiments. Twenty-five to forty rats were used in each experiment. Choice tests were performed for 6 days and the mean preference levels were calculated.

Morphine preference during prolonged choice tests (Fig. 2). Ten male and 10 female rats ingested morphine up to 340 mg/kg b.wt./day during 8 days and were kept on that dose for another 16 days. The animals were choice-tested during 12 days and the mean preferences for each day were calculated. After 4 days on morphine, the rats were again choice-tested for 14 days. Thereafter, the rats were treated with morphine and choice-tested with 550 mg/kg b.wt./day morphine (choice period III). The rats were again treated with morphine and choice tested with 225 mg/kg b.wt./day morphine (choice period IV).

Morphine preference and learning the preference behaviour (Fig. 3). Three groups with 10 or 11 animals in each ingested morphine according to the "experimental set-up" in the figure. One group received 4.0 mg/kg b.wt. IP naloxone at three different occasions during one week (group I). Abstinence symptoms were precipitated. The other group was going through a choice-test for 5 days while the third group was treated with morphine all the time. After 24 days all animals were choice-tested.

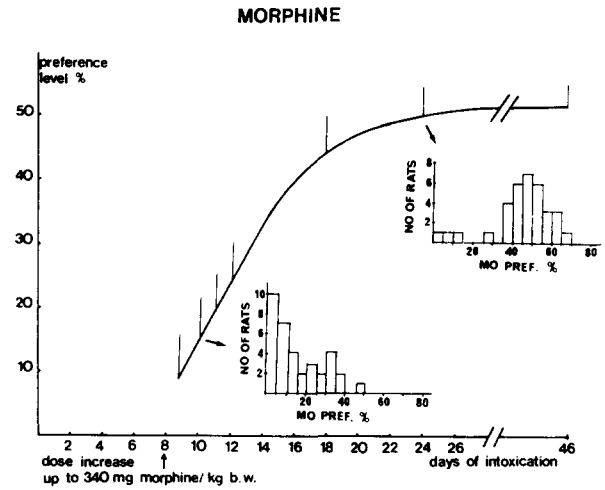


FIG. 1. Mean morphine preferences during 6-day choice tests after varying ingestion periods. Each point represents the mean preference ± S.E.M. (25–40 different male rats at each point). Preference increased after longer ingestion up to a mean level of 50%. Inset figures show the preference distribution of individual rats after short-term and long-term treatments, respectively.

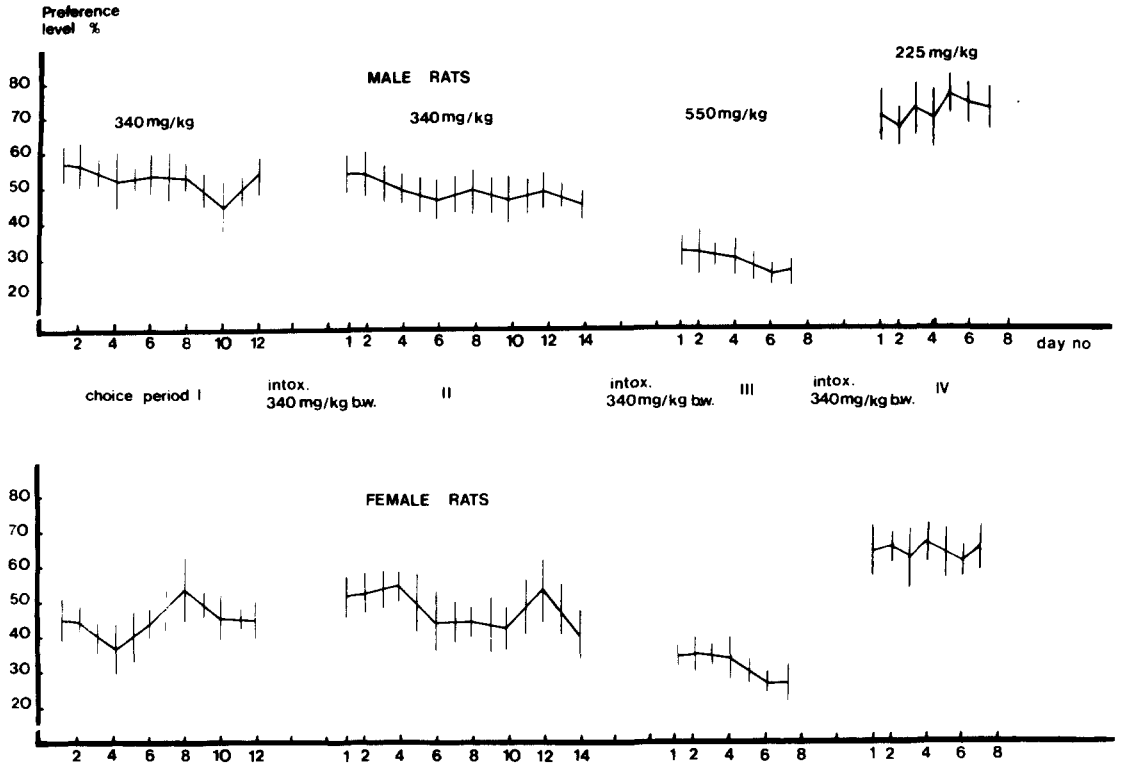


FIG. 2. Preference tests for long periods and on different morphine doses of physically dependent male and female rats. There were no statistically significant differences (variance analysis) in mean morphine consumption expressed as mg/kg b.wt./day comparing the different choice tests.

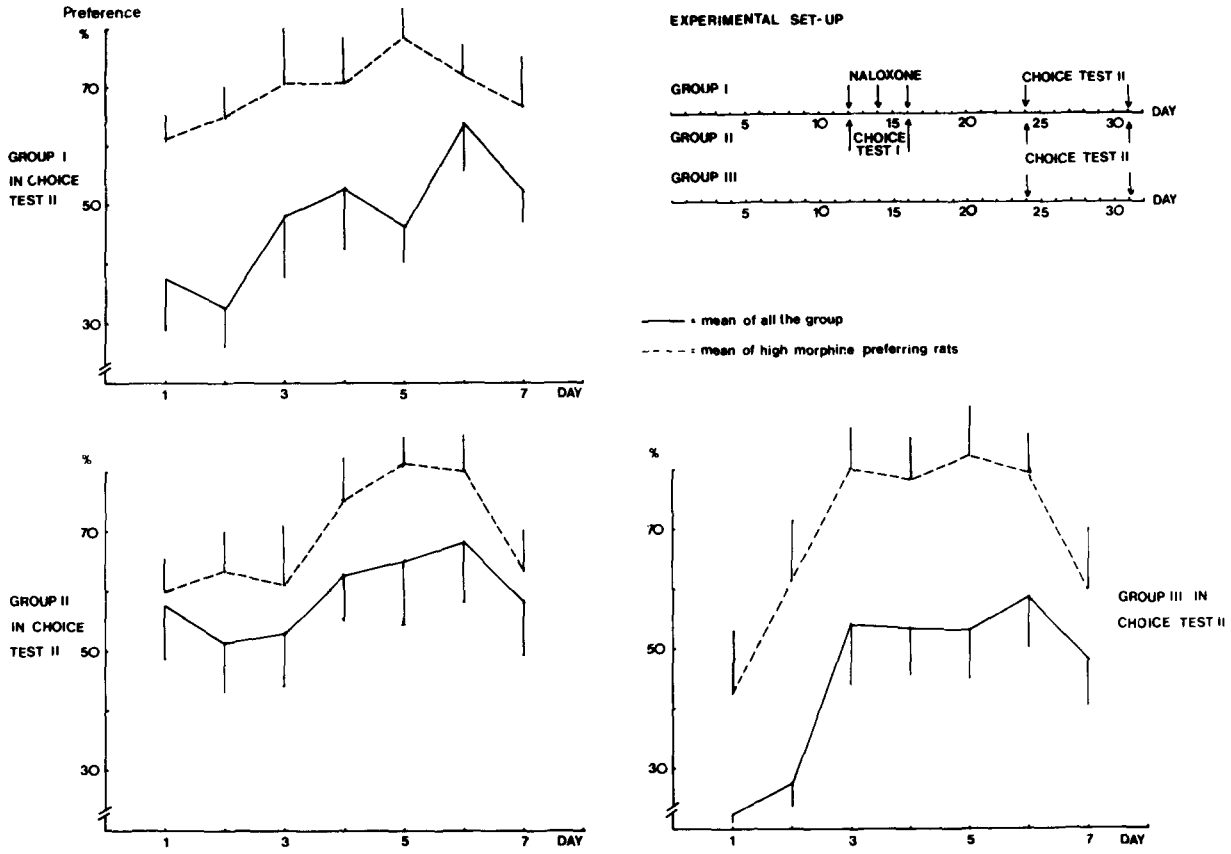


FIG. 3. Three groups of 10–11 rats in each were treated according to the "experimental set-up." In choice test II, group 2 (which had gone through one prior choice test) showed an initial high preference for the morphine diet. However, there is no difference in morphine consumption by the three groups of animals at the end of the choice tests. The 3 most high morphine consumers (= ---) from the different groups do not show any significant differences in morphine intake during the whole choice period (variance analysis).

Breeding high morphine preference rats (Fig. 4). Fifty-five male and 62 female Sprague-Dawley rats ingested morphine for 16 days on 340 mg/kg b.wt. (including the 8 day dose increase), and were choice tested for 6 days. The 10–12 most high preference male and female rats were paired and eight generations (F_0–F_8) were treated with morphine and choice-tested in a similar way. No sister and brother pairing was performed. The preferences of the offspring of the 3 highest preference male and female rats from each generation were calculated.

RESULTS

Morphine Preference (Fig. 1)

From Fig. 1 it is seen that the rats had a higher mean morphine preference after longer ingestion periods. A mean preference level of 50% was reached after 24 days of total treatment. Approximately 10% of the rats showed a relatively large preference already after a short treatment period, while a similar percentage of rats avoided morphine in choice situations even after a long ingestion (inset figures). Thus, there is a variability between different rats concerning the preference level.

Morphine Preference During Prolonged Choice Tests (Fig. 2)

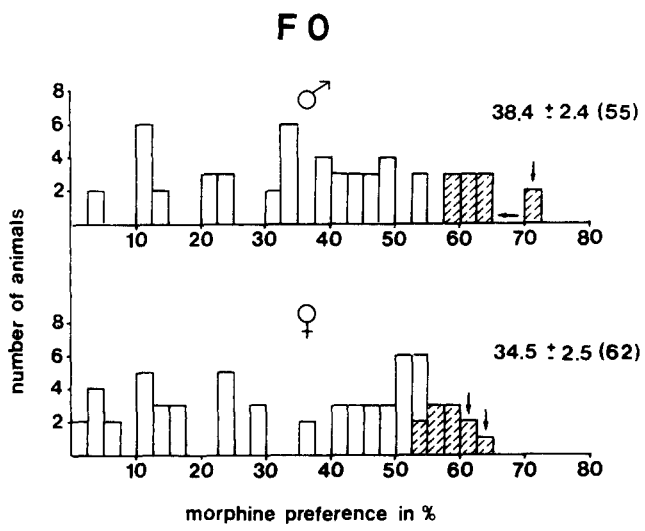
The mean preference level was not significantly different from one day to another during the choice tests, nor when comparing the two choice tests (I and II). When choice-tested on a higher dose (III), the mean preference level expressed in % decreased and when choice-tested on a lower dose (IV) the mean preference level increased. There were no statistically significant differences (variance analysis) in mean morphine consumption expressed as mg/kg b.wt./day comparing the different choice tests. The results indicate that the preference level is determined by the animal's respective pharmacological requirement for a certain amount of morphine.

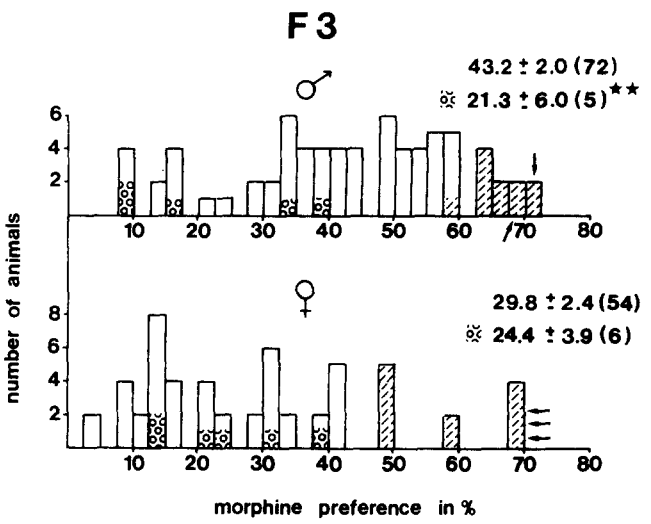
Morphine Preference and Learning the Preference Behaviour (Fig. 3)

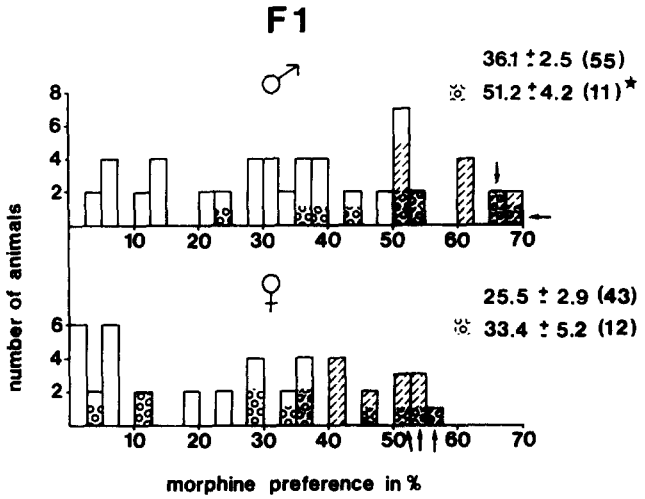
Those animals having undergone a previous choice test (group II) showed an initially high mean morphine preference, while the morphine consumptions of each group of animals were not significantly different from each other at the end of the choice situation (Student's *t*-test). Thus, the preference level is not due to unspecific factors. If the daily preference level is calculated for just high morphine consumers (mean >70% preference) from the different groups, no significant differences (two-way ANOVA) in morphine intake were found during the whole choice period.

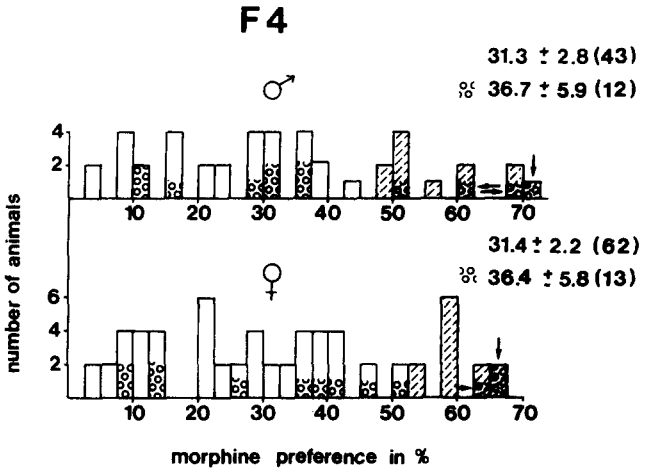
Breeding High Morphine Preference Rats (Fig. 4)

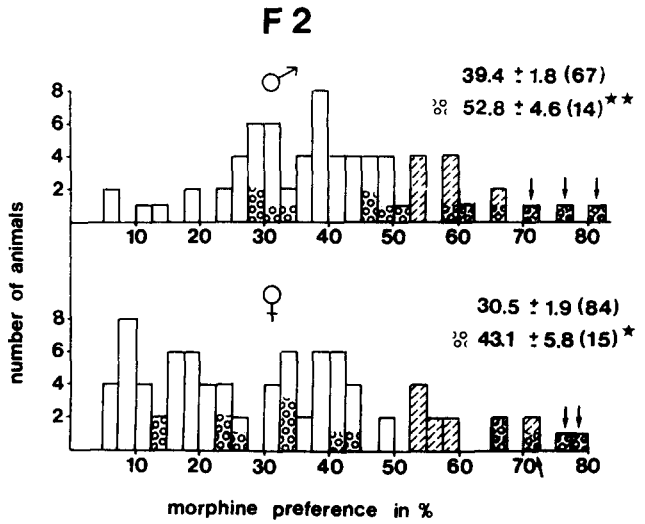
There were no significant differences in mean preference level

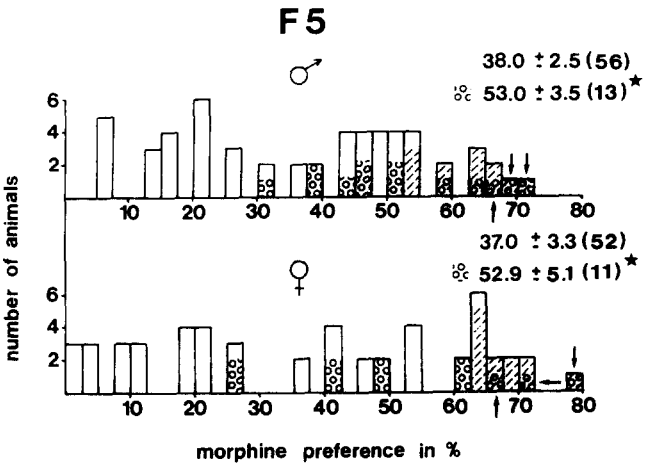

F 0
38.4 ± 2.4 (55)
34.5 ± 2.5 (62)
number of animals
morphine preference in %

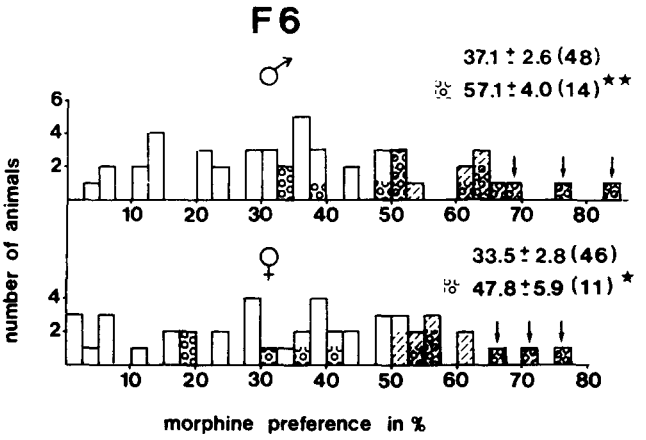

F 3
43.2 ± 2.0 (72)
♀ 21.3 ± 6.0 (5)**
29.8 ± 2.4 (54)
♀ 24.4 ± 3.9 (6)
number of animals
morphine preference in %

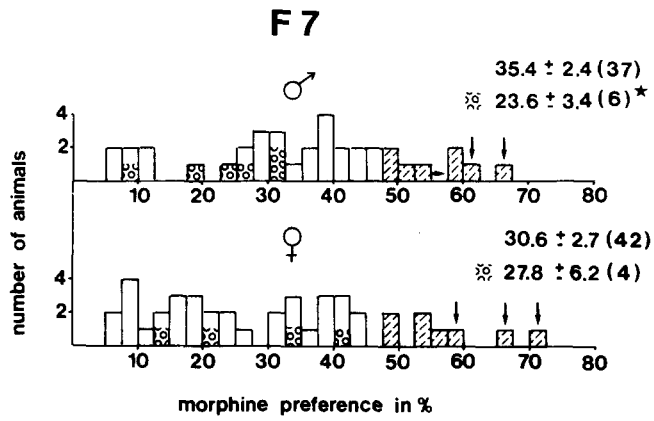

F 1
36.1 ± 2.5 (55)
♀ 51.2 ± 4.2 (11)*
25.5 ± 2.9 (43)
♀ 33.4 ± 5.2 (12)
number of animals
morphine preference in %

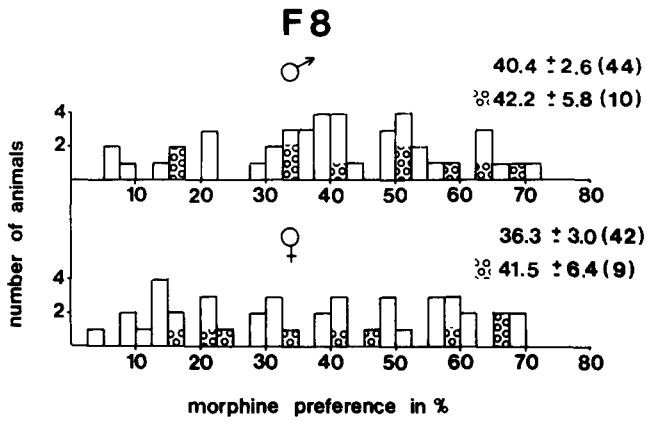

F 4
31.3 ± 2.8 (43)
♀ 36.7 ± 5.9 (12)
31.4 ± 2.2 (62)
♀ 36.4 ± 5.8 (13)
number of animals
morphine preference in %


F 2
39.4 ± 1.8 (67)
♀ 52.8 ± 4.6 (14)**
30.5 ± 1.9 (84)
♀ 43.1 ± 5.8 (15)*
number of animals
morphine preference in %


F 5
38.0 ± 2.5 (56)
♀ 53.0 ± 3.5 (13)*
37.0 ± 3.3 (52)
♀ 52.9 ± 5.1 (11)*
number of animals
morphine preference in %







ABOVE AND FACING PAGE

FIG. 4. Breeding high morphine preference rats for 8 generations. The mean preferences ± S.E.M. for male and female rats (number of rats in parentheses) in each generation are shown. No statistically significant differences of morphine preference were obtained when comparing the whole generations with Student's *t*-test or variance analysis. Columns with hatched marks = rats which were paired. The three highest preference male and female rats from each generation is marked with arrows and their offspring is marked as columns with circles. The male F_1 offspring of the 3 highest male and female morphine preference rats (marked columns with circles in the figure) showed a higher preference compared to the whole generation. The same result was obtained from the next generation (F_2) where the female offspring also had a higher preference (Student's *t*-test). In the third offspring generation (F_3), 65% of the pups from extremely high morphine preference male and female rats died within 24 hr after birth and the surviving rats showed a low preference when tested after morphine ingestion. Similar results were obtained in generations F_4 to F_8. $*p<0.05$: offspring from rats with highest preference (bars with circles) vs. whole generation (male and female separated). $**p<0.01$: offspring from rats with highest preference (bars with circles) vs. whole generation (male and female separated).

throughout the generations (31.3 up to 43.2% and 25.5 to 37.0% for male and female rats, respectively). However, the offspring of the 3 highest preference rats increased their preference over 2 generations (F_1 and F_2). In F_3, up to 65% of the pups of these extremely high morphine preference rats died within 24 hr after birth. The preference of the surviving F_3 rats (when adult) was significantly lower than that of the whole generation. In F_4 there was a restoration to a preference level which was not significantly different from the whole group. Similar results were obtained from generations F_5–F_8.

DISCUSSION

The rats were placed in individual cages and ingested morphine in the same environment and with the same vehicles as the preference tests were later performed. The amounts of calories, nutrients and fluid were similar whether or not the rats chose morphinized or control diets. The rats acquired a higher mean preference for morphine after a longer intoxication period, up to a preference level of approximately 50% (corresponding to a morphine consumption of 170 mg/kg b.wt.). Ten percent of the rats showed an even higher morphine preference, while other rats voluntarily went through an abstinence reaction. Furthermore, it was shown that the preference level is determined by the animal's respective pharmacological requirement for a certain amount of morphine. The mean preference level for a number of animals was affected by learning the specific behaviour. However, the very high morphine preference reached by some few percent of the rats, was not due to learning. Neither were there any correlations between this high morphine preference and the taste of the drug measured earlier as quinine diet preference, nor of serum morphine levels, food intake and body weight gain, differences in abstinence symptoms, precipitated by exclusion of morphine from the fluid diet or by the administration of the opiate antagonist naloxone (7,9).

Thus, previous and the present experiments demonstrate that, within one strain of rats, the individual animals vary in morphine preference after similar ingestion conditions (from some few percent up to 70–80% preference) with the specificity of the experimental model. This means that a differential pharmacological need for the drug exists between individual rats. It might be that these rats contain or lack, can activate or deactive some nonsex-related factor(s) which is (are) genetically transformed, and the presence or absence of which correlates with high morphine preference. This is partly in agreement with Nichols and Hsiao (6). The next step was to examine if extremely high morphine-preference rats could be genetically enriched. A heavy accumulation or depletion of such a factor(s) in F_3 and F_7 is not compatible with life. It could be claimed that the results of this study could be explained in terms of morphine affecting the chromatin of both male and female rats. There are, in fact, experimental results supporting the possibility of morphine affecting the genetic material of the sex chromosomes even in males (11). In the present study, all animals were treated in a similar

way, which is why such an explanation could not completely explain our results. The inheritance pattern could not be evaluated within this study, although our results strongly suggest that high morphine preference is under genetic control.

ACKNOWLEDGEMENTS

The study was supported by grants from The Swedish Medical Research Council (grant No. 25X-06005), from The Swedish Physicians' Foundation for Medical Research and from The Skandia Medical Foundation. The skilled technical assistance of Tomas Machek is highly appreciated.

REFERENCES

1. Beach, H. D. Morphine addiction in rats. Can. J. Psychol. (Toronto) 11:104–112; 1957.
2. Horowitz, G. P.; Whitney, G.; Smith, J. C.; Stephan, F. K. Morphine ingestion: genetic control in mice. Psychopharmacology (Berlin) 52:119–122; 1977.
3. Khavari, K. A.; Risner, M. E. Opiate dependence produced by ad libitum drinking of morphine in water, saline, and sucrose vehicles. Psychopharmacologia 30:291–302; 1973.
4. Kumar, R.; Steinberg, H.; Stolerman, I. P. Inducing a preference for morphine in rats without premedication. Nature 218:564–565; 1968.
5. Nichols, J. R.; Davis, W. M. Drug addiction II. Variation of addiction. J. Am. Pharm. Assoc. 48:259–262; 1959.
6. Nichols, J. R.; Hsiao, S. Addiction liability of albino rats: breeding for quantitative differences in morphine drinking. Science 157: 561–563; 1967.
7. Rönnbäck, L. Morphine preference in individual rats after morphine ingestion. Submitted.
8. Rönnbäck, L.; Eriksson, P.; Zeuchner, J.; Rosengren, L.; Wronski, A. Aspects of abstinence after morphine ingestion. Pharmacol. Biochem. Behav. 28:87–93; 1987.
9. Rönnbäck, L.; Zeuchner, J.; Rosengren, L.; Wronski, A.; Ögren, S.-O. Decreased morphine intake by opiate addicted rats administered zimelidine, a 5-HT uptake inhibitor. Psychopharmacology (Berlin) 82:30–35; 1984.
10. Schuster, C. R.; Thompson, T. Self administration of and behavioral dependence on drugs. Annu. Rev. Pharmacol. 9:483–502; 1969.
11. Smith, D. J.; Joffe, J. M. Increased neonatal mortality in offspring of male rats treated with methadone or morphine before mating. Nature 253:202–203; 1975.
12. Stolerman, I. P.; Kumar, R. Preferences for morphine in rats: validation of an experimental model of dependence. Psychopharmacologia 17:137–150; 1970.
13. Thompson, T.; Schuster, C. R. Behavioral pharmacology. Englewood Cliffs, NJ: Prentice-Hall; 1968.
14. Yanaura, S.; Suzuki, T. Preference for morphine and drug-seeking behavior in morphine dependent rats. Jpn. J. Pharmacol. 28:707–717; 1978.
15. 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.