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# Ethanol and Nicotine Consumption and Preference in Transgenic Mice Overexpressing the Bovine Growth Hormone Gene

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MELISKA, C. J., A. BARTKE, J. L. VANDERGRIFF AND R. A. JENSEN. *Ethanol and nicotine consumption and preference in transgenic mice overexpressing the bovine growth hormone gene.* PHARMACOL BIOCHEM BEHAV **50**(4) 563–570, 1995. – Transgenic mice overexpressing the phosphoenolpyruvate carboxykinase/bovine growth hormone (PEPCK/bGH) hybrid gene and normal (nontransgenic) littermate controls (10 males + 10 females/group) were given access to tapwater and an ascending series of concentrations of ethanol (1.0–22.0%), then a similar ascending series of concentrations of nicotine (1.0–40.0 μg/ml), in a two-bottle choice test. Male transgenic mice consumed more and exhibited greater preferences for ethanol and nicotine than control males; transgenic females consumed less and showed lower preferences for ethanol, but not nicotine, than control females. These results suggest that chronic exposure to high levels of bGH may modulate the rewarding effects of ethanol and nicotine in mice in a gender-specific fashion.

Alcohol Reward Self-administration Two-bottle choice Vulnerability Dopamine Sex differences

RECENT advances in gene transfer technologies have led to the creation of transgenic (T) mouse lines expressing a variety of "foreign" genes. We recently described the morphologic, reproductive, neuroendocrine, and aging characteristics of various lines of T mice overexpressing genes for human and bovine growth hormone (hGH and bGH) (1,5,6,38–41). Because receptors for GH are widely distributed throughout limbic and hypothalamic brain regions (21), significant effects of elevated GH levels on motivational and emotional behaviors might also be expected. However, few behavioral studies of the role of GH and GH excess have been reported.

Some lines of T mice overexpressing bGH genes display serum GH concentrations approaching 200 μg/l, or approximately 200 times the levels observed in nonT controls (37). The marked growth stimulation and increases of 50–100% in adult body weights of these T animals are associated with gender- and line-specific changes in basal content, as well as turnover, of dopamine (DA) in hypothalamic regions (39). Evidence suggests that central dopaminergic mechanisms may modulate the rewarding effects of ethanol (ETH) and other psychoactive substances (22,23,45). Furthermore, alterations in DA content and metabolism in some brain areas are associated with individual and strain differences in self-administration of ETH, opiates, and psychostimulants, in laboratory rodents (20,27,32,35).

In light of these facts, we became interested in whether T mice overexpressing the bGH gene might differ from non-T controls in self-administration of ETH. The two-bottle choice paradigm provides a convenient method for the rapid screening of ethanol preferences in rodents (2,28,34). We undertook the first experiment to characterize ETH consumption and preference in a line of T mice in which hypothalamic DA turnover was elevated in males, and reduced in females (39). On the basis of these gender-related differences in DA func-

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tion, we expected to find differences between T and non-T mice in ETH consumption and preference.

## EXPERIMENT 1: ETHANOL CONSUMPTION AND PREFERENCE IN TRANSGENIC MICE OVEREXPRESSING THE BOVINE GROWTH HORMONE GENE

### *Method*

*Animals.* The origin and maintenance of the transgenic mouse line used in these experiments has been described previously (30). Briefly, experimentally naive T and non-T mice were randomly selected from a line overexpressing the bGH gene in combination with rat phosphoenolpyruvate carboxykinase (PEPCK) promoter. The transgene is expressed in the liver, kidney, and adipose tissue of T animals (30), and thus bGH present in the circulation of these mice can be assumed to be derived from one or more of these sites. Transgene expression starts postpartum and continues throughout postnatal life. The line of T mice employed in the present study was maintained by crossing T males in each generation to C57BL/6J × $C^3H$/J (B6C3) F1 hybrid females. This mating system produces approximately equal numbers of T and non-T progeny. Thus, T and non-T mice are essentially genetically identical except for the presence or absence of the bGH gene. Pups were weaned at 21 days of age and housed with siblings of the same sex until 75 days of age, when they were separated and housed individually. Twenty T mice (10 male, 10 female) and 20 non-T littermate controls (10 male, 10 females) were assigned to the study in a 2 × 2 factorial design. Animals were approximately 110 days old at the start of testing, and were housed in individual 17.5 × 30.0 × 12.0 cm deep plastic cages in rooms with a 12 L : 12 D photoperiod and temperature of 22 ± 1°C. Mice had free access to food (Purina Formula 5008; Richmond, IN) throughout the study.

*Apparatus.* Standard wire cage tops were modified to accommodate two 250-cc water bottles, one on the left and one on the right of each cage. To minimize leakage of fluids, the standard sipper tube of each water bottle was replaced with a 16-ga, 19-mm special blunted tube (model E8B; Electrocap International, Columbus, OH), which was polished to smoothness at the tip. Mice drank readily from these tubes, and fluid leakage was typically < 1.0 g/day.

*Preparation of ethanol solutions.* Solutions were prepared by diluting 95% ethanol with tapwater to produce varying concentrations (1.0–22.0% v/v).

*Procedure.* The experimental protocol was approved by the institutional Animal Care Committee, in compliance with guidelines of the National Institutes of Health for Care and Use of Animals. After habituating mice to the two-bottle choice drinking conditions for 8 days, one bottle containing tapwater and one containing 1.0% ETH were placed one on the left and one on the right side of each mouse's home cage. Every 24 h the positions of the two bottles were reversed. Every 48 h bottles were weighed to the nearest 0.1 g, and the weight of solution consumed from each bottle was recorded. A "blank," calculated by determining the weight of solution lost due to leakage and evaporation from four identical pairs of bottles placed on empty cages, was subtracted from the weight of fluid weighed at each weighing. ETH concentrations were increased every 4–8 days to produce a logarithmically ascending series of concentrations (1.0, 2.2, 4.6, 10.0, and 22.0%). (Because tapwater was continuously available as an alternative to ETH, at no time during the experiment were mice deprived of water.)

*Dependent measures and statistical analyses.* Preliminary analyses indicated that T mice consumed substantially more total fluid (grams per kilogram of body weight per day of ETH plus water) than non-T mice ($p < 0.001$). To remove potential biases in measures of ETH consumption due to this difference, consumption was adjusted by calculating the quantity of ETH expected to be consumed under the null assumption that each mouse drank half of its fluid from the ETH bottle and half from the water bottle. Adjusted consumption (AC) was then calculated by subtracting expected consumption from the actual amount of ETH (grams per kilogram per day) consumed: AC = (actual consumption − expected consumption). A second measure, the preference ratio (PR), was defined as the ratio of ETH solution consumed (unadjusted grams per day) to total fluid consumed (ETH/ETH + Water). Both measures were analyzed with separate, 2-

TABLE 1

*F*-RATIOS (*df* = 1,36) AND *p* VALUES FOR ADJUSTED ETHANOL CONSUMPTION AND ETHANOL PREFERENCE RATIOS IN PEPCK/bGH T AND NON-T MICE

| | Males | | | | Females | | | |
|---|---|---|---|---|---|---|---|---|
| | T > Non-T? | | T Consump > 0.0? | | Non-T > T? | | Non-T Consump > 0.0? | |
| ETH (%) | *F* | *P* | *F* | *P* | *F* | *P* | *F* | *P* |
| *A. Adjusted Ethanol Consumption* | | | | | | | | |
| 2.2 | 2.89 | NS | 7.28 | 0.01 | 9.13 | 0.01 | 16.45 | 0.001 |
| 4.6 | 5.46 | 0.01 | 9.85 | 0.01 | 3.50 | 0.07 | 12.08 | 0.001 |
| 10.0 | 7.44 | 0.01 | 6.49 | 0.05 | 1.97 | NS | 2.81 | NS |
| 22.0 | 0.89 | NS | * | | 0.12 | NS | * | |
| *B. Ethanol Preference Ratios* | | | | | | | | |
| 2.2 | 2.51 | NS | 6.91 | 0.01 | 5.47 | 0.05 | 9.44 | 0.01 |
| 4.6 | 4.93 | 0.05 | 8.62 | 0.01 | 1.72 | NS | 8.41 | 0.01 |
| 10.0 | 6.96 | 0.01 | 4.10 | 0.05 | 1.42 | NS | 2.14 | NS |
| 22.0 | 2.81 | NS | † | | 0.12 | NS | † | |

The direction of effects is reversed for males and females.
*Adjusted consumption (actual − expected) < 0.0. †Preference ratio < 0.50.

between, 1-within (gender × genes × ETH concentration) analyses of variance (ANOVAs) with Geisser-Greenhouse correction for sphericity of repeated measures on the ETH concentration factor. Analyses of simple main effects were performed on significant interactions. Finally, to test whether observed PRs exceeded chance expectation, 0.50 was subtracted from individual PR values at each ETH concentration, and ANOVAs were performed to determine whether the mean remainder differed significantly from 0.00.

*Results*

Analysis of a significant gender × genes × ETH concentration interaction [$F(4, 108) = 4.36$, $p < 0.05$] showed that transgene expression produced opposite effects on ETH consumption in males and females: Male T mice ingested more ETH (adjusted grams per kilograms per day) than non-T mice when 4.6% and 10.0% ETH solutions were available (Table 1A and Fig. 1A). In contrast, female T mice consumed significantly less of the 2.2%, and marginally less of the 4.6% ETH solutions, than non-T females. Furthermore, as Table 1A indicates, T males consumed more ETH than would be expected by chance with 2.2, 4.6, and 10.0% ETH, but control males did not. In contrast, non-T females exceeded chance expectation in ETH consumption with 2.2 and 4.6% ETH, but T females did not exceed chance expectation with any concentration (all $ps > 0.05$).

By and large, preference ratios confirmed the consumption findings. Male T mice preferred 4.6 and 10.0% ETH solutions more than non-T males, whereas T females preferred 2.2% ETH less than non-T females (Table 1B and Fig. 1B). The preference for ETH of male T mice exceeded chance expectation (PR = 0.50) with the 2.2, 4.6, and 10.0% ETH solutions, whereas preferences of non-T males did not; in contrast, ETH preference of non-T females exceeded chance expectation when 2.2 and 4.6% ETH solutions were available, whereas T females' ETH preferences did not exceed chance expectation at any concentration. Thus, male T mice consumed more and preferred to drink moderate concentrations of ETH more than non-T mice, whereas female T mice consumed less and preferred to drink ETH solutions less than non-T control females.

*Discussion*

A number of studies report reliable differences in consumption of and preference for ETH among various inbred rodent strains (2,19,34). The present study is the first to characterize the effects of overexpression of GH genes on ETH consumption. We observed that chronic GH elevation is associated with gender-specific effects on ETH ingestion: elevated ETH consumption and preference by T males, and reduced ETH consumption and preference by T females.

Differences between rodent strains in substance ingestion may reflect both "preingestional" (peripheral/taste) as well as "postingestional" (central/pharmacologic) factors (19,33). For example, enhancing taste by sweetening solutions with saccharin increases ETH consumption in ethanol-preferring C57BL/6 mice; but ethanol-avoiding DBA/2 mice, which prefer saccharin solutions as much or more than C57 mice, avoid even sweetened ETH solutions (2), which suggests that postingestional effects of ETH are aversive to DBAs. Similarly, whereas C57 mice consume solutions of morphine plus quinine in preference to comparably bitter solutions of quinine alone, DBAs show no such preference for morphine plus quinine (14). This suggests that postingestional/pharmacologic effects modulate morphine-quinine consumption in C57 mice, but not in DBAs. Thus, although peripheral/taste factors may influence oral ingestion of ETH and other drugs, postingestional factors may account for at least some of the differences between strains in ETH and drug consumption. The relative importance of preingestional and postingestional effects in producing the observed differences between T and non-T mice in ETH consumption and preference remains to be determined.

A tentative interpretation of the present results is that chronic exposure to high circulating bGH concentrations makes ETH more rewarding for T males but less rewarding for T females, suggesting that gonadal hormones may modulate the rewarding effects ETH in T mice. Although the mechanisms of such effects is unclear, gonadal hormones have been shown to influence ethanol metabolism and elimination in humans (42). Estrogen may also increase DA metabolism (29), as well as DA uptake site density, and thus could modulate the rewarding effects of drugs acting at DA receptors (31). However, previous research examining gender effects on ETH consumption in laboratory rodents has produced contradictory findings; although some studies report greater oral ETH consumption by females than males (3,24), greater ETH consumption by males has also been found (44).

High-ethanol-preferring rodent strains often display increased self-administration of psychoactive substances other than ethanol (4,17,18,43). To the extent that bGH gene overexpression produces a generalized modification in drug-induced reward in T mice, consumption of other drugs of abuse could also be affected in a gender-specific fashion. Experiment 2 was designed to determine whether T mice also differed from controls in oral consumption of nicotine (NIC) solutions.

## EXPERIMENT 2: NICOTINE CONSUMPTION AND PREFERENCE IN TRANSGENIC MICE OVEREXPRESSING THE BOVINE GROWTH HORMONE GENE

Among humans, a high level of alcohol use tends to be associated with a high level of tobacco use (15,26). Research (8) indicates that a high-ethanol-consuming mouse strain, C57BL/6, also shows elevated oral consumption of NIC solutions. Experiment 2 was designed to test whether gene- and gender-related differences such as those observed with ETH in Experiment 1 would also occur with NIC in T mice. It was anticipated that mice that consumed more and showed greater preference for ETH in Experiment 1 would consume more and show greater preference for NIC in the two-bottle choice situation. Thus, a positive correlation between measures of ETH and NIC consumption was expected.

*Method*

*Subjects.* The same mice used in Experiment 1 served as subjects in Experiment 2, with the exception that three transgenic females and two transgenic males died before the completion of the experiment. Animals were approximately 200 days old at the start of testing, and were housed and maintained under the same conditions described previously.

*Preparation of nicotine solutions.* Solutions were prepared by diluting L-nicotine hemisulfate (400 μg/ml; Sigma Chemical Co., St Louis, MO) to varying concentrations (1.0 μg/ml L-nicotine base to 40.0 μg/ml).

*Procedure.* Four weeks after completion of the ETH preference studies, mice were presented with tapwater paired with an ascending series of NIC concentrations in tapwater (1.0,

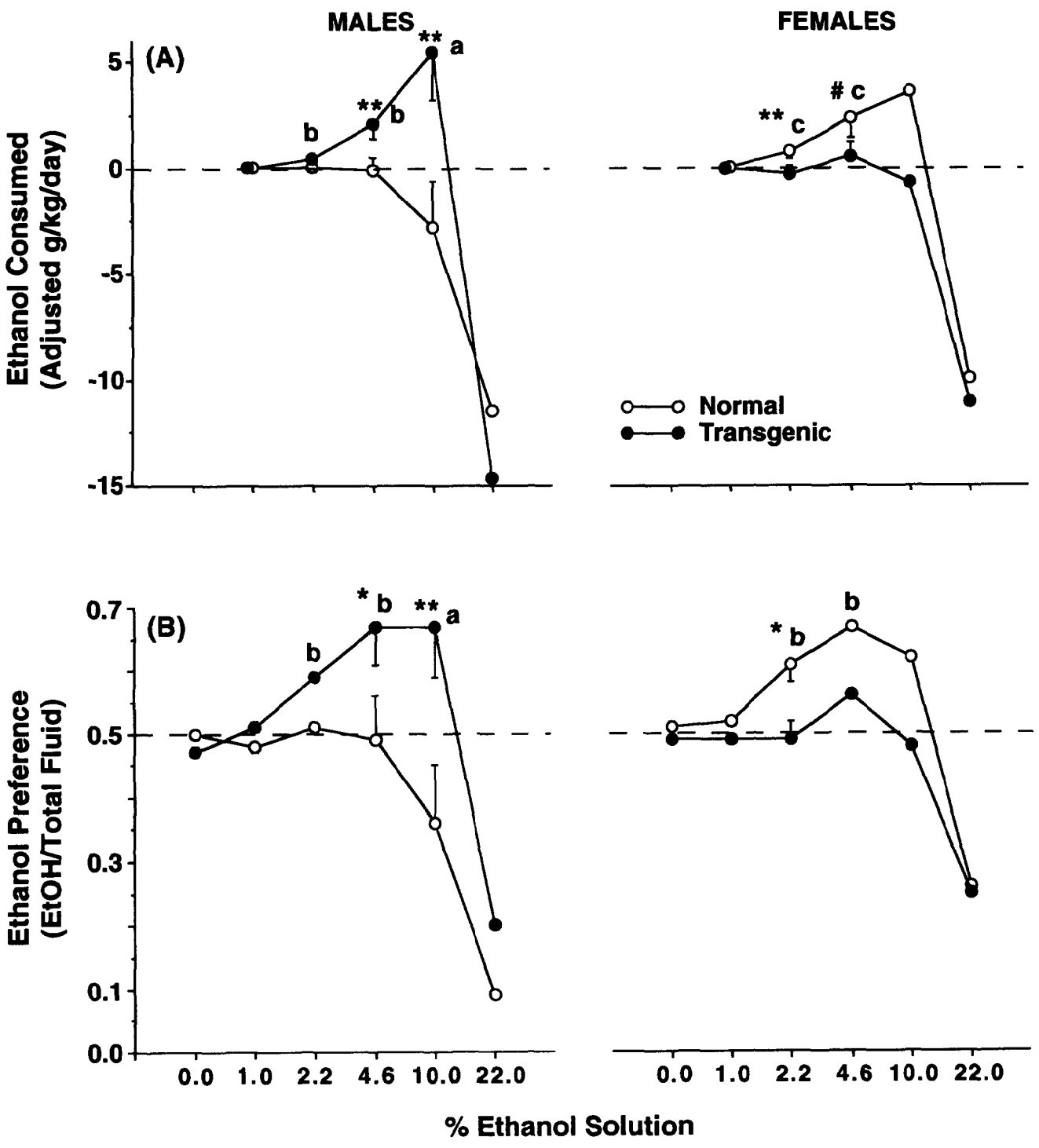


FIG. 1. (A) Adjusted ethanol consumption, and (B) ethanol preference ratios in normal (non-transgenic) and PEPCK-bGH transgenic mice in a two-bottle choice test. Vertical bars represent standard errors. Symbols denote significance of differences between transgenic and non-transgenic mice in adjusted ethanol consumption, and preference ratios: # = $p < 0.07$; *$p < 0.05$; **$p < 0.01$. Lower-case letters identify adjusted consumption means and preference ratios that exceeded chance expectation (0.00 and 0.50, respectively): a = $p < 0.05$; b = $p < 0.01$; c = $p < 0.001$.

TABLE 2

*F*-RATIOS (df = 1,31) AND *p* VALUES FOR ADJUSTED NICOTINE CONSUMPTION AND NICOTINE PREFERENCE RATIOS IN PEPCK/bGH T AND NON-T MICE

| | Males | | | | Females | | | |
|---|---|---|---|---|---|---|---|---|
| | T > Non-T? | | T Consump > 0.0? | | Non-T > ? | | Non-T Consump > 0.0? | |
| NIC (μg/ml) | *F* | *P* | *F* | *P* | *F* | *P* | *F* | *P* |
| *A. Adjusted Nicotine Consumption* | | | | | | | | |
| 10.0 | 6.65 | 0.01 | 5.83 | 0.05 | 0.01 | NS | 1.47 | NS |
| 16.0 | 1.49 | NS | 2.78 | NS | 1.34 | NS | 0.36 | NS |
| 25.0 | 11.21 | 0.01 | 8.77 | 0.01 | 1.67 | NS | * | |
| 40.0 | 0.02 | NS | * | | 1.25 | NS | * | |
| *B. Nicotine Preference Ratios* | | | | | | | | |
| 10.0 | 7.23 | 0.01 | 4.43 | 0.05 | 0.15 | NS | 1.68 | NS |
| 16.0 | 2.01 | NS | 0.18 | NS | 0.60 | NS | 0.67 | NS |
| 25.0 | 22.30 | 0.001 | 7.70 | 0.01 | 1.26 | NS | † | |
| 40.0 | 2.56 | NS | † | | 0.81 | NS | † | |

The direction of effects is reversed for males and females.
*Adjusted consumption (actual − expected) < 0.0. †Preference ratio < 0.50.

2.5, 4.0, 6.3, 10.0, 16.0, 25.0, and 40.0 μg/ml). As before, each concentration was presented for 4–6 days. Bottle positions (left vs. right) were alternated every 24 h, and bottles were weighed to the nearest 0.1 g every 48 h. Daily weight reduction from each bottle, corrected for the corresponding reduction in the weight of control bottles, was taken as a measure of consumption of that solution.

## *Results*

Consumption of NIC was analyzed as described earlier for ETH. For clarity of presentation, data on concentrations below 4.0 μg/ml were excluded, because effects of these lower NIC concentrations on consumption were negligible. Analysis of a significant gender × genes × NIC concentration interaction [$F(5, 155) = 7.32$, $p < 0.01$] showed that transgene expression produced different effects in males and females. T males consumed more NIC (adjusted milligrams per kilograms per day) than non-T control males when 10.0 and 25.0 μg/ml NIC solutions were available (Table 2A and Fig. 2A). Consumption of NIC by T males also exceeded chance expectation, with concentrations of 10.0 and 25.0 μg/ml NIC. In contrast, although non-T females consumed somewhat more NIC than T females, these differences were nonsignificant, and neither group of females exceeded chance expectation in NIC consumption with any of the concentrations tested (all *p*s > 0.05).

The preference ratio data essentially confirmed the consumption findings. Overall, T males preferred NIC more than non-T males [$F(1, 31) = 4.55$, $p < 0.05$]. Differences between T and non-T mice were most prominent with 10.0 and 25.0 μg/ml NIC solutions (Table 2B). The NIC preferences of T males also exceeded chance expectation (PR = 0.50) when mice had access to 10.0 and 25.0 μg/ml NIC solutions. As with consumption, female T and non-T mice did not differ significantly from each other, nor did their preference ratios exceed chance expectation at any NIC concentration presented (all *p*s > 0.05).

To assess the degree to which ETH and NIC self-administration covaried, partial correlation coefficients, controlling for gender and genetic background of experimental subjects, were determined for ETH and NIC consumption and preferences. After averaging across the concentrations, which produced the maximum differences between T and non-T mice (4.6 and 10.0% ETH, and 10.0, 16.0, and 25.0 μg/ml NIC), mean ETH consumption (adjusted grams per kilogram per day) was positively correlated with mean NIC consumption (adjusted milligrams per kilogram per day) [$r(31) = 0.402$, $p < 0.01$]. The correlation between ETH and NIC preference ratios was also positive [$r(31) = 0.436$, $p < 0.01$]. Thus, mice that consumed and preferred more ETH also tended to consume more and show greater preferences for NIC, when gender and line differences were controlled for statistically.

## *Discussion*

Although NIC self-administration via the intraperitoneal route has been demonstrated in rats (10), robust oral consumption of NIC by laboratory rodents is not widely reported. Oral self-administration has been achieved using schedule-induced polydipsia procedures, which produced NIC consumptions of approximately 4 mg/kg in rats, and subsequent behavioral stimulation lasting for at least 180 min (25). In a two-bottle choice study, a modest preference for oral consumption of a dilute NIC solution (1.0 μg/ml) was found to develop gradually in male Sprague–Dawley rats, after weeks of exposure (13). More recently, mice from the C57BL/6 strain, an inbred strain known to self-administer ETH readily (2,28), were reported to drink in excess of 12 mg/kg per day of NIC when presented with solutions of 100 μg/ml or more in a two-bottle choice paradigm (8). In that study, the C57BL/6 mice also showed greater preferences for NIC solutions than mice from the A/J strain. These findings were interpreted as evidence of strain differences in sensitivity to the rewarding effects of NIC (8). Results of the present study suggest that male bGH T mice may also experience greater reinforcement from NIC ingestion than non-T controls. A plausible alterna-

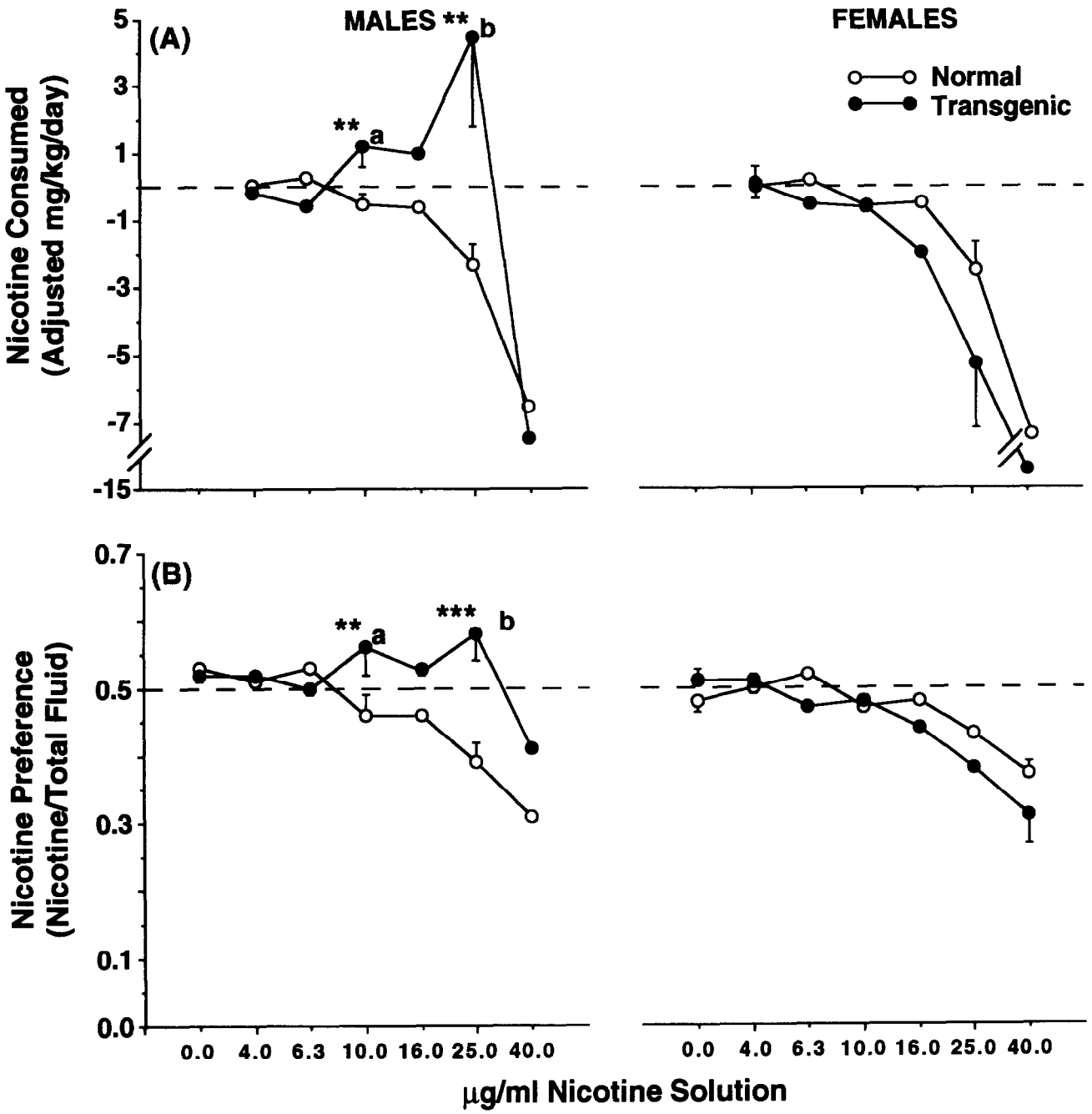


FIG. 2. (A) Adjusted nicotine consumption, and (B) nicotine preference ratios in normal (nontransgenic) and PEPCK-bGH transgenic mice in a two-bottle choice test. Vertical bars represent standard errors. Symbols denote significance of differences between transgenic and nontransgenic mice in adjusted nicotine consumption, and preference ratios: *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$. Lower-case letters identify adjusted consumption means and preference ratios that exceeded chance expectation (0.00 and 0.50, respectively): a = $p < 0.05$; b = $p < 0.01$.

tive interpretation is that increased NIC consumption reflects reduced aversive effects of NIC, or a combination of reduced aversion and increased reward. A caution to observe in making this interpretation is that prior experience with ETH in Experiment 1 could have modified the animals' responses to NIC in Experiment 2, as successive administrations of ETH and NIC could have produced an unanticipated interaction effect.

## GENERAL DISCUSSION

Rodent strains that ingest larger quantities of ETH also tend to self-administer larger quantities of opiates and psychomotor stimulants than strains that consume less ETH (4,18,36). This suggests that a common mechanism may un-

derlie reinforcement produced by ETH and other drugs (7,19). Results of the present experiments indicate that chronic bGH gene overexpression modifies consumption of both ETH and NIC, particularly in male mice. Dopaminergic systems have been implicated in the rewarding effects of both ETH and NIC (11,16), and individual differences in the rewarding effects of ETH and other abused substances may be linked to elevations in DA turnover in mesolimbic reward pathways (20,35). Interestingly, the gender-specific differences in ETH and NIC consumption reported here parallel differences in hypothalamic DA turnover noted previously in some bGH T lines that is, high-ethanol/high-nicotine-consuming T males displayed increased hypothalamic DA turnover relative to controls, whereas low-ETH-consuming T females showed reduced DA turnover (39). Studies to determine whether parallel differences in DA turnover occur in reward-relevant brain regions (e.g., nucleus accumbens) are currently under way in this laboratory.

As with other two-bottle choice tests, the results of the present study are open to alternative interpretations. For example, the role of taste/peripheral factors in mediating differences between T and non-T mice in ETH and NIC consumption remains unresolved. Relative to controls, T mice may tolerate unusual flavors more readily, or even prefer flavored water to tapwater. Systematic dose-response comparisons using salty, sweet, sour, and bitter substances could establish whether T and non-T mice differ in acceptance of, or preference for, common tastants. If T mice and controls display similar consumption profiles with substances known to have minimal pharmacologic impact (e.g., nonnutritive sweeteners, quinine), this would support the interpretation that postingestional/pharmacologic effects mediate differences in ETH and NIC consumption. In addition, it would be important to determine whether oral self-administration actually produces differences between T and non-T mice in plasma ETH and NIC concentrations, because pharmacokinetic differences between lines could alter distribution, absorption, and clearance of these substances. Furthermore, because cross tolerance to some behavioral effects of NIC and ETH has been noted (9; see below), pharmacokinetic factors may be particularly important when repeated tests with different substances are conducted with the same animals, as in the present study.

Noting the high association between alcohol and tobacco abuse, Collins (7) proposed that common (possibly genetic) mechanisms may mediate reinforcement from both substances. Our findings that male T mice consumed more ETH and NIC than controls, along with the positive correlations observed between ETH and NIC consumption and preferences, support that interpretation. Further support for the existence of a common mechanism derives from work showing that sensitivity to ETH in mice selectively bred for differential response to barbiturates, the long-sleep and short-sleep lines, is moderately to highly correlated with sensitivity to NIC in a number of behavioral tests (12). Furthermore, long-sleep mice made tolerant by exposure to high doses of NIC exhibit cross tolerance to some of the behavioral effects of ETH (9).

Transgenic mice overexpressing GH genes may be useful in studies of the neurobiologic bases of susceptibility to self-administration of psychoactive substances. That both ETH and NIC consumption were enhanced in male bGH T mice, a concordance of drug preferences noted previously in human alcoholics and tobacco abusers (7,26), suggests that this transgenic line might represent a valid model for studies of vulnerability to substance abuse in humans.

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