Morphine Ingestion: Genetic Control in Mice

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Abstract. C57BL/6J mice will drink large amounts of, and display a highly positive preference for, morphine sulfate when it is dissolved in an aqueous solution of sodium saccharin. In identical test situations DBA/2J mice will drink very little of, and display a strong avoidance toward, the morphine-saccharin solution. This clear separation between morphine-accepting and morphine-rejecting animals within a single species combined with a quick and simple method of inducing high levels of morphine ingestion could facilitate the discovery of causal factors in opiate addiction.

Key words: Morphine ingestion — Mice — Behavior genetics — Saccharin.

Considerable attention is being 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 abuse and addiction. Historically, procedures for generating self-administration of opiate compounds by laboratory animals have taken one of two forms: either the animal performs some response to receive an injection of the drug, or ingests the drug with food or drink (Kumar et al., 1968; Thompson and Schuster, 1968; Schuster and Thompson, 1969; Stolerman and Kumar, 1970; Khavari and Risner, 1973). Unfortunately, many of the procedures yet reported for inducing self-administration of opiate compounds present serious practical obstacles in terms of preliminary time and effort, and the eventual result is often a rather low level of drug self-administration. The method of administering the opiate in an adulterated solution has the definite advantage of reducing preliminary time and effort, and the eventual result is sometimes a quite high level of drug ingestion (Risner and Khavari, 1973; Khavari et al., 1975). With few exceptions, studies using opiate ingestion have not investigated genetic factors, and the few genetic studies of opiate ingestion (Nichols and Hsiao, 1967; Erikson and Kiianmaa, 1971) have not employed adulterated solutions. Here we report simple procedures involving no pretreatment by which mice (Mus musculus) can be induced to drink large (in some cases, lethal) amounts of morphine sulfate.

The two variables of central importance are (A) the genotype of the subject and (B) the vehicle in which the drug is presented. When mice of the C57BL/6J inbred strain were presented with morphine sulfate in an aqueous solution of sodium saccharin they ingested large quantities of morphine. Indeed, 11 of the first 40 C57BL/6J mice that we exposed to morphine in a saccharin vehicle drank lethal amounts of the solution. C57 mice have consumed lethal amounts of morphine in both forced and choice situations (Horowitz, 1976). The central importance of genotype as a variable contributing to this profound morphine acceptance was indicated by the very different results obtained with mice of another inbred strain. Under conditions identical to those in which C57 mice consumed lethal amounts, animals of the DBA/2J strain not only avoided the morphine solution, but also came to avoid the normally preferred saccharin vehicle (Horowitz, 1976; further unpublished results in our laboratory).

In the present experiment C57 and DBA mice were given two-tube preference tests for morphine in a saccharin vehicle versus tap water. Here the animals were never forced to consume morphine to overcome thirst since an alternative fluid was always available. In addition the concentration of saccharin employed as an adulterant to the morphine solution was systematically decreased to see what level of morphine preference would be maintained across a gradual reduc-
tion of adulterant. To indicate the role of the saccharin and morphine in determining the pattern of preference for each genotype, separate animals were also preference tested with morphine (no saccharin) versus water and with saccharin (no morphine) versus water.

METHODS

The subjects were 24 C57BL/6J and 30 DBA/2J male mice, 55 to 62 days of age at the start of the experiment. Throughout the experiment they were individually housed in standard 25 x 13 x 10 cm metal cages, with front and floor of hardware cloth. Standard Purina lab chow was available ad libitum from a hopper on the back wall of the cage. Fluids were presented through the cage front from stainless steel sipper tubes attached to inverted 25 ml graduated cylinders.

Three groups of mice, each group consisting of 8 C57s and 10 DBAs, were given two-tube preference tests. For the first group the test was morphine in saccharin vehicle versus tap water. The concentration of morphine remained constant (0.375 mg/ml) while the concentration of saccharin in the vehicle was sequentially halved every 2 days. For the first 2 days the morphine was in a vehicle of 0.24%, (w/v) saccharin solution, then 2 days in 0.12%, saccharin and so on, until a final concentration of 0.015%, saccharin was reached. The second group received the decreasing concentrations of saccharin, without morphine, in one tube and tap water in the other. The third group received 0.375 mg/ml morphine in aqueous solution from one tube, tap water in the other tube throughout the experiment. For statistical analysis individual 2-day preference scores were transformed such that: X' = arcsine in radians [X⁰⁵]. Such arcsine-square root transforms are often recommended to normalize raw score ratio data (Snedecor, 1962; Whitney et al., 1970; Winer, 1971). The transformed data were subjected to a three-way repeated measures analysis of variance.

RESULTS

As illustrated in Figure 1, the results were quite different for the two genotypes. In the analysis of variance all main effects were significant beyond the P < 0.00001 level (Genotype, F = 180.44, df = 1.48; Groups, F = 288.84, df = 2.48; Saccharin Concentration, F = 18.02, df = 4.192). In addition, the interactions were statistically significant with the important Genotype x Groups interaction having a value of F = 129.64 (df = 2.48; P < 0.00001). Post-hoc Tukey B tests were applied to specify further the effects obtained.

Both genotypes avoided morphine in aqueous solution (Fig.1), with no significant difference between strains for this group (P > 0.05). This finding is consistent with previous reports of low ingestion of unadulterated morphine by rats and mice (including C57 mice), after similarly short periods of access (Kumar et al., 1968; Stolerman and Kumar, 1970; Eriksson and Kiianmaa, 1971). Similarly, there was no significant difference between the two strains in saccharin preference in the absence of morphine when the data were combined across the saccharin concentrations employed in this study (P > 0.05). The C57 genotype displayed a strong preference for the morphine in saccharin (for comparison of C57 saccharin vs. morphine + saccharin, P > 0.05; either of these groups vs. aqueous morphine, P < 0.01). High levels of voluntary ingestion of sucrose adulterated morphine have similarly been reported for rats (Khavari et al., 1975). However, for DBA mice the results were quite different. The morphine + saccharin group was not different from the aqueous morphine group (P > 0.05), and both of these groups were significantly lower than the saccharin group (P < 0.01 for each comparison). As is evident in Figure 1, the morphine + saccharin group differed significantly between the two strains (P < 0.01). Since between-strain comparisons yielded no significant differences between preferences for saccharin across the concentrations included in this study, or in avoidance of aqueous morphine, these comparisons demonstrate
the central importance of both genotype and vehicle in considerations of morphine ingestion.

In an adjunct experiment following the 2-day test period for the last concentration of saccharin, all animals were subjected to 4 days of aqueous morphine vs. water to see if morphine-directed behavior could be generated in the absence of the saccharin solution. These data were transformed, then analyzed separately for each genotype by repeated measures analyses of variance followed by orthogonal contrasts between groups of interest.

The results of this final morphine alone vs. water test paralleled those of the earlier saccharin fading procedure for both strains. Within the C57 strain, the group that had previously had morphine + saccharin consumed considerable amounts of morphine alone, drinking an average of 75% of their daily intake from the morphine tube. The group whose previous exposure to morphine had always been in an aqueous solution preferred the solution significantly less than did either of the other two groups ($F = 36.55, df = 1,21; P < 0.001$). Surprisingly, the group that had previous exposure to saccharin alone did not differ significantly from the group whose previous exposure was to morphine + saccharin ($F < 1$).

Within the DBA strain, no group drank over 50% of their mean fluid intake from the morphine tube. The group with the highest morphine intake was the previous saccharin alone group which was significantly higher than either the previous morphine + saccharin group or the morphine alone group ($F = 54.38, df = 1,27; P < 0.001$). These latter two groups were not significantly different from each other ($F < 1$).

**DISCUSSION**

It had previously been found that C57 mice would drink large, and for some individuals lethal, doses of morphine over a relatively broad range of concentrations of morphine. The high intake, including lethal doses, occurred in both single-bottle no-choice tests and in two-bottle voluntary intake situations when the morphine was in a saccharin vehicle (Horowitz, 1976; further unpublished pilot experiments). The results of the present experiment demonstrate that with an alternative fluid of plain tap water available C57 mice display a strong preference for morphine sulfate in a sodium saccharin vehicle ranging from 0.015 to 0.24% (w/v) saccharin solution. Through this same range of morphine and saccharin concentrations, DBA mice will drink only negligible quantities of morphine and will avoid morphine solutions when provided with an alternative.

An early concern that was quickly settled was whether our morphine ingestion procedures were sufficient to induce morphine dependence. To investigate this related issue, 12 mice that had drunk a 0.75 mg/ml aqueous morphine solution over a period of days were abruptly withdrawn. Upon removal of all morphine tubes, half of the animals were also treated with levallorphan, a narcotic antagonist. Ten water control animals were treated with the same antagonist or simply put on water deprivation. All animals were observed for indices of withdrawal (defecation and urination) at 15 min and 1 h by two raters who were blind with regard to treatment condition. Additionally, the soiled plastic home cages of each of these mice were rated by two other observers by the method of paired comparisons for number and consistency of fecal bolus 24 h after treatment. Both pairs of raters clearly differentiated previous morphine animals from water controls on the basis of quantity and consistency of fecal bolus (Mann-Whitney U tests, $P < 0.01$). These findings indicate that oral ingestion of a morphine solution can lead to physical dependence in mice, consistent with reports by others from oral ingestion of morphine by rats (e.g., Khavari and Risner, 1973; Risner and Khavari, 1973; Khavari et al., 1975; McMillan, 1975).

The specific factors responsible for the high level of morphine self-administration by C57 mice, and the great difference between C57 and DBA mice, remain to be identified. Differential responsibility to taste factors may well be involved in that morphine in the concentrations used is bitter to humans while saccharin is sweet. C57s have been reported to be less sensitive to another substance (P.T.C.) that is bitter to humans than are a number of other inbred strains of mice (Klein and DeFries, 1970). However, taste factors alone are unlikely to account totally for a difference so extreme that 1 animal will ingest lethal amounts of a substance while another will even come to avoid the substance's normally preferred vehicle. Suggestions have been made concerning possible commonalities in the genetics and biochemistry of alcohol and opiate addictions (Nichols and Hsiao, 1967; Davis and Walsh, 1970; Eriksson and Kianmara, 1971; Nichols, 1972), and C57 and DBA mice are well known for their widely different alcohol preferences (Rodgers and McClearn, 1962; Rodgers, 1966; Whitney et al., 1970). The results of ethanol metabolism are different enough across these genotypes so that DBA mice will form a conditioned aversion to the taste of substances associated with ethanol injection while C57 mice will not (Horowitz and Whitney, 1975). These genotypes are also quite different in their pattern of responsiveness following opiate injections. At several time intervals after morphine sulfate injection, C57 mice were found to increase greatly their activity (heightened "running
fit") while activity of DBA mice was unaffected (Oliverio and Castellano, 1974; Collins and Whitney, submitted to this journal 1974). Similarly, C57 mice displayed a large effect of morphine on an analgesia test while DBA mice displayed no effect (Collins and Whitney, submitted to this journal 1974). These strain differences across measures were evident in both initial sensitivity and tolerance tests, strongly suggesting that opiate action is quite different in these two genotypes (Collins et al., 1976).

The potential for a clear separation between addiction-prone and addiction-resistant animals within a single species combined with a quick and simple method of inducing high morphine self-administration in addiction-prone animals could facilitate the identification of causal factors in opiate addiction.

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REFERENCES


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