ELSEVIER

Alcohol 41 (2007) 557–566

ALCOHOL

Short-term selection for acute ethanol tolerance and sensitization from an F_2 population derived from the high and low alcohol-sensitive selectively bred rat lines

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Received 19 July 2007; received in revised form 18 September 2007; accepted 1 October 2007

Abstract

Previous studies have identified quantitative trait loci (QTL) in the inbred high and low alcohol-sensitive rat (IHAS1 and ILAS1) strains. The original development of the strains involved selection for ethanol sensitivity based on duration of the loss of the righting reflex (LORR) after a standard dose of ethanol. This paper confirms some of these QTL using a short-term selection procedure based on the difference between the blood ethanol level at LORR and regain of the righting response. An F_2 population of rats was developed by a reciprocal cross of IHAS1 and ILAS1 rats. Selection for five generations was carried out using delta-blood ethanol concentration (dBEC) as the selection trait, where dBEC = BECLR (BEC at loss of righting reflex) − BECRR (BEC at regain of righting reflex). The lines were labeled tolerant (TOL) or sensitive (SENS). Approximately one-third of the offspring for each generation in each line were genotyped using DNA markers that had been previously found to be linked to QTL on chromosomes 1, 2, 5, 12, and 13. By the fifth generation of selection, the lines showed a very large difference in dBEC, BECRR, and duration of LORR; BECLR showed little segregation during the selection, and latency to lose the righting reflex showed none. IHAS allele frequency increased in the SENS line for markers on chromosomes 1, 5, 12, and 13 while ILAS allele frequency increased in the TOL line. These results were in good agreement with the two previous QTL studies. On chromosome 2, the selection resulted in an accumulation of ILAS alleles in both lines. This study provides independent confirmation of the location of QTL on chromosomes 1, 5, 12, and 13 for ethanol sensitivity. It also suggests that genetic differences in duration of LORR are mediated primarily by the dBEC phenotype.

Keywords: Genetics; Quantitative trait loci; Ethanol tolerance; Selected lines; Inbred rats

Introduction

One of the best predictors of alcohol abuse and alcoholism in humans is their acute sensitivity to ethanol (Schuckit, 1992, 1994; Schuckit et al., 2000, 2001; Schuckit and Smith, 1996). Much research has gone into development of genetic animal models of acute ethanol responses in both mice (Boehm et al., 2000; Crabbe et al., 1979, 1989; Erwin and Deitrich, 1996; McClearn and Kakihana, 1981) and rats (Draski et al., 1992; Eriksson and Rusi, 1981). The goal of the development of animal models is twofold. First, studies were aimed at defining the pharmacological, biochemical, and electrophysiological underpinnings of the responses of selected lines of rats and mice (Allan et al., 1988; Avdulov et al., 1995; Collins, 1981; Deitrich and Baker, 1995; Deitrich and Spuhler, 1984; Deitrich et al., 1988). Second, more recent studies were aimed at locating and identifying the specific genes that influence or control the behavioral reactions to ethanol (Bennett et al., 2002, 2007; Kirstein et al., 2002). In this regard, this laboratory has developed the high and low alcohol-sensitive (HAS and LAS) rats from the nNIH heterogeneous population of rats by selective breeding for the duration of the loss of righting reflex (LORR) (Draski et al., 1992). The lines were selectively bred for 25 generations and subsequently inbred. Using these inbred lines, studies were undertaken to map quantitative trait loci (QTL) as a first step toward the goal of identifying genes that contribute to genetic variation for the duration of LORR

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0741-8329/07/$ – see front matter
doi: 10.1016/j.alcohol.2007.10.001

response. QTL initially were mapped on chromosomes 1, 2, 5, 12, and 13; follow-up studies confirmed QTL on chromosomes 2, 5, and 13 (Radcliffe et al., 2004, 2006a).

Acute ethanol sensitivity is often measured as the blood ethanol concentration (BEC) at the time at which a specific behavioral or other endpoint is achieved. As such, a single endpoint measurement of an acute response, such as the duration of LORR, is often taken as a measure of *initial* sensitivity. However, such measurements can be obscured by neuroadaptive processes that occur on both the ascending and descending limbs of alcohol distribution; for example, acute functional tolerance (AFT) (Mellanby, 1919; Newlin and Thomson, 1990). AFT is an acute pharmacodynamic adaptation that counteracts the cellular disturbance created by the presence of ethanol. For behaviors or other neuronal responses that show AFT, true initial sensitivity is difficult to accurately assess, at least within the limits of experimental error, because neurons start adapting virtually immediately after they come into contact with ethanol; this would represent the early stages of AFT (Goldstein, 1989; Palmer et al., 1985; Radlow, 1994). Thus, ''sensitivity'' for most acute ethanol responses is typically comprised of the combined effects of true initial sensitivity and AFT. The specific contribution of each of these is dependent on the time course of the response and AFT kinetics, and modified by genetic and environmental factors. Note that acute sensitization is also theoretically possible; indeed, acute sensitization is an important issue in the current study.

The fact that acute neuroadaptive processes such as AFT do occur has important implications for the study of acute ethanol responses in both humans and model organisms. For example, two experimental groups might show a similar response if it is measured soon after ethanol administration, while the response could diverge later on as a result of differential acquisition of AFT (see Newlin and Thomson, 1990). As mentioned above, QTL were mapped for the duration of LORR in crosses derived from the inbred HAS– and LAS (IHAS and ILAS)-selected rat lines; yet, it is not known for which component of ''acute sensitivity'' the QTL represent: initial sensitivity, AFT, or some combination (Radcliffe et al., 2004, 2006a). AFT is known to occur for the LORR response, at least in mice (Ponomarev and Crabbe, 2002), and it was speculated that a substantial portion of the genotype-dependent difference in duration of LORR in mouse lines selected for a differential LORR response was related to acquisition of AFT rather than initial sensitivity (Radcliffe et al., 2006b). Thus, the current experiment was undertaken to test the hypothesis that at least some of the duration of LORR QTL in the IHAS and ILAS lines would also control AFT. A bidirectional short-term selection was carried out using the development of acute tolerance or acute sensitization after a standard hypnotic dose of ethanol as the selection trait. The founding population was an F_2 derived from an intercross of IHAS1 × ILAS1 rats. At each generation of selection, rats were genotyped at the previously mapped QTL to test for significant cosegregation of IHAS and ILAS alleles with the selection trait. In this way, we hoped to dissect the relationship between alleles controlling initial sensitivity and those controlling the acute neuroadaptation which develops during the LORR response.

Materials and methods

Animals

All rats were bred at the Center for Laboratory Animal Care at the University of Colorado at Denver and Health Sciences Center (UCDHSC; Denver, CO) and were 51–88 days of age at the time of testing (mean: 65.0 ± 0.2). The founding population was an F_2 intercross created from reciprocal matings of IHAS1 × ILAS1 F_1 rats. This was the same type of cross used in earlier QTL mapping studies (Radcliffe et al., 2004, 2006a), but from a completely independent group of F_2 rats. The animals were maintained on a 12-h light/dark cycle in an environment of constant temperature and humidity (22°C, 40% humidity) and given access to normal rodent chow (Harlan Teklad 22/5) and water ad libitum. The procedures described in this report have been established to ensure the absolute highest level of humane care and use of the rats, and have been reviewed and approved by the UCDHSC Institutional Animal Care and Use Committee (IACUC).

Acute sensitivity and tolerance

The F_2 rats were tested for hypnotic sensitivity to ethanol using the same procedure that was used to select the IHAS and ILAS with a slight modification (Draski et al., 1992). After alcohol administration (3.5 g/kg; 15% wt/vol ethanol in normal saline; intraperitoneal [ip]), rats were placed on their back in a V-shaped trough and the time at which they could no longer right themselves was recorded. A 40-μl retro-orbital blood sample was drawn at this time for determination of the BEC at loss of righting (BECLR). The BECLR sample was not obtained in the original selection of the HAS and LAS nor in the subsequent QTL studies using those lines. Duration of LORR was the elapsed time from the LORR until the time at which they could right themselves at least three times within a 1-min span. A second 40-μl retro-orbital blood sample was drawn for determination of the BEC at regain of righting (BECRR). Delta BEC (dBEC) was the quantitative difference between BECRR and BECLR (i.e., dBEC = BECLR − BECRR) and was used as the sole selection trait (see below). Positive or negative dBEC indicated acute tolerance or sensitization, respectively. Values for duration of LORR are expressed in minutes and BEC measures as mg ethanol per dl blood (mg%). BECs were determined using a reliable spectrophotometry-based enzyme assay with comparison against a linear standard curve (Lundquist, 1959). Values shown in figures are mean ± S.E.M.