AN EXPERIMENTAL APPROACH TO UNDERSTANDING THE GENETIC AND NEUROBIOLOGICAL BASIS OF ALCOHOLISM* **

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INTRODUCTION

There is now a mounting strength of evidence that genetic/biological factors influence not only individual differences in susceptibility to abusive and alcoholic drinking, but also in patterns of social drinking. Alcohol ingestion or self-administration is a metric trait. More likely than not, multiple genes and mechanisms contribute to its expression, as do a number of environmental factors.

As reviewed recently by Devor and Cloninger (1), twin, adoption and family studies have concluded that there is genetic predisposition to alcoholism (alcohol dependence). Genetic heterogeneity is discernible from age of onset, patterns of aberrant drinking and alcohol-related problems, personality characteristics, and pattern of inheritance. A male-limited subtype (Type II) of alcoholism appeared to be particularly heritable. Recently published twin studies (2, 3) comparing monozygotic and dizygotic same-sex twin pairs for concordance of alcohol abuse and/or dependence suggest that heritability of liability to alcoholism may be as high as 50%, the remainder being shared and nonshared environmental influences.

Since alcohol abuse and alcoholism are abnormal patterns of alcohol-seeking behavior, it is relevant to know the extent to which normal or socially acceptable drinking practices might be genetically influenced. In other words, is alcohol-drinking/seeking behavior itself influenced by genes or is genetic influence limited to susceptibility for abuse potential and dependence? Until recently, the most convincing data in support of a genetic influence on drinking behavior itself have come from animal studies in experimental animals through selective breeding for high and low voluntary alcohol intake or preference (vide infra). Publications have now appeared indicating that drinking behavior in humans is also influenced by genetic factors. An analysis of the inheritance of alcohol consumption patterns in a general population twin sample by model

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fitting concluded that abstinence is strongly influenced by shared environmental effects but not much by genetic effects in Caucasian populations (4, 5). On the other hand, consumption in drinkers is determined by quantity and frequency dimensions, both of which are moderately influenced by genetic effects, as well as by an abstinence dimension. Heritability estimates for frequency ranged between 42 and 75% and those for quantity ranged between 24 and 57%, depending upon the assumptions of the model. In a less complicated analysis of a different data set on male twins (6), significant genetic variances for quantity, frequency and density of drinking were also found, and heritability estimates were in the range of 0.35–0.40.

Prevalence of Alcohol Abuse and Alcoholism and Etiological Domains

In the U.S., about 35% of the population are abstainers, 55% drink and experience no problems, 4% can be classified as alcohol abusers (drinking leading to social and medical problems, but no dependence), and 6% have alcohol dependence (alcoholism). Questions fundamental to our understanding of the etiology of alcohol abuse and alcoholism are, therefore:

Why do people drink?
Why do some drink more than others?
And, why do some drink despite negative consequences?

Answers to these questions have been sought through the study of factors that influence exposure, personality, the metabolism of alcohol, and the pharmacological effects of alcohol. In general terms, we know that why and when people begin drinking are strongly influenced by social and cultural factors such as family traditions, peer pressure, and stage of psychosocial development (adolescence). Personality dimensions such as sensation seeking may be important factors as well. Why some drink more than others may relate to differences in sociocultural norms and personality, to individual differences in ethanol metabolism, and to individual differences in response to the psychoactive effects of ethanol. Ethanol's action is biphasic; it is usually behaviorally reinforcing at low doses but becomes aversive at high doses. Finally, why some people drink despite negative consequences may have its roots in key neuroadaptive responses to chronic exposure, such as tolerance, and psychological and physical dependence.

In addition to alcohol drinking behavior, a number of other immediate and long-term responses to ethanol ingestion exhibit a wide range of interindividual variation that is, in part, genetically determined. In humans, these include alcohol elimination rate, the innate sensitivity of

the brain to alcohol as measured by the electroencephalogram, alcohol-induced aversive reactions and, possibly, susceptibility to alcohol liver disease and alcohol dementia. In experimental animals, genetic factors contribute to the variances observed in alcohol metabolic rate, alcohol-induced stimulation and sedation, acute and chronic tolerance development, and susceptibility to physical dependence as measured by ethanol withdrawal reactions.

These and other observations have suggested specific hypotheses on mechanisms that promote or deter drinking, now amenable to testing in experimental animals and humans. These are:

1. The aversive effects of high concentrations of ethanol (and/or acetaldehyde) deter heavy drinking.
2. The reinforcing features of low-to-moderate doses of ethanol (euphorigenic, anxiolytic, antiwithdrawal) encourage increased frequency of use.
3. Tolerance developed to the aversive effects of ethanol/acetaldehyde promote increased quantity of drinking.
4. Genetic differences among individuals in these responses to ethanol contribute to differences in susceptibility to alcohol abuse and dependence.

Biochemical genetic studies of the enzymes of alcohol metabolism have substantiated the first hypothesis in humans. It has been found that certain genetic variant forms of alcohol and aldehyde dehydrogenase are responsible for an aversive reaction to alcohol, the alcohol-flush reaction, and the inheritance of these genes is protective against heavy drinking and alcoholism (7, 8, 9). The mediator of the flush reaction is elevated acetaldehyde levels in blood and tissues (10), arising from decreased acetaldehyde removal and/or accelerated acetaldehyde production in the course of ethanol oxidation (11). The testing of hypotheses two and three is difficult to perform in humans and, as described below, experimental animals have been employed to address these research questions. Longitudinal studies in humans testing the fourth hypothesis building upon what we have learned in experimental animals are currently underway.

Development of Genetic Animal Models for Studying Abnormal Alcohol-Seeking Behavior and Alcoholism

Until recently, there has been doubt whether studying alcohol consummatory behavior in subhuman primates and lower animal species would make a major contribution to our understanding of the human condition alcoholism (12). The principal reason for this skepticism is that voluntary oral consumption of alcohol-containing solutions by common stock ani-

mals rarely results in pharmacologically meaningful blood ethanol concentrations unless experimental manipulations such as weight reduction, schedule-induced polydipsia and secondary conditioning procedures are undertaken. However, it has long been known that experimental animals such as rodents exhibit a wide range of ethanol-drinking preference (13), and this behavior is genetically influenced (14). By use of selective breeding, several high and low alcohol-drinking rat lines have been raised. These are the University of Chile UChA/UChB lines (15), the Alko (Finland) AA/ANA lines, (16), the Indiana University P/NP lines and the HAD/LAD lines, (17, 18), and the Sardinian sP and sNP lines (19).

All these lines were developed by assessing alcohol preference with a two-bottle choice test (20). In this procedure, the rats are housed individually after the onset of puberty and are given an aqueous 10% (v/v) ethanol solution as the sole source of fluid for the first four days. Thereafter, the animals are given free access to the 10% ethanol solution and to water for three weeks. The locations of these fluids are changed on a random basis daily after the volumes of ethanol solution and water consumed have been recorded. Food is provided *ad libitum*. Male and female rats with high alcohol preference are then mated to start the high preference lines and male and female rats with low alcohol preference are mated to start the nonpreferring lines (17).

Selection for mating in subsequent generations is performed in the same manner. At Indiana University, the P (preferring) and NP (nonpreferring) lines developed from a Wistar stock are now in the 35th generation. The voluntary alcohol intakes (g ethanol/kg body weight/day, mean ± SD) of the animals are: P male, 5.7 ± 0.16; P female 6.6 ± 0.19; NP male 0.5 ± 0.08 and NP female 0.4 ± 0.08. The HAD and LAD lines were developed later from a genetically more heterogeneous foundation stock of rats, the N/Nih rat (21). As with the P and NP lines, divergence of ethanol intake emerged quickly after a few generations and continued to diverge at a slower pace thereafter. This pattern suggests the involvement of a few major genes influencing drinking behavior and several or many minor genes. The HAD and LAD lines are now in the 14th selected generation and their drinking scores are: HAD males, 5.64 ± 0.34; HAD females 4.53 ± 0.45; LAD males 0.33 ± 0.06; LAD females 0.69 ± 0.12. The realized heritability estimate for divergence in the drinking scores has been in the vicinity of 0.3–0.4.

The principal objective for developing the P line of rats was to test the hypothesis that animals selectively bred for alcohol preference can satisfy all the perceived criteria for an animal model of alcoholism (12). This goal has been accomplished to the satisfaction of the alcohol-research community and the animals are now being used by investigators throughout the country. To summarize, the P rats:

1. voluntarily consume 5–8 g ethanol/kg body weight/day, and attain blood alcohol concentrations (BACs) of 50–200 mg% with free-choice drinking (22, 23).
2. work by bar-pressing to obtain the ethanol orally when food and water are freely available, demonstrating that ethanol is behaviorally reinforcing (24). In fact, although the P rats were selected using 10% ethanol, concentrations of ethanol as high as 35–40% are as reinforcing (same amount of ethanol consumed/24 h) as 10% ethanol (25).
3. consume ethanol for its pharmacological effects and not because of its taste, smell or caloric properties. It has been shown that the P and NP rats react to the taste and smell of ethanol similarly (26) and that P rats will self-administer ethanol intragastrically (27, 28). They voluntarily drink the same or greater amounts of ethanol even in the presence of other highly palatable fluids and caloric sources (29). Finally, recent studies have shown that the P rats will self-administer, through operant responding, nanoliter quantities of ethanol in concentrations of 50–200 mg % directly into the ventral tegmental area of brain (30).
4. develop with chronic free-choice drinking metabolic (23) as well as physiological tolerance toward the motor-impairing effects of ethanol (31).
5. develop physical dependence with chronic free-choice drinking (32).

Differences in Responses to Ethanol Between the Alcohol-Preferring and Alcohol-Nonpreferring Lines

Several discovered line differences between P and NP rats and between HAD and LAD rats may have importance in understanding the mechanisms underlying ethanol preference, and serve to substantiate hypotheses two and three stated above. At this time, more data are available for P vs. NP than for HAD vs. LAD comparisons:

1. Ethanol solutions in moderate to high concentrations (10–40%) are able to maintain reinforced responding in P rats, but not in NP rats (25). Ethanol is clearly rewarding to the P rats, but not to the NP rats.
2. P and HAD rats exhibit increased spontaneous locomotor activity with the administration of low-to-moderate doses of ethanol, but NP and LAD rats do not (33, 34). This response has been interpreted by some investigators as a manifestation of the euphorigenic (activating) effect of ethanol.
3. P rats are less sensitive to the aversive and sedative/hypnotic effects of ethanol (high dose) than are NP rats (35, 36).

4. Acute tolerance developed with exposure to a single large dose of ethanol is more robust and persists 3–4 times longer in P than in NP rats (37, 38).
5. Tolerance to the aversive effects of alcohol develops with 14 days of chronic free-choice alcohol intake. Concomitantly, voluntary alcohol intake increases 50% (39).

Heretofore, initial sensitivity and acute (within-session) tolerance are by far the most generalizable and robust responses to ethanol found in association with ethanol preference in rodents. Differences in either or both of these responses have been described for the alcohol-preferring C57BL and alcohol-nonpreferring DBA mouse strains (40), for the HS/Ibg heterogeneous stock mice with high and low ethanol preference (41), for the inbred rat lines used to constitute the N/Nih heterogeneous stock rat (42), and for the selectively bred AA and ANA rat lines (43).

There are a limited number of studies to date indicating that the alcohol preferring and nonpreferring rats differ in behaviors that are unrelated to alcohol. The P rats exhibit higher spontaneous motor activity than NP rats in a novel environment, but there is no line difference after habituation (22). HAD rats also exhibit higher spontaneous motor activity than LAD rats (34). P rats appear more anxious and/or emotional than NP rats in a variety of test measures (44, 45) and the P rats exhibit a preference for oral consumption of highly palatable, nondrug solutions (e.g. sucrose, saccharin) compared with NP rats (46).

Neurobiological Differences

Neurochemical and neuroanatomical studies conducted on the P/NP and HAD/LAD rats have implicated the serotonin (5HT), dopamine (DA) and gamma-aminobutyric acid (GABA) systems in controlling alcohol-seeking behavior. The 5HT systems are known to affect mood, consummatory behaviors and the development of tolerance to alcohol. 5HT recently has been shown to modulate the release of DA, particularly through the 5HT-3 receptor. DA systems play a major role in locomotor activity, drug reinforcement and reward. GABA is the major inhibitory neurotransmitter in the brain and may interact with DA and other neurotransmitter systems in alcohol reinforcement. Based on neuropharmacologic and brain stimulation reward studies, 5HT, DA, GABA as well as opioids are implicated in the circuitries that connect the brain reward pathway, i.e., the raphe nuclei, ventral tegmental area (VTA), lateral hypothalamus, olfactory tubercle, nucleus accumbens (Acc), the medial prefrontal cortex and other limbic areas.

One of the most consistent neurochemical and neuroanatomical findings observed in the P/NP and HAD/LAD rats is a deficiency of 5HT in

the alcohol-preferring lines (47). Compared with NP and LAD rats, the contents of 5HT and 5-hydroxyindoleacetic acid (5-HIAA) are 12–26% lower for the P and HAD rats in several brain regions of the P and HAD rats, including the frontal cortex, hippocampus, corpus striatum, thalamus, hypothalamus, pons-medulla and the nucleus accumbens. An association of a deficiency of the brain 5HT system with alcohol preference has been reported also in other animals that prefer alcohol, e.g., the inbred C57BL mice (48) and the inbred Fawn-Hooded rats (49). While lower contents of 5HT and 5-HIAA can be caused by decreased synthesis, lowered functional activity and/or decreased 5HT innervation, the abnormality in the P rats appears to result from decreased number of 5HT-containing fibers, e.g., in the anterior frontal cortex, nucleus accumbens, and portions of the ventral hippocampus (50), and a decrease in number of 5HT immunostained neurons in the dorsal and raphe nucleus of the P rats as compared with NP rats (51). As a result of the decreased 5HT innervation, there is an up-regulation of the number of $5HT_{1A}$ receptors as seen in the frontal cortex and hippocampus (52, 53).

An abnormality in the VTA-Acc dopamine system has also been found in association with high alcohol preference. A 10–30% decrease in DA and its metabolites in the Acc and anterior striatum has been reported in the P and HAD lines, as compared with the NP and LAD lines (54, 55). Low doses of ethanol stimulate the release of DA in the Acc and the P rats are more sensitive than are Wistar rats in this regard. Since the 5HT system plays a role in regulating the DA system in the brain reward pathway, and no neuroanatomical differences are seen in the VTA of P and NP rats, we postulate that the lowered 5HT innervation alters the DA functioning of the VTA projecting to the other limbic region in the P rats. Ethanol driving the hypersensitive, hypofunctioning VTA DA system toward normal becomes the mechanism of ethanol reward in the P rats (56).

In addition to abnormalities in the 5HT and DA neurotransmitter systems summarized above, other studies in the P/NP and HAD/LAD lines have demonstrated a higher density of GABAergic terminals in the Acc of the alcohol-preferring lines (57). How this neuroanatomical finding relates to the tension-reducing or anxiolytic property of ethanol is unclear. However, P rats are more "anxious" and more sensitive to stress-producing stimuli (45). Other studies have suggested that several peptidergic systems, e.g., corticotropin releasing factor (58) and enkephalins, may also be related to alcohol-drinking in the P rats. Importantly, P and NP rats differ in basal as well as ethanol-stimulated enkephalinergic activity in mesolimbic regions of the brain (59).

The functional significance of the 5HT, DA, GABA and enkephalin differences between the alcohol-preferring and -nonpreferring lines have

been demonstrated in a number of neuropharmacological studies. A variety of agents that block the uptake and metabolism or the release of 5HT and DA decrease voluntary ethanol consumption in the P rats, as does also the GABA inverse agonist Ro 15-4513, and the opioid antagonist naltrexone (60, 61).

Relevance of the Animal Model Studies to Human Alcoholism

The finding that alcohol-preferring animals are less sensitive and/or more tolerant to intoxicating and aversive effects of ethanol may have an intriguing counterpart in humans. Schuckit and coworkers have been studying the reaction to alcohol of sons of alcoholic fathers (family history positive, FHP) and comparing them with sons of nonalcoholic fathers (family history negative, FHN) as controls. By a variety of measures, the FHP group is less sensitive to an intoxicating dose of alcohol than the FHN group (62). In long term followup, preliminary studies indicate that this insensitivity is predictive of future alcoholism (63). The neuropharmacological studies in the P rats also have encouraging analogies in humans. Serotonin reuptake inhibitors such as fluoxetine and citalopram decrease alcohol consumption, but the effect is short-lived, lasting only 1–2 weeks (64, 65). Clinical trials with the opioid antagonist have yielded more promising results. In two double-blind placebo-controlled trials, naltrexone, 50 mg/d, given as an adjunct to standard treatment following alcohol detoxification, reduced relapse rate, increased abstention rate, decreased number of drinking days and amounts consumed per session. Patients on naltrexone experienced less craving and less desire to drink heavily (66, 67). The continued study in experimental animals and humans of more specific pharmacotherapeutic agents to lessen the craving for alcohol that is central to the problem of relapse is clearly indicated. The convergence of these kinds of findings in humans and experimental animal models is heartening that rational and effective treatment and prevention measures (e.g., through early identification) can be found in the not-too-distant future.

SUMMARY

The development and characterization of an animal model to study mechanisms underlying abnormal alcohol-seeking behavior have been described. Raised by genetic means, it demonstrates the importance of genetic factors in this behavior. It has allowed the elucidation of neural pathways and neurotransmitter systems that subserve alcohol-seeking behavior. It offers the potential for screening new medications for the treatment of alcoholism, based upon these kinds of newly discovered knowledge.

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DISCUSSION

Fisher, Gainesville: It is my recollection that in animal models and also humans manifesting aggressive and violent behavior, one finds a decrease in brain serotonin reactivity. Does this overlap with the alcohol phenomenon you are observing?

Li: Yes, this certainly does. The alcohol preferring rats are considerably more aggressive than the non-preferring rats and, also, are hyperactive in terms of their spontaneous motor activity. People have said that this might be a model of the so-called type II alcoholic that you just alluded to.

Schrier, Colorado: Thanks for your exciting work, T.-K. In alcoholic patients, about 50% of alcoholic patients have hypertension and when they stop drinking, the hypertension goes away. Could you hypothesize whether any of the neuropathways that you've seen in your animals as far as the addiction or the alcohol-seeking behavior goes, may mediate alcohol-induced hypertension? Have you measured blood pressure in these animals? Lastly, there are data that alcohol will up-regulate calcium channels in the brain and also in vascular smooth muscle. Has anyone examined the effect of calcium-channel blockers on the alcohol-seeking behavior?

Li: We have not measured blood pressures on these animals. The effect of alcohol on blood pressure, I think, is a long term effect and, of course, there may be genetic susceptibilities to it. There is an interesting relationship between drinking behavior in these rats and the renin angiotensin system. The P rats have low basal renin activity and ACE inhibitors lower voluntary ethanol drinking. The precise mechanisms underlying these relationships have not been explored. The effect of alcohol on calcium channels is really very interesting in that it is one of the primary sites of alcohol action. Ethanol inhibits the NMDA-activated calcium channel and calcium-channel blockers have been tried in treatment of alcohol withdrawal. No relationship to alcohol preference is known.

Schrier: So you don't know if these animals get hypertensive or not?

Li: We have not looked at that specifically because that has not been one of our primary goals.

McCarty, Milwaukee: Fascinating story. One wag in our audience wondered aloud whether these rats also prefer cigarettes in addition to the alcohol. It wasn't quite clear to me how similar genetically the two groups are, the group that prefers alcohol and the group that doesn't.

Reply: As far as smoking cigarettes is concerned, we are looking to see if they will also show seeking or preferring behaviors for other drugs of abuse and this is a study currently in progress. How similar or different the lines are is an issue that pertains to all animal studies because animals, especially laboratory animals, are not genetically heterogeneous like humans. That was one reason why we started the second selection. The first one is an outbred Wistar, which is an outbred animal of uncharacterized genetic background. The second selection is from the N/Nih rat, which has been developed by crossing eight inbred strains, so we know what the genetic background is. Hopefully, that will give us more

information. As far as trying to find out how many genes are involved, we know that there is no single major gene effect, but there may be three or four major ones with several other minor ones.

Schenker, San Antonio: Beautiful paper, T.-K. It has been suggested that children of alcoholics who are prone to develop the disorder may manifest certain abnormal evoked potentials and may be identifiable by that. Have you tried to see whether an electrophysiologic recording from your animals in the preferred and non-preferred groups might show similar types of characteristics?

Li: Cindy Ehlers has done so and they are different. The animals can be characterized by their evoked potential response as well. How this relates to their drinking behavior, obviously, we don't yet know. We do know the response of the EEG, as well as the evoked potential, are quite different, as you would expect from just looking at their behavior because the non-preferring animals at a relatively low dose will be falling asleep, whereas the preferring animals are excited by the alcohol. I ought to point out, Steve, that in looking at the children of alcoholics and their responses to alcohol, this is work done by Mark Schuckit, he has now done a 10 year follow-up on subjects he had tested before, and by looking at their reaction to alcohol, he can predict future alcoholism. There is a very interesting parallel that is now coming out between human studies and animal studies.

Abboud, Iowa City: Very interesting work. How early in the development of these rats do you find the defect in the serotonergic pathways? Is it present when they are newly born, or before that, or does it develop afterward?

Li: It is developmental. We currently are studying this. The serotonin system in the rat brain develops in the first two weeks after birth and during this period, you can track their behavior looking at differences in activity. In terms of what we have done, we see a difference in behavior, in spontaneous activity. We have also looked at their brain serotonin content at three weeks after birth and the difference is there. Shortly after that, they begin to show a difference in alcohol preference. This is seen during development.

Weissler, Rochester: I too enjoyed your paper. I was wondering whether or not you have observed any parallel genetically determined variations in the rats in their propensity to develop liver or cardiac disorders?

Li: There are some twin studies in humans that suggest that cirrhosis has a difference in genetic susceptibility. We have not looked at that in the rats. That was not the basis of the selection. I think to look at that question, we would really want to select for differences in liver pathology, for example, with a constant dose of alcohol given involuntarily.

Weissler: Is there any parallelism between the alcohol-seeking rat and the seeking of other narcotic, addictive agents?

Li: We are currently looking at that and the only thing we've looked at so far is cocaine and there seems to be a difference.

Middleton, Buffalo: A few years ago, there was an interesting theory that went something like this, that acetaldehyde could condense with certain endogenous amines to produce precursors of addicting molecules. I wonder whether or not that thought could fit at all in the data you've been finding?

Reply: That hypothesis just doesn't go away, it keeps coming back. We thought it went away because people couldn't measure it and then there are some people like Kym Faull in UCLA whose better methods are beginning to detect it again. Now, if it has a role, I think it has a role in the persistence of drinking behavior or the addictive aspects, but not in the initial alcohol reward features. What our research tried to do is separate some of these. Some of the people have our animals to study those questions that you have brought up.