Pharmacology Biochemistry & Behavior, Vol. 9, pp. 107–110. Printed in the U.S.A.

Addiction Liability of Tryon Rats: Independent Transmission of Morphine and Alcohol Consumption[1]

SHIRLEY Y. HILL[2]

Department of Psychiatry, University of Pittsburgh School of Medicine, Pittsburgh, PA 15261

(Received 23 January 1978)

HILL, S. Y. *Addiction liability of Tryon rats: Independent transmission of morphine and alcohol consumption.* PHARMAC. BIOCHEM. BEHAV. 9(1) 107–110, 1978.—Two inbred strains of Tryon rats were tested for consumption of morphine (0.5 mg/ml) and alcohol (10% w/v) solutions in both free and forced choice situations. Statistically significant differences in intake by strain were found both for morphine and alcohol. The Tryon S_3 strain consumed significantly more morphine than the S_1 strain in two of the four phases of the experiment. The Tryon S_1 strain consumed significantly more alcohol than the S_3 in two of the four phases. Factors affecting consumption of drug solutions including sex and activity level were assessed using analysis of covariance. Strain differences were apparent even when these factors were removed from the analyses. These results are discussed in relation to previous reports suggesting a common addiction liability for both morphine and alcohol in inbred strains of animals.

Ethanol dependence Morphine dependence Consumption Genetic influences Activity Sex differences

A COMMON genetic factor influencing alcohol and morphine consumption in rodents has been suggested. Evidence has been presented [14] showing that in a comparison of two strains of rats, the strain exhibiting a higher consumption of morphine also consumes the most alcohol in a choice situation. Similar evidence has been presented in mice [2]. These data have been interpreted to indicate that a common addiction proneness is present within strains.

These data are open to other interpretations including the possibility that genetic differences in taste sensitivity or emotionality predispose animals to drink more fluid when that fluid is given a distinctive taste. Animals that are more emotional do not approach novel stimuli as readily as non-emotional ones and tend to show reduced activity in the open field test [7]. Introduction of drug solutions having totally different taste characteristics and post-ingestional effects presents the animal with novel stimulation. The emotional animal might be expected to avoid the drug solutions because of their novelty while the non-emotional animal would tend to approach it.

Given the possible effects of these variables, the present study was designed to test the genetic independence of alcohol and opiate consumption in two inbred strains of rats, controlling for taste factors and statistically covarying out the influence of activity level or emotionality in the open field.

METHOD

Animals

A total of 50 animals were used, 26 from the Tryon S_3 strain and 24 from the Tryon S_1 strain. Among the 26 Tryon S_3s, 13 were female and 13 male; among the 24S_1s, 16 were female and 8 were male.

All animals were born in our laboratory from inbred stock obtained from a colony maintained at the University of Northern Iowa. These animals were originally derived from selective inbreeding for maze learning ability [16]. Animals in the present study were born in our laboratory and were the offspring of animals inbred for at least 38 generations (brother-sister matings).

Consumption Procedures

Animals were reared with the mothers until weaning (23 days of age). At weaning the animals were reared with like sex litermates until an average of 103 days (range 98–106), at which time they were individually caged. The animals were given a month to acclimate to the drinking tubes and presented with free access to food and water.

At approximately 135 days of age the animals were assigned to either morphine or alcohol presentation conditions. Approximately half of the S_3s were assigned to the morphine

[1]Supported in part by USPHS Grants AA–00209 and DA–4RG008 while the author was in the Department of Psychiatry, Washington University School of Medicine, St. Louis, MO.

[2]Send reprint requests to: Department of Psychiatry, University of Pittsburgh School of Medicine, 3811 O'Hara Street, Pittsburgh, PA 15261.

condition (N=12) and the remainder to the alcohol condition (N=14). Similarly, half of the S_1s were assigned to the morphine condition (N=12) and half to the alcohol condition (N=12).

The morphine condition consisted of a two bottle choice of either distilled water in one bottle and morphine solution (0.5 mg/ml) in the other. The alcohol condition consisted of a two bottle choice of distilled water and alcohol (10% by weight). The alcohol and morphine solutions were prepared frequently throughout the experiment with distilled water. Presentation of fluids was accomplished using 100 ml graduated tubes fitted with ball-bearing stoppers.

The design of the experiment consisted of four phases. In the first phase animals were presented with the drug solution or distilled water. In the second phase, the drug solutions were prepared with a 1% sodium saccharine solution (distilled water and saccharin, 1% by weight). This method of adulterating morphine solutions has been shown to substantially increase consumption of morphine over repeated trials [9]. The sweetened drug solutions were presented in a two-bottle choice with a 1% saccharine solution as the alternative fluid during this phase. The third phase consisted of forced presentation of the drug solutions prepared with 1% saccharine in a single bottle. The final phase reinstated the choice condition of the second phase using saccharine-drug solution and saccharine solution without drug as the alternative fluid.

The study was designed to include four phases in order that the effect of forced consumption (Phase III) on subsequent choice consumption (Phase IV) could be evaluated in a comparison with intakes prior (Phase II) to forced consumption, all employing saccharine adulterated drug solutions. The purpose of Phase I was to determine the initial preference of the two strains using non-adulterated taste solutions. It was expected that differences in consumption might be apparent in Phase I but that this difference might reflect only differences in taste aversiveness of the two substances employed. Therefore, a comparison of Phase I and II was used to detect differences in consumption that could be attributed to taste qualities of the drug solutions.

Activity Measures

Activity was assessed in an open field before animals were presented with drugs at approximately 107 days of age. Measurement consisted of placing each animal in the same corner of the open field and counting the number of squares traversed in 3 min. Lighting was constant throughout testing and was placed directly above the field. Two independent observers were used to insure the reliability of the activity measurements. Also, the open field was cleaned between each animal's test to insure that the scent of the preceding animal would not contaminate the results obtained.

RESULTS

Consumption of drug intake was assessed by calculating mg/kg intake of morphine or g/kg intake of alcohol. In addition, the percent preference, drug intake divided by total fluid intake, was calculated and found to accurately represent drug intake in most cases. However, in Phase IV the S_3s showed a higher preference for the ethanol-saccharine solution than did the S_1s (64% versus 39%). This higher preference was due to a decrease in placebo intake in Phase IV as compared to Phase II and not due to changes in the amount of ethanol consumed. Therefore, the mg/kg and g/kg intakes of morphine and alcohol, respectively, were used to characterize the obtained results.

Analysis of Phase I intakes showed no significant difference in intakes of alcohol and morphine as a function of strain or sex. When the taste of the two drug solutions was masked with saccharine and a saccharine solution offered in a two-bottle choice, differences in intake by strain were manifest. Phase II analysis revealed a significant difference in alcohol intake by strain, $F(1,22)=4.88$, $p<0.04$. The S_1 animals consumed significantly more alcohol than did the S_3 animals. In contrast, no difference in morphine intake was noted for the second phase of the experiment (Fig. 1). At this point in time, intakes of morphine remained very low (2.5–2.7 mg/kg).

During the third phase, when animals were forced to consume the drug solutions prepared with saccharine and offered in a single bottle presentation, a significant difference in alcohol intake as a function of strain was found, $F(1,22)=33.43$, $p<0.001$. The S_1s drank significantly more alcohol than did the S_3s. It may be noted that alcohol intake varied little from the second to the third phase of the experiment within strains, though differences across strains were clearly evident. Also, in the third phase, significant differences in morphine intake were found, $F(1,22)=6.65$, $p<0.02$. The S_3 strain showed the greatest morphine intake. This is in direct contrast to the greater intake of alcohol found for the S_1 animals when compared with S_3s (Fig. 1).

In the final phase of the experiment (IV), in which the choice condition was reinstated, a significant strain difference was again apparent. Significantly more morphine was consumed by the S_3 animals during Phase IV than was consumed by the S_1 animals, $F(1,20)=27.22$, $p<0.001$. While higher consumption of morphine was noted for the S_3 animals, no difference in consumption was noted for alcohol during this phase of the experiment. Both the S_1 and S_3 animals consumed approximately 5.0 g/kg/day of ethanol. Consumption of alcohol during Phase IV showed a drop in intake from that observed in Phase II for both strains. The reason for this decrease in intake of alcohol following the forced consumption period is uncertain. It is interesting to note, however, that the forced consumption period (Phase III) served to magnify the strain differences in morphine consumption during Phase IV, substantially increasing morphine consumption among the S_3s as compared to that observed for the S_3s during Phase II.

The present data do not support the notion that strains showing the highest intake of morphine will necessarily exhibit greater intake of alcohol. The S_1 strain showed statistically higher intakes of alcohol during two phases of the experiment (II and III), while the S_3 strain showed statistically higher intakes of morphine during two phases (III and IV). During the forced consumption period (Phase III) the S_3 animals consumed significantly more morphine than the S_1s while the S_1s consumed significantly more alcohol than did the S_3 animals. The present data are in direct contrast to those obtained by Nichols and Hsiao [14]. In that study selective breeding resulted in two strains differing in susceptibility to morphine addiction. The morphine susceptible strain was found also to be the alcohol susceptible strain. Similarly, the morphine resistant strain appeared to be alcohol resistant, showing a lesser consumption of alcohol.

Activity Measures

Analysis of results obtained in the open field test, per-

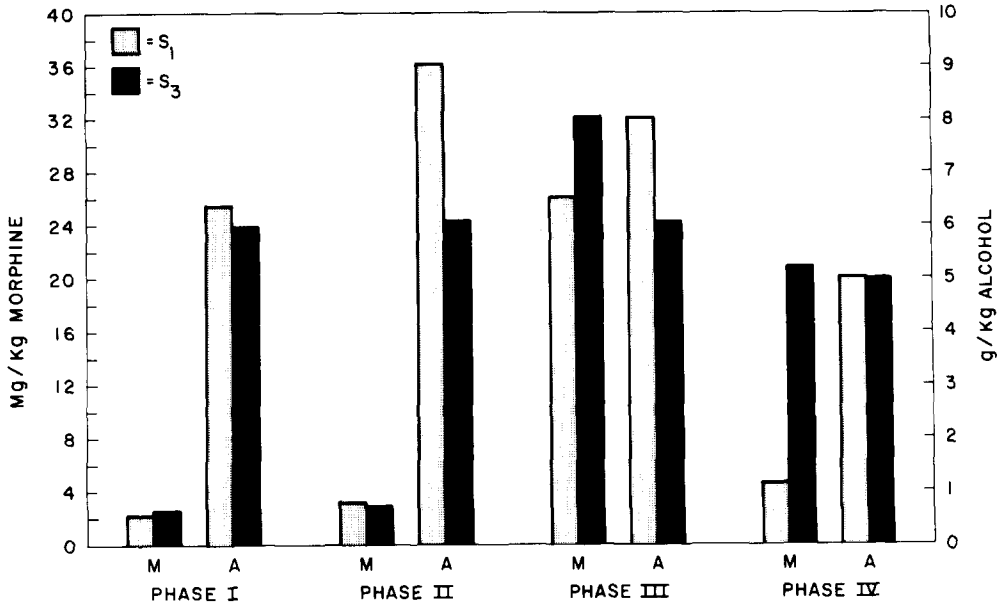

FIG. 1. Alcohol and morphine intake in Tryon S_1 and S_3 strains during four drug presentation phases.

formed before drug presentation was begun, revealed highly significant differences in activity between the two strains. The S_3 animals were significantly more active than the S_1s, $t(48)=3.88$, $p<0.001$. This finding is in agreement with a previous report by Harrington [7].

Due to the significant differences found for activity, it appeared appropriate to determine what effect, if any, differences in activity might contribute to the observed differences in the two strains' consumption of ethanol and morphine. The open field test is a standard procedure for assessment of emotionality in rodents and has been demonstrated to be substantially influenced by hereditary influences in rats [6]. A covariate analysis was performed using the activity score as the covariate. This analysis revealed the same level of significance for all previously performed analyses of variance of drug intake.

Low activity in the open field has been characterized as emotional behavior for the rodent. Therefore, it might be expected that the S_1s would consume less alcohol and less morphine than the S_3s due to their greater avoidance of novel stimulation. However, the present data do not support this prediction. The S_1s consumed either more alcohol in particular phases of the experiment or showed no difference by strain (Phase IV). The present data do, however, verify that hereditary factors contribute to the observed differences in activity though these differences in activity are not predictive of differential drug intake.

Sex Differences

To insure that differences observed by strain were not a result of differences in sex, further analyses were performed using sex as the covariate. These analyses, like those performed for activity, showed no change in the level of statistical significance when the influence of sex was covaried out.

Analyses of variance did reveal differences in drug intake by sex during Phases III and IV of the experiment. For alcohol, females displayed greater intake than males in both strains, $F(1,22)=33.96$, $p<0.001$; $F(1,22)=10.02$, $p<0.004$, Phase III and IV, respectively. A similar pattern was observed for morphine, females drinking more morphine solution than did males in both strains, $F(1,20)=30.07$, $p<0.001$; $F(1,20)=8.21$, $p<0.01$, Phase III and IV, respectively. However, using covariate analysis, effects of strain were clearly manifest even when differences in sex were removed.

DISCUSSION

The present study provides evidence for independent transmission of morphine and alcohol consumption in animals. Employing two independent inbred strains of Tryon rats S_1 and S_3, originally derived for differences in maze solving ability [16] and later observed to vary in open field activity [7], allowed for determination of genetic differences in consumption patterns. Every effort was made to reduce genetic variability within strains through inbreeding for 38 generations and by splitting the available litters so that approximately half of each litter was assigned to either the morphine or alcohol condition. Genetic differences in morphine and alcohol consumption have previously been studied in two strains of rats [14] and in two strains of mice [2]. In both studies it was found that the strain exhibiting the highest consumption of alcohol also showed a higher consumption of morphine solutions.

Procedural differences may explain the disagreement between the present results and those reported earlier [2,14]. In the Eriksson and Kiianmaa study [2] morphine consumption was determined in C57BL and CBA/Ca mice whose alcohol preference was known from previous work. Although C57 mice have been shown to exhibit relatively high alcohol consumption in a number of studies [3, 4, 12, 13, 17], failure to test for alcohol consumption within the same study may be a serious drawback. As Broadhurst [1] has pointed out heritability is a characteristic of a population and not a trait. Values obtained for the environmental component of the observed phenotypic variations are not applicable to other en-

vironments than the one in which the population is reared and tested.

While the Nichols and Hsiao study [14] did attempt to determine phenotypic variation (alcohol and morphine consumption) in the same laboratory, other aspects of their procedure may have varied the environmental component. Different procedures were used for training and testing of alcohol and morphine consumption. Morphine training consisted of five 3-day cycles: deprivation, morphine only, and water only with choice tests interspersed between the cycles. In that study the procedure used for training animals to drink alcohol included forced drinking of 10% alcohol as the sole source of fluid for 68 days followed by seven choice tests at two-week intervals.

The present study attempted to minimize environmental differences by using the same schedule of presentation for both morphine and alcohol. Also, four different consumption phases were included, allowing for any similarities in consumption of these strains to be manifest. Three of these phases included use of 1% saccharine in order to minimize the differences in taste qualities of alcohol and morphine.

The present investigation demonstrating independent transmission of alcohol and morphine consumption in two inbred Tryon strains would appear to have implications for the question of whether a common genetic liability to alcoholism and opiate abuse exists in man.

Whether there is a common genetic liability for both alcoholism and heroin addiction, such that certain individuals have a greater vulnerability for both, is a question that has received increasing attention. A greater vulnerability for alcoholism has been demonstrated among children of alcoholics raised apart from the biological alcoholic parent [5], and in studies of drinking patterns among twins [11,15] in which genetic factors appear to have a role. Also, recent data demonstrating little overlap in the familial transmission of opiate and alcohol abuse within each family studied further suggest a possible genetic independence in man [8].

ACKNOWLEDGEMENT

I wish to thank Douglas Fishel for help in testing the rats and Renato Reyes for excellent help in the statistical analysis of data, and Lou Sedlock for typing the manuscript. Also, I am grateful to Dr. Gordon Harrington and Ms. Joyce Schima for helping us establish a Tryon colony.

REFERENCES

1. Broadhurst, P. L. Methods of psychogenetic analysis. International Symposium Biological Aspects of Alcohol Consumption, 27–29 September 1971, Helsinki. *The Finnish Foundation for Alcohol Studies* **20:** 97–103, 1972.
2. Eriksson, K. and K. Kiianmaa. Genetic analysis of susceptibility to morphine addiction in inbred mice. *Ann. Med. exp. Biol. Fenn.* **49:** 73–78, 1971.
3. Eriksson, K. and P. H. Pikkarainen. Strain and sex differences in voluntary alcohol consumption, liver ADH activity and aldehyde-oxidizing capacity in inbred strains of mice. *Jap. J. Stud. Alcohol* **5:** 1, 1970.
4. Fuller, J. L. Measurements of alcohol preference in genetic experiments. *J. comp. physiol. Psychol.* **57:** 85–88, 1964.
5. Goodwin, D. W., F. Schulsinger, L. Hermansen, S. B. Guze and G. Winokur. Alcohol problems in adoptees raised apart from alcoholic biological parents. *Arch. gen. Psychiat.* **28:** 238–243, 1973.
6. Hall, C. S. The genetics of behavior. In: *Handbook of Experimental Psychology*, edited by S. S. Stevens. New York: Wiley, 1951, pp. 304–330.
7. Harrington, G. M. Strain differences in open-field behavior of the rat. *Psychon. Sci.* **27:** 51–53, 1972.
8. Hill, S. Y., C. R. Cloninger and F. R. Ayre. Independent familial transmission of alcoholism and opiate abuse. *Alcoholism: clin. exp. Res.* **1:** 335–342, 1977.
9. Hill, S. Y. and B. J. Powell. Cocaine and morphine self-administration: effects of differential rearing. *Pharmac. Biochem. Behav.* **5:** 701–704, 1976.
10. Isbell, H., H. F. Fraser, A. Walker, R. E. Belleville and A. J. Eisenman. An experimental study of the etiology of "Rum" fits and delirium tremens. *Q. Jl Stud. Alcohol* **16:** 1, 1955.
11. Kaij, L. *Studies on the Etiology and Sequels of Abuse of Alcohol.* Lund: University of Lund, Department of Psychiatry, 1960.
12. McClearn, G. E. Genetics and motivation of the mouse. *Neb. Symp. Motiv.* **16:** 47–83, 1968.
13. McClearn, G. E. and D. A. Rodgers. Differences in alcohol preference among inbred strains of mice. *Q. Jl Stud. Alcohol* **20:** 691–695, 1959.
14. Nichols, J. R. and S. Hsiao. Addiction liability of albino rats: breeding for quantitative differences in morphine drinking. *Science* **157:** 561–563, 1967.
15. Partanen, J., K. Bruun and T. Markkanen. *Inheritance of Drinking Behavior. A Study on Intelligence, Personality and Use of Alcohol in Adult Twins*, Vol. 14. Helsinki: The Finnish Foundation for Alcohol Studies, 1966.
16. Tryon, R. C. Genetic differences in maze learning ability in rats. *Natn Soc. Stud. Educ.* **39:** 111–119, 1940.
17. Whitney, G. Relationships between alcohol preference and other behaviors in laboratory mice. International Symposium Biological Aspects of Alcohol Consumption, 27–29 September 1971. Helsinki: The Finnish Foundation for Alcohol Studies **20:** 151–161, 1972.