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Insulin-like growth factor I expression alters acute sensitivity and tolerance to ethanol in transgenic mice

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Abstract

We compared some biobehavioral effects of ethanol in transgenic mice that overexpress insulin-like growth factor I (IGF-I) in brain and in those that exhibit ectopic brain expression of IGF binding protein 1 with those in non-transgenic littermate controls. Ethanol-induced sleep in IGF-I transgenic mice was significantly shorter, and in IGF binding protein 1 transgenic mice significantly longer, than in controls. A similar tendency, though not significant, was observed for ethanol-induced hypothermia. The groups did not differ in the degree of ethanol-induced ataxia. IGF-I transgenic mice did not acquire tolerance to either the hypothermic or hypnotic effects of ethanol following 7-day ethanol treatment. In contrast, tolerance in IGF binding protein 1 transgenic mice was significantly more pronounced than in controls. There were no significant differences among the three groups in the peak blood alcohol concentrations or the overall blood alcohol curves following acute ethanol challenge. In general, these data support the prediction made that chronically elevated exposure to IGF-I in IGF-I transgenic mice renders them less susceptible to the effects of ethanol than their non-transgenic siblings, and that overexpression of IGF binding protein 1 has the opposite effect.

Keywords: Ethanol; IGF-I (insulin-like growth factor I); IGF binding protein 1; Ataxia; Hypothermia; Hypnosis; Tolerance; Blood alcohol concentration; (Transgenic mouse)

1. Introduction

Although alcoholism is considered to have a polygenic basis, changes in expression of single genes may modify susceptibility to specific biobehavioral effects of alcohol. The primary goal of this study was to evaluate whether overexpression of a single gene, insulin-like growth factor-I (IGF-I), modifies the acute and chronic effects of alcohol. IGF-I was initially discovered as a growth promoting factor in serum (see reviews: Jones and Clemmons, 1995; Rotwein, 1991). It is an anabolic peptide that is critical to cell proliferation and differentiation in virtually every organ, including brain (Raizada and LeRoith, 1993; Rotwein, 1991). IGF-I, the type 1 IGF receptor, and high affinity IGF binding proteins, are all widely expressed throughout the body, including the central nervous system (cf. Raizada and LeRoith, 1993). Multiple high affinity IGF binding proteins modulate the actions of IGF in both serum and tissues (see review Jones and Clemmons, 1995). One of them, IGF binding protein 1, is acutely regulated by diet and limits the bioavailability of IGFs when present in molar excess (Lemozy et al., 1994; Pucilowska et al., 1993). IGF binding protein 1, when present in sufficiently high concentrations, inhibits the in vivo actions of IGF-I (D'Ercole et al., 1994).

Considerable evidence indicates that ethanol exerts profound and complex effects on cellular Ca^{2+} homeostasis. Acute exposure to ethanol causes a concentration-dependent decrease in neuronal Ca^{2+} uptake, whereas chronic exposure to ethanol leads to increased intracellular Ca^{2+} (Leslie et al., 1990; Little, 1995). Acute effects of ethanol (hypothermia, ataxia, sleep) are potentiated by drugs that block either receptor- or voltage-gated Ca^{2+} channels (Little, 1995; Pucilowski, 1992), whereas the L-type Ca^{2+} channel activator, 1,4-dihydro-2,6-dimethyl-5-nitro-4-[2-(trifluoromethyl)-phenyl]-3-pyridine carboxylic acid methyl ester (Bay K 8644), antagonizes the acute effects of ethanol (Dolin et al., 1988). Conversely, Ca^{2+} channel blockers

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suppress development of tolerance to ethanol (Dolin and Little, 1989; Pucilowski et al., 1989; Wu et al., 1987), symptoms of ethanol withdrawal (Pucilowski et al., 1989; Rezvani et al., 1993b) and alcohol seeking behavior (e.g., Pucilowski et al., 1992, 1994; Rezvani et al., 1993a,b). IGF-I may affect responsiveness to alcohol by virtue of modifying intracellular Ca^{2+} concentrations. IGF-I has been demonstrated to facilitate high-threshold (L-type) Ca^{2+} influx in cultured neurons (Kleppisch et al., 1992). However, when noxious concentrations of intraneuronal Ca^{2+} are generated by hypoglycemia, hypoxia or excitatory amino acid receptor activation, IGFs appear to have protective properties against Ca^{2+}-related neuronal damage as do Ca^{2+} channel blockers. The Ca^{2+}-stabilizing effects of IGF may include reduction of Ca^{2+} influx and/or enhancement of Ca^{2+} extrusion/buffering (Mattson and Cheng, 1993). The independent effects of ethanol and IGFs on Ca^{2+} homeostasis suggest that IGF-I could modify the biobehavioral consequences of acute and chronic ethanol exposure.

To test this hypothesis we evaluated some biobehavioral effects of ethanol in transgenic mice that overexpress IGF-I in brain (Mathews et al., 1988) and in those that exhibit ectopic brain expression of IGF binding protein 1 (Dai et al., 1994), and compared these with the effects seen in non-transgenic littermate controls. An alteration of the biobehavioral effects of ethanol in IGF-I transgenic mice would be expected to represent an exaggeration of the effects of endogenous IGF-I, while those observed in IGF binding protein 1 transgenic mice would be expected to represent an inhibition of endogenous IGF-I activity, and therefore, a diminution of endogenous IGF activity. Specifically, we compared (a) acute hypothermic, ataxic and hypnotic effects of ethanol, (b) tolerance to ethanol-induced hypnosis and hypothermia, and (c) blood pharmacokinetics of ethanol. We predicted that chronically elevated exposure to IGF-I in IGF-I transgenic mice would render them less susceptible to the effects of ethanol than their non-transgenic siblings, and that overexpression of IGF binding protein 1 would have the opposite effect.

2. Materials and methods

2.1. Animals

Heterozygous IGF-I transgenic mice were generated by classical microinjection methodology using the same construct as previously reported to create the original line of these mice (Mathews et al., 1988) in the Transgenic Core Facility at the University of North Carolina at Chapel Hill. Two lines of IGF-I transgenic mice (lines 32 and 43), i.e. the progeny of different founder mice, were used in this study. Because the parameters measured did not differ between mice from these lines, data were pooled. Heterozygous IGF binding protein 1 transgenic mice were generated in the same fashion and in the same facility. IGF binding protein 1 transgenic mice have been described in detail elsewhere (Dai et al., 1994; D'Ercole et al., 1994). As with the IGF-I transgenic mice, two lines of IGF binding protein 1 transgenic mice were used in this study (B and C lines). Again because the parameters measured did not differ, data were pooled.

The transgenes used to generate both IGF-I transgenic and IGF binding protein 1 transgenic mice are driven by the mouse metallothionein I promoter. In both groups of mice the transgenes are expressed in many tissues including brain. The overexpression of IGF-I in the brains of these transgenic mice results in brain overgrowth, while the ectopic expression of IGF binding protein 1, a protein not normally expressed in brain, results in brain growth retardation. In the present study, the brain weights of IGF-I transgenic mice were significantly ($P < 0.001$) higher (0.58 ± 0.01 g), and IGF binding protein 1 transgenic mice lower (0.37 ± 0.004 g), than those of control mice (0.46 ± 0.01 g). All lines of transgenic mice were bred with normal, non-transgenic C57BL/6J mice. This approach allowed the use of non-transgenic littermates as controls. Transgenic mice were identified by PCR analysis of a tail DNA, as previously described (Dai et al., 1994).

The mice were housed 2–4 per cage (acrylic plastic, $18 \times 28 \times 12$ cm), under 12 : 12 h light : dark cycles (light on at 7:00 a.m.) and temperature of 21 ± 1°C. They had unlimited access to food (Prolab 3000, Agway) and tap water containing 25 mM zinc (in the form of $ZnSO_4$) to promote transgene expression. Control animals were also on 25 mM zinc water. Experiments were carried out in mice between 80–110 days of age, typically during 10:00–14:00 h, in an experimental room with the same light and temperature conditions as the animal room. Mice were injected with ethanol in random order, both within and between the experimental groups. Behavioral scoring was done either automatically or by an observer unaware of the group assignment of tested animals.

In all experiments, three groups of animals were compared, i.e.: non-transgenic controls; IGF-I transgenic mice; and IGF binding protein 1 transgenic mice. The control group included approximately equal number of non-transgenic littermates of the IGF-I transgenic and IGF binding protein 1 transgenic mice used in a given experiment. All transgenic mice were at least F5 progeny of the respective founder strain and C57BL/6J line. All experimental protocols were approved by an institutional review committee ID No. 93-261 A and B.

2.2. Experiment 1: sedative effect (loss of righting reflex) and hypothermic effect

Baseline temperature was examined by inserting a lubricated probe 2.5 cm into the rectum (ambient temperature of 20 ± 1°C). Temperature was recorded with a BAT-12 digital thermometer (Sensortek, Clifton, NJ), with a resolu-

tion of 0.1°C. The baseline temperature (T0) was calculated as the average of two readings taken 10–15 min apart. The mice were then injected with ethanol (3 g/kg i.p., 20% v/v) and placed individually in a cage similar to the home cage. The time of loss and regaining of the righting reflex was subsequently recorded as follows. Mice were placed on their backs in a trough made in the sawdust bedding. If a mouse failed to right itself twice within 30 s, it was scored as being asleep. Upon awaking, each mouse was placed again on its back, and only if it righted itself within 30 s, was the mouse scored as being awake. The difference between the times of losing and regaining the righting reflex served as the measure of ethanol-induced sleep. The temperature reading was taken again 60 min after ethanol injection (T60) and the difference from T0 reading was the measure of ethanol-induced hypothermia.

2.3. Experiment 2: ethanol-induced ataxia

Mice were first familiarized with the apparatus by placing them on a rod (Basile, Italy; 5 cm in diameter), rotating at a constant speed of 8 rpm. Ten consecutive trials, with 2–3 min inter-trial interval, were given on this training day. Mice that failed to remain on the drum for a mean minimum of 20 s during a minimum of seven trials, were excluded from the study. On the first day of the experiment, the mice were given two additional 180 s trials. After approximately 30 min each mouse was injected i.p. with ethanol, and 20 min later was tested for ataxia during three consecutive trials on a drum rotating at a rate of 8 rpm. The test continued until the mouse fell from the drum or for a maximum of 180 s. A two min break was allowed between consecutive trials. Two doses of ethanol, 1 and 2 g/kg (16% v/v), were tested in the same mice following a randomized crossover design, with the injections spaced 4 days apart.

2.4. Experiment 3: development of chronic tolerance

On the first day of experiment mice were challenged with 3 g/kg of ethanol (i.p., 20% v/v). The length of loss of righting reflex and the hypothermic response upon awaking were recorded. For the next seven days, mice were administered ethanol, 1.5 g/kg i.p. once daily at about 11:00 a.m. On the eight day, the change in the hypothermic and hypnotic response to a challenge 3 g/kg dose of ethanol was re-examined. After the final temperature reading, 6 mice from each transgenic line and 4 from each non-transgenic control line were randomly chosen and killed for collection of trunk blood samples for measurement of blood alcohol concentration (Rezvani et al., 1993b).

2.5. Blood alcohol curves

A single dose (2 g/kg, i.p.) of ethanol (as 16% v/v) was administered to both groups of transgenic and control mice. Blood samples (20 μl) were collected from the tail at 5, 10, 15, 30, 60 and 120 min after ethanol administration. Samples from five different animals of each experimental group were collected into microcentrifuge tubes containing 180 μl tetrabutanol as an internal standard and analyzed by gas chromatography using techniques described elsewhere (Rezvani et al., 1993b).

2.6. Statistical analysis

The results were analyzed by either one-way or factorial analysis of variance (ANOVA) using line and dose, time or treatment as factors. $P < 0.05$ was used as a significance limit. Post hoc comparisons were made for significant main effects by the Newman-Keuls test (Tallarida and Murray, 1987).

3. Results

3.1. Sedative and hypothermic effects

All IGF binding protein 1 transgenic mice lost the righting reflex following ethanol challenge whereas three mice in the IGF-I group did not. However, these three mice were otherwise markedly affected by ethanol as judged by a profound drop in T60 body temperature which was at the low end of readings for this experimental group. In the IGF binding protein 1 transgenic group, three mice slept longer than the predetermined temperature measurement time and their T60 reading was taken immediately upon awaking i.e. around 76 min after loss of righting reflex. Overall, the effects of ethanol (3 g/kg), as shown by the duration of loss of righting reflex and possibly the intensity of hypothermic response, were less pronounced in

Table 1
Ethanol-induced ataxia in IGF-I transgenic mice, IGF binding protein 1 transgenic mice and non-transgenic siblings (means ± S.E.M.)

Group	n	Average time (s) on a drum after i.p. ethanol	
		1 g/kg	2 g/kg
Control	6	28.5 ± 8.5	1.4 ± 0.1
IGF-I transgenic	6	38.2 ± 10.3	4.6 ± 2.4
IGF binding protein 1 transgenic	6	31.3 ± 17.5	1.2 ± 0.2

n = number of animals. There was no statistically significant overall group effect on ethanol-induced ataxia for either of the two doses.

Table 2
Ethanol-induced sleep and hypothermia in IGF-I transgenic mice, IGF binding protein 1 transgenic mice and non-transgenic siblings (means ± S.E.M.)

Group	n	Sleep time (min)	Temperature drop (°C)
Control	15	37 ± 3.3	−4.0 ± 0.3
IGF-I transgenic	12	15 ± 3.4 [a]	−3.2 ± 0.4
IGF binding protein 1 transgenic	13	57 ± 4.1 [a,b]	−4.1 ± 0.3

n = number of animals. [a] $P < 0.01$ vs. Controls; [b] $P < 0.01$ vs. IGF-I transgenic (Newman-Keuls test).

IGF-I transgenic mice, and more pronounced in IGF binding protein 1 transgenic mice, than in the controls (Table 1). Only differences in the hypnotic effect, however, were found significant, $F(2,39) = 31.34$, $P < 0.001$. These data suggest that mice with genetically altered expression of IGF-I and IGF binding protein 1 differ in opposing ways from control mice in their sensitivity to the acute effects of ethanol.

3.2. Ethanol-induced ataxia

There were no differences among genotypes in the overall times on the rotarod during the pretest when all animals, i.e. those that qualified further and those that failed to reach the criterion, were included. The mean scores (± S.E.M.) were 45.5 ± 14, 46.3 ± 17 and 38.9 ± 22 s for controls, IGF-I transgenic and IGF binding protein 1 transgenic mice, respectively. There were also no significant differences among the groups of animals that met the 20 s cut-off criterion in baseline activity on the rotarod: non-transgenic mice spent 62.5 ± 11 s walking (mean ± S.E.M.), IGF-I transgenic and IGF binding protein 1 transgenic mice 70.9 ± 27 and 49.3 ± 20 s, respectively. Ethanol pretreatment had a dose-dependent suppressant effect on ability of mice from all three groups to remain on the rotating drum. The lower dose of ethanol impaired ability of mice to remain on the drum, decreasing walking time by 34 ± 9%, 33 ± 18% and 18% ± 4% in non-transgenic controls, IGF-I transgenic and IGF binding protein 1 transgenic mice, respectively (Table 2). The higher dose of ethanol (2 g/kg) nearly abolished the ability of mice to remain on the moving drum. ANOVA revealed that the time on drum was not different among the mice for either 1 g/kg, $F(2,17) = 0.15$, or 2 g/kg of ethanol, $F = 0.58$.

3.3. Tolerance to ethanol

After 8 days of ethanol, ANOVAs revealed that the groups of mice differed with respect to both the hypnotic effect, $F(2,34) = 26.33$, $P < 0.001$, and the hypothermic effect of ethanol, $F(2,34) = 5.12$, $P < 0.05$ (Table 3). For each effect tolerance formation was significantly attenuated in IGF-I transgenic group, whereas it was augmented in IGF binding protein 1 transgenic mice. No significant differences were noted in blood alcohol concentrations tested at 60 min after ethanol injection between the randomly selected groups of mice on the last day of experiment, $F(2,19) = 0.26$, suggesting that the differences among the groups are not due to differences in blood alcohol concentrations.

3.4. Blood alcohol concentration after acute ethanol challenge

The peak blood alcohol concetrations were similar in all groups, 223 ± 18, 230.2 ± 15 and 230.9 ± 16 mg/dl for controls, IGF-I transgenic and IGF binding protein 1 transgenic mice, respectively (Fig. 1). Repeated measures ANOVA revealed that there was no significant difference in overall blood alcohol concentration values between the three groups, $F(2,72) = 1.66$ for the group effect.

4. Discussion

These experiments were intended to test the prediction that overexpression of IGF-I will attenuate, while overexpression of IGF binding protein 1 will enhance the acute central depressant effects (ataxia, hypnosis, hypothermia) effects of ethanol. The theoretical basis for this hypothesis was the observation that acute ethanol administration prevents (Leslie et al., 1990), while IGF-I facilitates, Ca^{2+} influx in neurons (Kleppisch et al., 1992). Although the present results do not offer an unequivocal support for the above hypothesis, they suggest that overexpression of IGF-I attenuates some central depressant effects of ethanol. This suppression is more profound in case of the hypnotic effect and less so for the hypothermic effect of ethanol. Overex-

Table 3
Tolerance to ethanol in IGF-I transgenic mice, IGF binding protein 1 transgenic mice and non-transgenic siblings

Group	n	Hypnotic effect (min)	Hypothermic effect (°C)
Control	14	−19.5 ± 3.7	−1.2 ± 0.33
IGF-I transgenic	10	−4.6 ± 3.6 [a]	−0.4 ± 0.36
IGF binding protein 1 transgenic	11	−46.1 ± 4.2 [b,c]	−2.1 ± 0.33 [c]

Values (means ± S.E.M.) express the difference in readings on Day 8 vs. Day 0 in each response to a challenge dose (3 g/kg) of ethanol before and after 7 days of ethanol treatment. n = number of animals. [a] $P < 0.05$, [b] $P < 0.01$ vs. controls; [c] $P < 0.01$ vs. IGF-I transgenic (Newman-Keuls test).

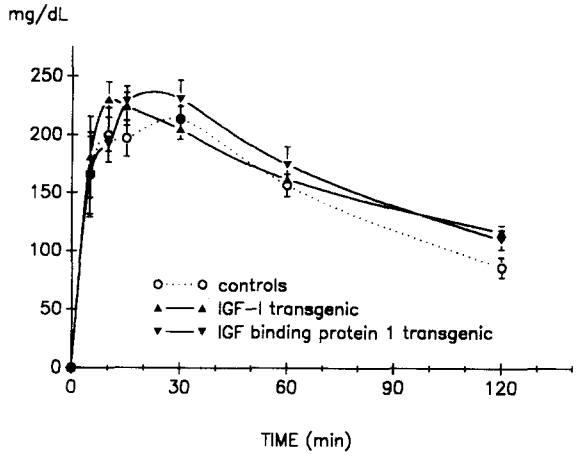

Fig. 1. Mean (±S.E.M.) blood alcohol concentration in IGF-I transgenic mice, IGF binding protein 1 transgenic mice and non-transgenic siblings (controls) as tested at different time points following a single i.p. injection of 2 g/kg of ethanol.

pression of IGF binding protein 1, on the other hand, resulted in changes opposite to those seen in IGF-I transgenic mice. The latter finding is consistent with the modulatory role of this binding protein on the actions of IGF-I (Jones and Clemmons, 1995). Neither IGF-I transgenic nor IGF binding protein 1 transgenic mice differed from non-transgenic controls in the intensity of ethanol-induced ataxia. This might have been due to generally low baseline activity in all three groups when tested on the rotarod or to a selection bias. The latter is not likely the case because the average activity scores in all three groups prior to elimination of marginally active individuals were almost identical (approximately 33 ± 6 s). It is, however, not uncommon that biologically active substances affect different actions of ethanol with varying potency. For example, some Ca^{2+} channel blockers have been found to exert variable modulatory effects on different central depressant effects of ethanol (Little, 1995).

Following an acute alcohol challenge, there were no differences in blood alcohol concentrations among groups detected by ANOVA. In the tolerance experiment, and despite differences among groups in the degree of tolerance formation, the blood alcohol concentration tested 60 min after alcohol challenge were virtually identical. This suggests that pharmacokinetic differences are not responsible for the above mentioned behavioral differences in the acute hypnotic response or the acquisition of tolerance to the physiological effects of alcohol.

Chronic treatment with ethanol led to tolerance in non-transgenic controls both to the hypnotic and hypothermic effects of ethanol. Chronic ethanol exposure made IGF binding protein 1 transgenic mice less responsive to both effects of ethanol, i.e. more tolerant, than the controls. In contrast, overexpression of IGF-I appears to prevent acquisition of protracted tolerance to either effect of ethanol (IGF-I transgenic mice were equally responsive to an acute ethanol challenge before and after 8-day alcohol treatment).

The mechanisms responsible for these opposing effects of overexpression of IGF-I and IGF binding protein 1 on ethanol tolerance are not clear at the present time. One possible mechanism may involve IGF-I interaction with Ca^{2+} trafficking in the cell. IGF-I has been demonstrated to protect against neuronal damage caused by an elevation in the intracellular Ca^{2+} content due to hypoxia, hypoglycemia or excitatory amino acid receptor activation (Mattson and Cheng, 1993). Furthermore, involvement of receptor-gated Ca^{2+} channels is suggested by the recent findings that IGF-I inhibits glutamate-induced release of GABA in cerebellar Purkinje cells (Castro-Alamancos and Torres-Aleman, 1994). Because changes in neuronal intracellular Ca^{2+} concentrations are considered to underlie several of the biobehavioral effects of ethanol (Little, 1995), we hypothesized that IGF-I may modify the physiological and behavioral effects of both acute and chronic ethanol administration. These changes may in turn affect other mechanisms conducive to the biobehavioral outcome of IGF-I/ethanol interaction. One common link could be brain myelin content and consequently changes in neuronal impulse propagation (Mithen et al., 1990; Zoeller et al., 1994). The latter possibility is consistent with the findings that the brains of IGF-I transgenic mice have a marked increase in myelin content (Carson et al., 1993) that is characterized by an increased percentage of myelinated axons and thicker myelin sheath (Ye et al., 1995). In contrast, IGF binding protein 1 transgenic mice exhibit a decrease in brain myelination and a decreased percentage of myelinated axons (Ye et al., 1995).

The present studies provide the initial validation of the utility of IGF-I/IGF binding protein 1 transgenic mice as genetic models for further behavioral, pharmacological and molecular research of interactions between ethanol and the IGF system. While much more work remains to be done in order to clarify the mechanisms involved in the described effects, the results indicate that modification of IGF-I system activity can substantially change responsiveness to acute and chronic ethanol. Although further actions of ethanol, particularly those related to tolerance/dependence should be studied, this evidence points towards the intriguing possibility that altering the expression of a single gene may substantially change the effects of chronic alcohol exposure.

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