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Effects of sweetened ethanol solutions on ethanol self-administration and blood ethanol levels

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Abstract

The enhancement of voluntary self-administration of ethanol by sucrose or saccharin was tested in conjunction with measurements of blood ethanol levels. Adult male rats were given access to both tap water and one of five solutions: 0.125% saccharin, 10% sucrose, ethanol, saccharin + ethanol, or sucrose + ethanol. The rats receiving the sucrose + ethanol solution drank consistently more ethanol (> 5 g/kg/day) than did the rats receiving the saccharin + ethanol solution (< 3 g/kg/day) or ethanol only (< 2 g/kg/day). Both sweetened solutions produced higher ethanol consumption during these periods than ethanol alone. However, no significant differences in blood ethanol levels were found between the sucrose + ethanol and saccharin + ethanol conditions, when tested at different intervals on Day 44 or Day 45 of ethanol consumption. Following 45 days of consumption, no change in the bicuculline seizure threshold was observed in the ethanol-consuming rats compared to the controls. In a separate study using 90 naive rats, rats were gavaged with ethanol (1, 2, or 3 g/kg) containing either 10% sucrose ($n = 10$ for each dose of ethanol), 0.125% saccharin ($n = 10$ for each dose of ethanol), or ethanol alone ($n = 10$ for each dose of ethanol), and blood was collected from the tip of the tail 30, 60, 180, 300, and 540 min later and analyzed for ethanol concentrations. Sucrose significantly decreased the resultant blood ethanol levels at several time points following gavage. These results indicate that sucrose can significantly alter blood ethanol levels and that chronic self-administration of a sweetened ethanol solution for 6 weeks does not produce ethanol dependence.

Keywords: Ethanol self-administration; Sucrose; Saccharin; Blood ethanol levels; Bicuculline seizure thresholds

Voluntary persistent ethanol consumption produces ethanol tolerance and dependence in humans (Schwarz-Stevens et al., 1991). However, research investigating the mechanisms producing ethanol tolerance and dependence has been hindered by lack of an animal model that dissociates the parameters associated with forced ethanol administration from the effects of ethanol per se. Al-though, ethanol tolerance and dependence are reliably produced by forced ethanol administration in animals (Becker and Hale, 1993; Devaud et al., 1995; Morrow et al., 1992), it has been proven difficult until recently to induce tolerance and dependence in animals that voluntarily self-administer ethanol (Cicero, 1979; Waller et al., 1982; Kampov-Polevoy et al., 2000).

Several experimental procedures have been developed to increase voluntary ethanol self-administration in rats in an attempt to more closely mimic human experience. One procedure, ''sucrose fading'' (Samson, 1986), has been successful in increasing ethanol consumption. Rats are initially trained to respond for sucrose (usually 10–20%) at stable rates before ethanol, in increasing concentrations, is added to the sweetened solution. Following stable responding to the sucrose + ethanol solution, the sucrose concentra-

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tion is gradually faded until the animal is responding for ethanol alone.

The sucrose-fading procedure is a method that has been proven successful in producing ethanol self-administration in a variety of studies (Files et al., 1997; Hodge et al., 1993; Samson, 1986; Sedman et al., 1976). However, only small amounts of ethanol are typically consumed (e.g., ~0.8 g/kg/session (Samson et al., 1989)) thereby resulting in rather low blood ethanol concentrations (~range 40–110 mg/dl). Consequently, the sucrose-fading procedure is a useful model of ethanol reinforcement but has not been proven to be useful in studying mechanisms underlying ethanol tolerance and dependence.

Recently, the sucrose-fading procedure has been modified so as to increase daily ethanol intake (Gatto et al., 1990; Samson et al., 1997; Slawecki, 1997). These procedures use either sucrose or Polycose (with the possible addition of NaCl and/or saccharin) throughout the experimental procedure. The sweeteners are not faded once the concentration of ethanol reaches its maximum. Use of a continuous sucrose + ethanol solution increases the daily amount of ethanol intake. In a preliminary report, Samson et al. (1997) found that ethanol self-administration for 90 days produced ethanol intake ranging from 8 to 10 g/kg/day. Consequently, using a sucrose + ethanol solution throughout self-administration might result in high enough ethanol intake to produce ethanol dependence and tolerance.

Although use of a sucrose + ethanol solution may increase ethanol intake, it might not produce ethanol dependence and tolerance if the intake is not sufficiently high and/or if sucrose has other physiological effects that lower blood ethanol levels. For example, a high sucrose level (e.g., 10% w/v) might impede absorption of ethanol from the gut, alter gastric emptying and/or increase ethanol metabolism thereby producing low blood ethanol levels. It has been shown that sugars can reduce the blood ethanol levels following oral (Sellers and Kalant, 1976) or intravenous administration of ethanol (Mascord et al., 1988). In addition, several recent reports have provided conflicting evidence suggesting that sucrose either has no effect on blood alcohol concentrations (Czachowski, 1999; Gauvin, 1999) or that sucrose can significantly reduce blood alcohol concentrations (Roberts et al., 1999). To test if self-administration of a sucrose + ethanol solution produces blood ethanol levels sufficient to produce ethanol dependence, the present study was conducted.

Rats self-administered ethanol (0–7.5% v/v) either alone or in a 10% sucrose solution or a 0.125% saccharin solution for 45 days or either of the two sweetened solutions without ethanol. Twice during the procedure, 20 μl of tail blood was collected to assay blood ethanol concentrations. However, routine blood collection was not conducted in order to avoid potential stress effects that might confound ethanol self-administration behavior. Following the 45 days of self-administration, ethanol dependence was measured by determining bicuculline seizure thresholds 6–8 h after removal of the ethanol solutions (i.e., during ethanol withdrawal). Furthermore, a separate study was conducted to investigate if 10% sucrose alters blood ethanol concentrations following intragastric administration of one of three ethanol doses (1.0, 2.0, or 3.0 g/kg).

1. Materials and methods

1.1. Animals

All animal care and use were conducted in accordance with IACUC guidelines at the University of North Carolina. Adult male Sprague–Dawley rats (Charles River, NC) weighing approximately 150–180 g were housed individually in hanging steel wire cages under constant temperature (21 ± 1°C) on a reverse day/night light cycle (lights off at 10:00 AM and on at 10:00 PM). Rats had constant access to standard rat lab chow and water throughout the experiment.

1.2. Ethanol self-administration procedure

Rats were randomly assigned to one of five self-administration conditions: 10% sucrose, w/v, (Suc, $n = 4$), 0.125% saccharin, w/v, (Sacc, $n = 6$), 10% sucrose + ethanol (Suc/Etoh, $n = 9$), 0.125% saccharin + ethanol (Sacc/Etoh, $n = 9$), or ethanol only ($n = 9$). These concentrations of sucrose and saccharin have been used successfully to increase ethanol self-administration in previous experiments (Gatto et al., 1990; Roberts et al., 1999; Samson et al., 1997). Prior to the availability of the sweetened solutions, animals were allowed free access to tap water for 1 day in graduated Richter tubes. Animals were then initiated to the appropriate sweetened solutions for 6 days. Specifically, animals in the Suc and Suc/Etoh conditions could consume tap water or 10% sucrose while animals in the Sacc or Sacc/Etoh conditions could consume tap water or 0.125% saccharin using a standard two-bottle choice method (Wedel et al., 1991). Following the 6-day sweetened water initiation period, the ethanol concentration was gradually increased in the Suc/Etoh, Sacc/Etoh, and ethanol only conditions as follows: Days 1–5, 0% ethanol (initiation period); Days 6–10, 2.5% ethanol; Days 11–15, 5% ethanol; Days 16–20, 7.5% ethanol; Days 21–30, 5% ethanol; Days 31–45, 6% ethanol. The concentration of ethanol was decreased on Day 21 due to low drinking levels by animals when the ethanol concentration was 7.5%. All animals had free access to water and food and were weighed at least three times per week.

To ascertain blood ethanol concentrations at specific times following the initiation of the dark cycle, blood was collected from the tip of the tail at two time points. On Day 44, blood was collected 60 min following the beginning of the dark cycle while on Day 45 blood was collected 180 min following the beginning of the dark cycle for rats in the Suc/Etoh, Sacc/Etoh, and the ethanol only condition. The first

hours following the beginning of the dark cycle is the time when rats self-administer the greatest amount of ethanol (Gatto et al., 1990). No attempt was made to control for food consumption prior to the collection of blood samples.

1.3. Ethanol gavage procedure

To investigate whether sucrose or saccharin alters blood ethanol levels that might reflect changes in ethanol absorption, gastric emptying or metabolism, naive male Sprague–Dawley rats ($n = 90$) were administered a solution of sucrose + ethanol, saccharin + ethanol, or ethanol only by intragastric gavage. Animals were administered either 1.0, 2.0, or 3.0 g/kg ethanol (20% v/v) in a 10% sucrose solution ($n = 10$ per ethanol dose), a 0.125% saccharin solution ($n = 10$ per ethanol dose), or ethanol alone ($n = 10$ per ethanol dose). Animals were rapidly gavaged by inserting a ball-tipped 16-gauge stainless-steel needle (Becton-Dickenson) into the stomach via the esophagus and the ethanol solution was delivered by gentle pressure on a syringe. Blood was collected via the tail at 30, 60, 180, 300, and 540 min following ethanol administration for determination of blood ethanol concentrations. All animals were food deprived for 10 h prior to ethanol administration.

1.4. Blood ethanol determination

Blood samples from the self-administration studies were immediately transferred to a snap-cap microcentrifuge tube containing 180 μl of *tert*-butanol (0.3 mg/ml) as an internal standard. After shaking, each tube was stored at 4°C until gas chromatography analysis. Each tube was centrifuged ($14{,}000 \times g \times 10$ min) and 20 μl of the supernatant was injected into a gas chromatograph (Varian Aerograph Model 2400) equipped with a flame ionization detector and a 60/80 Carbopack B/5% Carbowax 20 M, 6 ft × 2 mm i.d. glass column (Supelco). The chromatographic conditions were: carrier gas (N_2) flow rate 20 ml/min; 60–110°C at 10°C/min temperature program; injector temperature 120°C; detector temperature 140°C. Blood ethanol levels were determined by regression of the ratio of the alcohol peak and the *tert*-butanol peak to the ratio of known ethanol standards. Blood ethanol concentrations are expressed as milligrams per deciliter (Rezvani and Gradi, 1994). Because animals were able to self-administer ethanol continuously (i.e., 24 h/day), the resultant blood ethanol concentrations may be related to, but not determined by, the amount of ethanol consumed during the time elapsed between the initiation of the dark cycle and the time tail blood was collected.

Blood samples collected following the ethanol gavage procedure were processed in heparinized capillary tubes and plasma was separated by centrifugation. Blood ethanol concentrations were determined via the Analox GL-5 (Analox Instruments, USA). For each determination, 5 μl of plasma was injected and the blood ethanol concentration was expressed as milligrams per deciliter.

1.5. Bicuculline-induced seizure threshold procedure

Ethanol dependence is characterized by an increase in CNS excitability during ethanol withdrawal that can be demonstrated by a significant reduction in bicuculline induced seizure thresholds (Devaud et al., 1995; Kampov-Polevoy, 2000). To determine if chronic ethanol self-administration produced ethanol dependence, seizure thresholds were determined at the beginning of the light cycle. Approximately 6 h before seizure threshold determinations, the ethanol solutions were removed to ensure that animals would be completely withdrawn from ethanol. Threshold determination was made by constant tail vein infusion of bicuculline (Sigma, St. Louis, MO) dissolved in 0.1 N HCl and diluted with isotonic saline to a concentration of 0.05 mg/ml. Bicuculline was infused via a lateral tail vein at a rate of 1.6 ml/min until the first myoclonic twitch of the face and/or neck was observed. Seizure thresholds were calculated by the time of infusion × dose of bicuculline/body weight. Seizure severity was not measured.

1.6. Data analysis

Twenty-four hour ethanol intake was analyzed using a two-way ANOVA with repeated measures (Sweetener Type - × Time Block) with Newman–Keuls post hoc tests where appropriate. Blood ethanol levels from the gavage experiment were analyzed using a two-way ANOVA with repeated measures (Sweetener Type × Time) followed by Newman–Keuls post hoc tests. The main effect of sweetener was also analysed (i.e., blood ethanol levels collapsed over time) using a one-way ANOVA followed by planned orthogonal comparisons. If the blood alcohol concentration was below the level of detection for a particular time point, data from that time point was not used in the calculation of the two-way ANOVA or any subsequent post hoc test. Finally, weight gain and bicuculline seizure thresholds were analyzed using a one-way ANOVA with Newman–Keuls post hoc tests.

2. Results

Data for ethanol intake were collapsed into blocks of days according to the ethanol concentration (Time Block 1 — 2.5% ethanol (4 days); Time Block 2 — 5% ethanol (5 days); Time Block 3 — 7.5% ethanol (5 days); Time Block 4 — 5% ethanol (5 days); Time Block 5 — 5% ethanol (5 days); Time Block 6 — 6% ethanol (6 days); Time Block 7 — 6% ethanol (6 days); Time Block 8 — 6% ethanol (5 days)). Once stable ethanol self-administration was established, animals in the Suc/Etoh condition consumed approximately 5.0 g/kg ethanol/day, animals in the Sacc/Etoh condition consumed < 2.0 g/kg/day and animals in the ethanol only condition consumed < 1.0 g/kg/day (Fig. 1). Rats in the Suc/Etoh condition had significantly greater

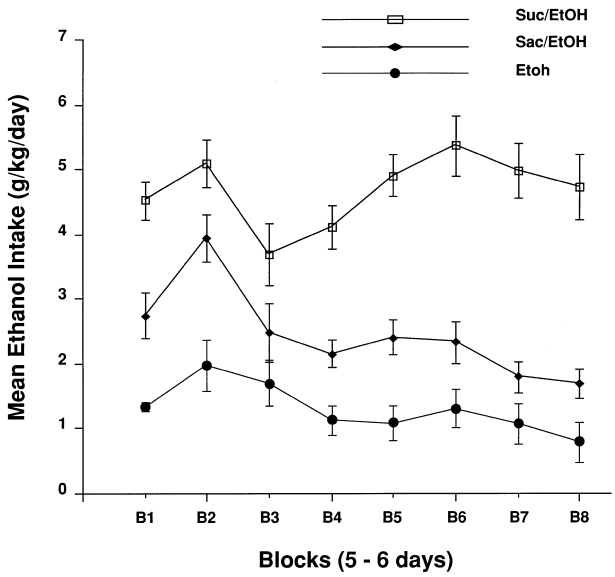


Fig. 1. Mean 24-h ethanol intake by rats in the Suc/Etoh, Sacc/Etoh, and ethanol only condition. Time blocks are mean ethanol intake collapsed over days within a particular ethanol concentration (Time Block 1 — 2.5% ethanol (4 days); Time Block 2 — 5% ethanol (5 days); Time Block 3 — 7.5% (5 days); Time Block 4 — 5% ethanol (5 days); Time Block 5 — 5% ethanol (5 days); Time Block 6 — 6% ethanol (6 days); Time Block 7 — 6% ethanol (6 days); Time Block 8 — 6% ethanol (5 days)). Due to experimenter error, 4 days of ethanol intake during the 6% ethanol concentration were lost. Data are plotted as means ± standard error of the mean (S.E.M.). Suc/Etoh, $n=9$; Sacc/Etoh, $n=9$; ethanol only, $n=9$.

ethanol intake (g/kg) compared to rats in the other two conditions while rats in the Sacc/Etoh condition had significantly greater ethanol intake than rats in the ethanol only condition (two-way ANOVA with repeated measures, $F(14,192)=1.99$, $P<0.02$; main effect of sweetener $F(2,192)=206.28$, $P<0.0001$; main effect of Time Block $F(7,192)=4.31$, $P<0.001$). Individual comparisons by time block reveal that rats in the Suc/Etoh condition had significantly greater ethanol intake compared to rats in the Sacc/Etoh condition on every block except Time Block 3 with significantly greater intake compared to rats in the ethanol only condition on every time block (Newman–Keuls test, P's <0.05). Rats in the Sacc/Etoh condition consumed significantly greater ethanol than rats in the ethanol only condition except for Time Blocks 3, 6, and 8 (Newman–Keuls test, P's <0.05).

To investigate if either sweetened solution (sucrose or saccharin) altered blood ethanol levels, data were analyzed on two different days, Day 44 and Day 45. No significant difference in blood ethanol concentrations was found between animals in either sweetened conditions on either day although animals in the sucrose + ethanol condition consistently consumed greater amounts of ethanol than animals in either the saccharin + ethanol or ethanol alone conditions (see Figs. 1 and 2). Finally, it was found that a significant difference existed between the amount of weight gained by animals in the three ethanol conditions (one-way ANOVA, $F(2,24)=3.6$, $P<0.05$). Specifically, rats in the Suc/Etoh condition gained significantly less weight than animals in either the Sacc/Etoh or ethanol only conditions (Newman–Keuls test, P's <0.05).

The addition of 10% sucrose to the ethanol solution significantly decreased blood ethanol concentrations compared to ethanol alone or saccharin + ethanol when rats were administered 1.0 g/kg ethanol (two-way ANOVA with repeated measures, main effect of sweetener $F(2,54)=3.36$, $P>0.05$). Specifically, animals treated with sucrose + ethanol had lower blood ethanol concentrations compared to animals treated with ethanol alone or saccharin + ethanol (orthogonal comparison (sucrose + ethanol compared to

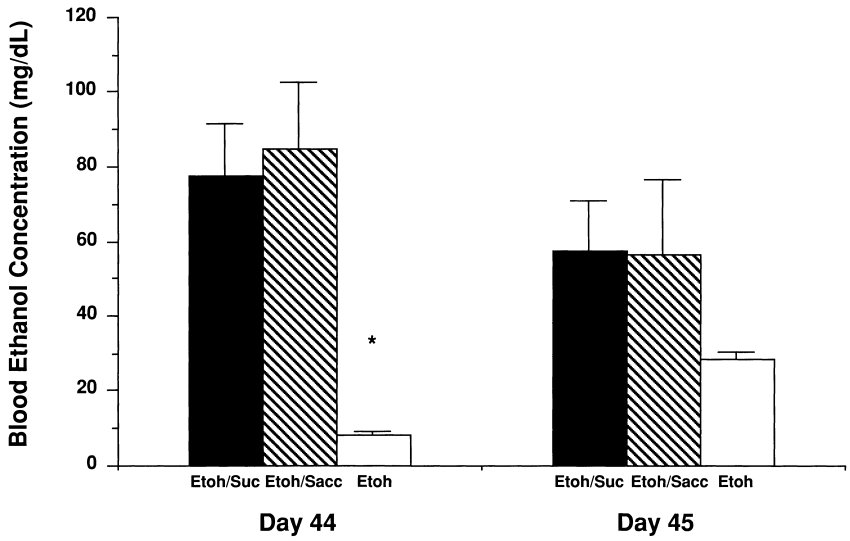
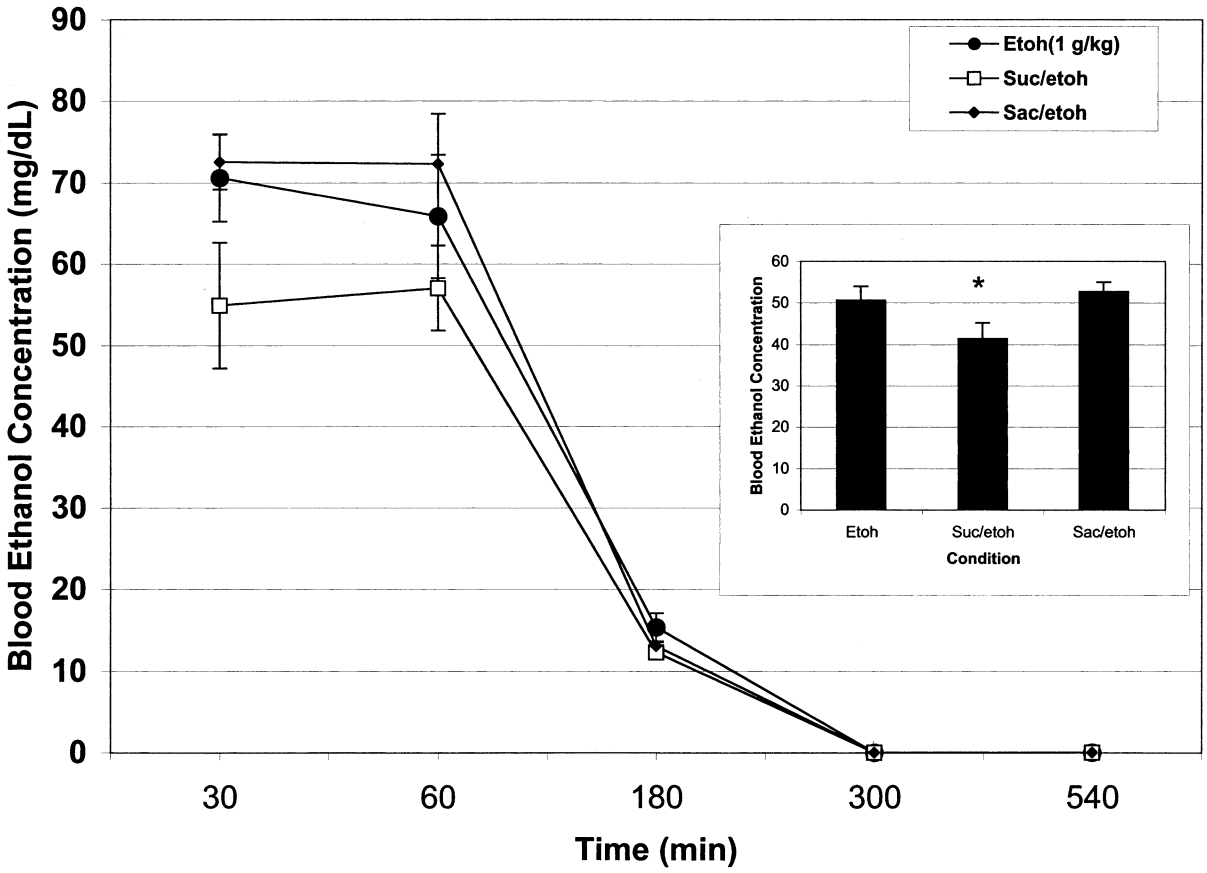


Fig. 2. Mean blood ethanol levels obtained on 2 separate days by rats in the Suc/Etoh, Sacc/Etoh, and ethanol only conditions. Rats in the three conditions had significantly different blood ethanol conditions on the Day 44 measurement, but no significant differences in BAC were found on the Day 45 measurement even though animals in the sucrose + ethanol condition had greater ethanol intakes. Data are plotted as means ± standard error of the mean (S.E.M.). * $P<0.05$.


Etoh(1 g/kg)
Suc/etoh
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Blood Ethanol Concentration (mg/dL)
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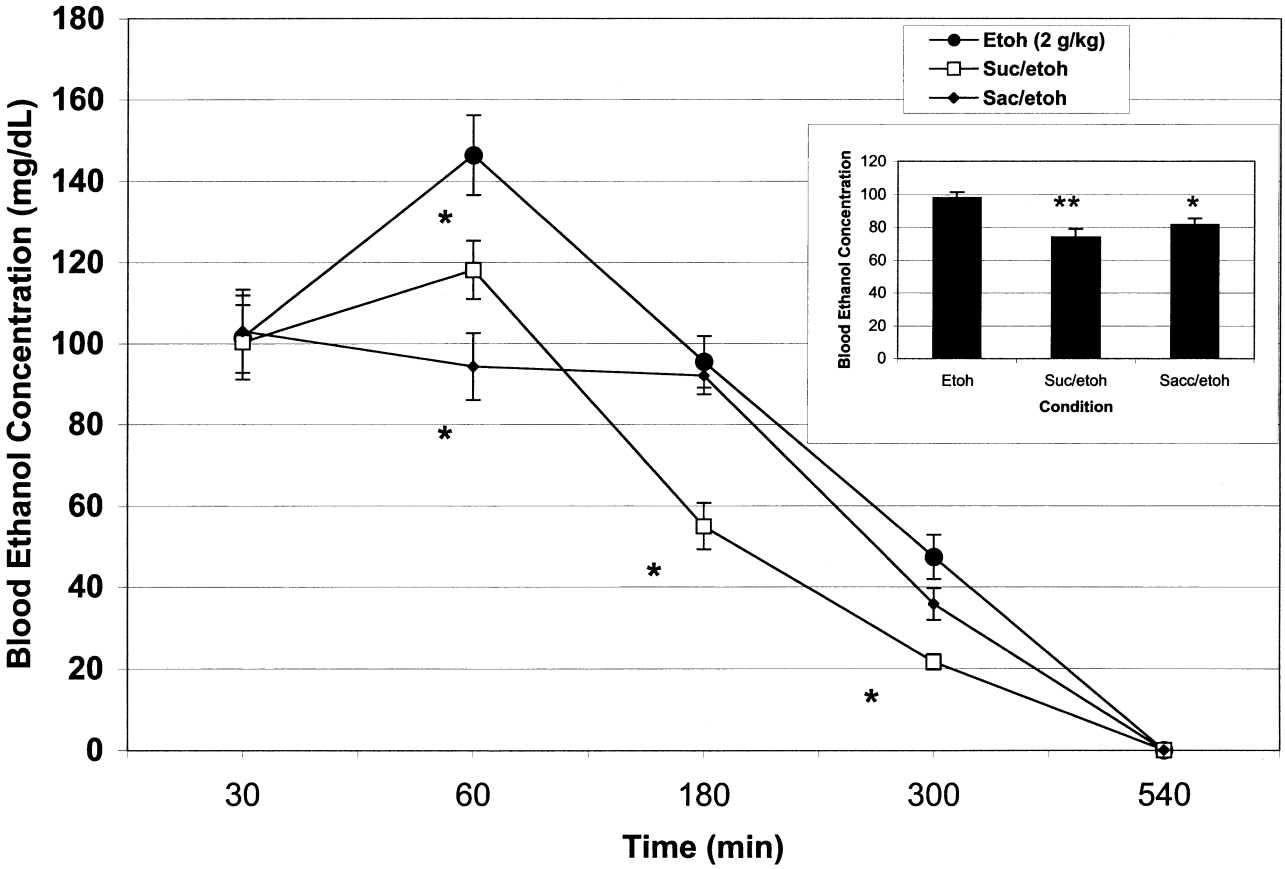
Etoh (2 g/kg)
Suc/etoh
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Blood Ethanol Concentration (mg/dL)
Time (min)
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Condition
Etoh
Suc/etoh
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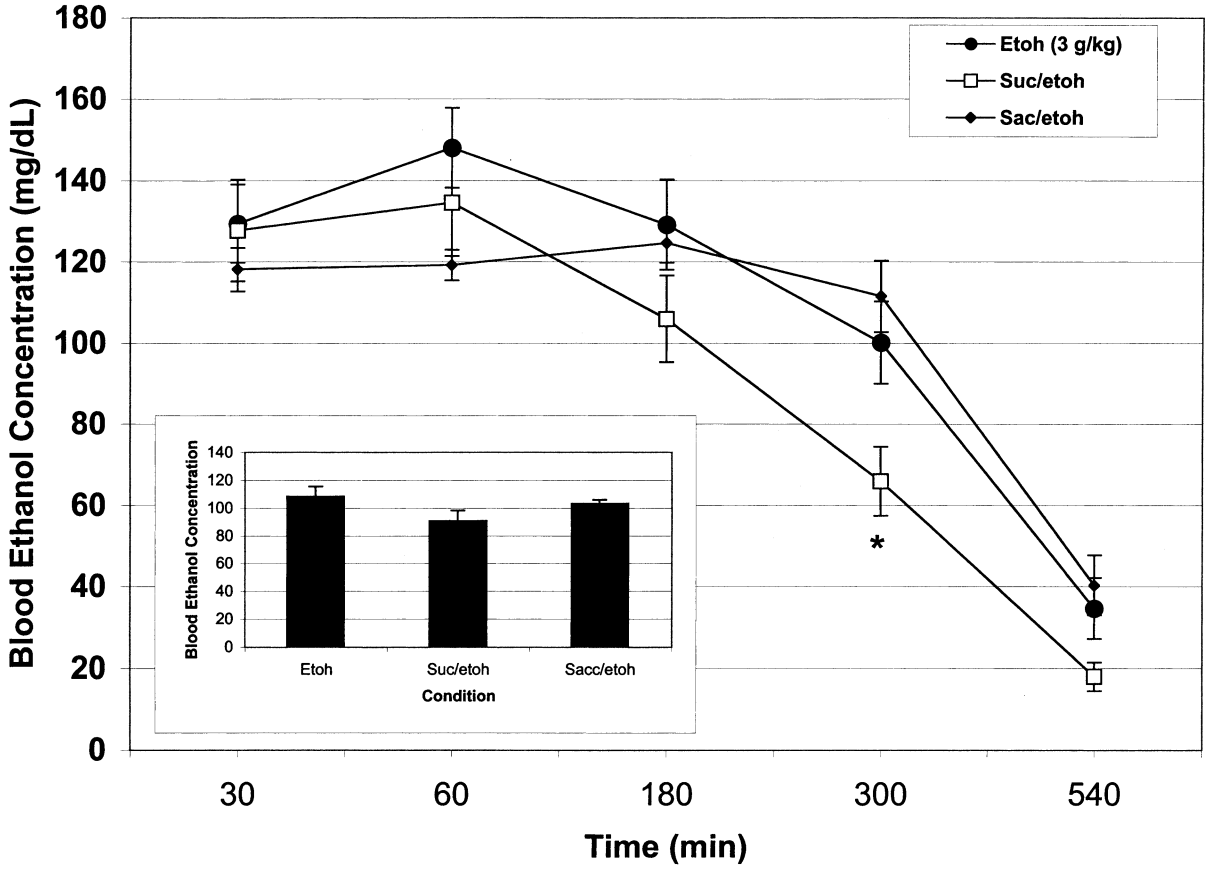

Fig. 3. (A) Mean blood ethanol levels following gavage of 1.0 g/kg ethanol in either 10% sucrose or 0.125% saccharin. The addition of sucrose lowered the blood ethanol consumption at all time points. Insert: Sucrose produced an overall reduction in blood ethanol concentrations compared to animals consuming ethanol only or ethanol + saccharin. Suc/Etoh, $n = 10$; Sacc/Etoh, $n = 10$; ethanol only, $n = 10$. * $P < 0.05$. (B) Mean blood ethanol levels following gavage of 2.0 g/kg ethanol in either 10% sucrose or 0.125% saccharin. A significant difference was found in blood ethanol levels depending upon sweetener (sucrose, saccharin, or tap water). Suc/Etoh, $n = 10$; Sac/Etoh, $n = 10$; ethanol only $n = 10$. * Indicates significantly different from ethanol only, $P < 0.05$. Insert: Sucrose and saccharin produced an overall decrease in blood ethanol concentrations compared to animals consuming ethanol alone. * $P < 0.05$, * * $P < .01$. (C) Mean blood ethanol levels following gavage of 3.0 g/kg ethanol in either 10% sucrose or 0.125% saccharin. A significant difference was found in blood ethanol levels depending upon sweetener (sucrose, saccharin, or tap water). Insert: Sucrose produced an overall reduction in blood ethanol concentrations compared to animals consuming ethanol only or ethanol + saccharin. Suc/Etoh, $n = 10$; Sac/Etoh, $n = 10$; ethanol only, $n = 10$. * Indicates significantly different from ethanol only, $P < 0.05$.

ethanol only and saccharin + ethanol combined); $t(28) = 2.598$, $P < 0.02$). The addition of saccharin did not alter blood ethanol concentration compared to ethanol alone ($t(18) = 0.50$, $P > 0.50$) (see Fig. 3A).

The addition of sweeteners significantly reduced the blood alcohol concentrations when rats were gavaged with 2.0 g/kg ethanol (two-way ANOVA with repeated measures, $F(6,72) = 4.616$, $P > 0.0006$) (see Fig. 3B). The addition of sweeteners significantly effected the resultant blood alcohol concentration (main effect sweetener, $F(2,72) = 10.92$, $P < 0.0003$). No significant difference in blood alcohol concentration was found at 30 min postgavage indicating that the addition of sucrose or saccharin did not alter ethanol absorption. However, the addition of sucrose resulted in significantly lower blood alcohol concentrations at 60 min (one-way ANOVA, $F(2,29) = 9.37$, $P < 0.0009$), 180 min (one-way ANOVA, $F(2,29) = 13.61$, $P < 0.0002$), and 300 min postgavage (one-way ANOVA, $F(2,29) = 10.42$, $P < 0.0005$). Specifically, the addition of both sucrose (Newman–Keuls test $q = 3.308$, $P < 0.05$) and saccharin (Newman–Keuls test $q = 6.115$, $P < 0.01$) in the ethanol gavage solution reduced blood alcohol concentrations at 60 min compared to ethanol alone. Sucrose also reduced blood alcohol concentrations at 180 min (Newman–Keuls test $q = 6.87$, $P < 0.001$ compared to ethanol only and $q = 5.767$, $P < 0.001$ compared to saccharin and ethanol). Finally, sucrose reduced blood alcohol concentrations at 300 min (Newman–Keuls test $q = 6.444$, $P < 0.001$ compared to ethanol only and $q = 3.543$, $P < 0.05$ compared to saccharin and ethanol). Hence, the addition of 10% sucrose increased ethanol metabolism when animals were gavaged with 2.0 g/kg ethanol.

The addition of sweeteners also significantly reduced the blood alcohol concentrations when rats were gavaged with 3.0 g/kg ethanol (two-way ANOVA with repeated measures, $F(8,90) = 2.91$, $P > 0.007$) (see Fig. 3C). The addition of

sweeteners significantly altered the resultant blood alcohol concentration at some time points (main effect sweetener, $F(2,90) = 3.345$, $P < 0.05$). No significant difference in blood alcohol concentration was found at 30, 60 or 180 min postgavage indicating that the addition of sucrose or saccharin did not alter ethanol absorption. However, the addition of sweeteners significantly decreased blood ethanol concentrations at 300 min postgavage (one-way ANOVA, $F(2,29) = 6.613$, $P < 0.005$) and marginally decreased blood ethanol concentrations at 520 min postgavage (one-way ANOVA, $F(2,29) = 3.242$, $P < 0.06$). Specifically, at 300 min postgavage, the addition of 10% sucrose resulted in a significant decease in blood alcohol levels (Newman–Keuls test, $q = 3.706$, $P < 0.05$ compared to ethanol only and $q = 4.941$, $P < 0.01$ compared to saccharin and ethanol).

Finally, ethanol self-administration for 45 days did not produce ethanol dependence as assessed by the determination of bicuculline seizure threshold during ethanol withdrawal. Specifically, no significant reduction in the amount of bicuculline (mg/kg) needed to induce seizures was observed following withdrawal of the sweetened ethanol solution. The mean threshold to seize was 0.187 (±0.017 (S.E.M.)) and 0.20 (±0.21) mg/kg bicuculline for animals in the saccharin and sucrose conditions, while the mean threshold for the Suc/Etoh, Sacc/Etoh, and ethanol only conditions were 0.218 (±0.03), 0.208 (±0.01), and 0.21(±0.03), respectively.

3. Discussion

Rats self-administered significantly more ethanol contained in a sucrose-sweetened solution than ethanol contained in a saccharin-sweetened solution or in tap water. However, no significant difference was found in blood ethanol levels regardless of whether the ethanol was mixed with sucrose or saccharin. Furthermore, gavaging animals with either 1.0, 2.0, or 3.0 g/kg ethanol mixed with 10% sucrose resulted in lower blood alcohol levels compared to blood alcohol levels obtained from animals gavaged with either ethanol mixed with saccharin or tap water.

Several mechanisms might account for the paradox between significantly greater ethanol intake of the sucrose + ethanol mix without a corresponding increase in the resultant blood ethanol levels. One possibility is that sucrose, compared to saccharin, impedes the absorption of ethanol from the small intestine, a portion of the gastrointestinal tract in which ethanol absorption is significant (Halsted et al., 1973; Israel et al., 1969). However, it is unlikely that this is the case. Blood ethanol levels from rats gavaged with 1.0, 2.0, or 3.0 g/kg ethanol were not significantly different at 30 min postgavage regardless of whether ethanol was mixed with sucrose, saccharin or tap water. Hence, it is not likely that sucrose decreases ethanol absorption from the gut. Furthermore, if a sucrose + ethanol solution is infused directly into the small intestine of a rat, ethanol absorption is actually augmented compared to infusion of ethanol alone (Broitman et al., 1976).

A second possibility is that sucrose increases ethanol metabolism, a mechanism that could account for a sucrose + ethanol solution resulting in significantly lower blood ethanol at some time points greater than 30 min postgavage. This possibly has received mixed support. For example, it has been shown that high levels of sucrose can increase ethanol metabolism in man (Pawan, 1968) and possibly rats (Broitman et al., 1976), perhaps by "buffering" the alcohol-oxidizing system (Wedel et al., 1991). However, it has also been shown that sucrose has no effect on ethanol metabolism in rats (Rawat, 1977). These disparate results may be due to procedural differences. Rawat (1977) administered ethanol via a stomach tube then infused sucrose into the blood after peak blood ethanol levels were obtained. It is possible that sucrose must be present in the gut during the rising phase of the blood ethanol curve to affect blood ethanol levels. The results obtained from gavaging animals with either 1.0, 2.0, or 3.0 g/kg ethanol support the hypothesis that 10% sucrose increases ethanol metabolism. Specifically, blood alcohol levels were significantly decreased at 60, 180, and 300 min following gavage of 2.0 g/kg ethanol + sucrose indicating that the addition of sucrose increased overall metabolism thereby reducing blood ethanol concentrations. When animals were gavaged with 3.0 g/kg ethanol, the resultant blood alcohol concentrations from the sucrose condition were also significantly decreased following 300 min and marginally decreased following 520 min.

A third possibility is that sucrose decreases gastric emptying, a phenomenon that has been shown to lower blood ethanol levels (Kalant, 1971; Miller et al., 1966; Wedel et al., 1991). Therefore, one model explaining the present data is that ingestion of ethanol with sucrose delayed gastric emptying and increased ethanol metabolism relative to ingesting ethanol by itself. If such were the case, then it would be predicted that while a greater ethanol intake level would be obtained with a sucrose solution, compared to an unsweetened solution, no difference in blood ethanol levels would result. This prediction is also consistent with the present results.

Rats self-administering ethanol + sucrose displayed lower blood ethanol concentration compared to rats self-administering either ethanol + saccharin or ethanol alone when BAC was determined on Day 44 or Day 45. However, because ethanol intake was only recorded every 24 h, it is unclear if this difference represents differences in drinking patterns or difference in ethanol metabolism. Consequently, we determined BACs following gavage of three ethanol concentrations. While the addition of 10% sucrose significantly decreased BAC at each concentration of ethanol, the BACs following gavage of 1.0 g/kg ethanol are of particular interest because the BACs most closely match those obtained by self-administering animals. As previously described, the addition of 10% sucrose signifi-

cantly decreased BACs following gavage of 1.0 g/kg ethanol (see Fig. 3A). This suggests that even though animals will self-administer greater amounts of a sucrose + ethanol solution, the pharmacological impact of the greater intake is dubious.

Several recent studies have investigated the effect of sucrose on blood alcohol levels (Czachowski et al., 1999; Gauvin, 1999; Roberts et al., 1999). These studies have provided contradictory evidence indicating that sucrose significantly decreases blood alcohol levels (Roberts et al., 1999) or that sucrose does not alter blood alcohol levels (Czachowski et al., 1999; Gauvin, 1999). We believe that the data reported herein provide a more complete investigation of the effect of sucrose on blood alcohol concentrations. Czachowski et al. (1999) and Gauvin (1999) both report that sucrose does not alter blood alcohol concentrations 30 min following either moderate ethanol intake ($\sim$ 1.6 g/kg per 30 min operant session) or 2.0 g/kg intragastric administration. The gavage data reported herein also revealed no significant difference 30 min following gavage of either sucrose + ethanol, saccharin + ethanol, or ethanol alone (see Fig. 3). However, a significant difference is found at time points longer than 30 min. Furthermore, Czachowski et al. (1999) and Gauvin (1999) never used sucrose concentrations greater than 5%. Hence, 10% sucrose may alter blood alcohol levels to a greater extent than 5% sucrose.

The present study supports recent data obtained using a limited ethanol access procedure (Roberts et al., 1999). In this study, rats could operantly respond for ethanol mixed in sucrose, saccharin or tap water for 30 min. They found that the resultant blood ethanol levels were significantly lower in rats that were responding for the ethanol/sucrose mix compared to the other two groups while the blood ethanol levels from the ethanol/saccharin group did not differ from the ethanol only group.

Forced chronic ethanol consumption for 14 days has been shown to produce ethanol dependence as determined by a significant decrease in bicuculline-induced seizure thresholds 6 h following ethanol withdrawal (Devaud et al., 1995). Furthermore, long-term (6 weeks) voluntary chronic ethanol self-administration by alcohol preferring P rats produce ethanol dependence as measured by a significant decrease in bicuculline-induced seizure thresholds (Kampov-Polevoy et al., 2000). However, the present data show that chronic ethanol self-administration for 45 days in rats that do not prefer alcohol does not alter bicuculline seizure thresholds, even in rats self-administering relatively high amounts of ethanol (i.e., the ethanol + sucrose group). This result suggests that using sucrose + ethanol as a model to produce physical dependence has severe limitations.

In summary, rats self-administered significantly more ethanol (i.e., greater ethanol intake) in a sucrose solution compared to ethanol in a saccharin solution. However, no significant differences in blood ethanol levels were found. Furthermore, it was demonstrated that a 10% sucrose concentration significantly decreased blood alcohol concentrations following gavage of 1.0, 2.0 or 3.0 g/kg ethanol. The reduced blood ethanol concentrations are likely due to increased metabolism of ethanol. These results suggest that a complex interaction between ethanol dose, sweeteners, and ethanol metabolism.

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References

Becker HC, Hale RL. Repeated episodes of ethanol withdrawal potentiate the severity of subsequent withdrawal seizures: an animal model of alcohol withdrawal "kindling". Alcohol: Clin Exp Res 1993;17:94–8.

Broitman SA, Gottlieb LS, Vitale JJ. Augmentation of ethanol absorption by mono- and disaccharides. Gastroenterology 1976;70:1101–7.

Cicero TJ. A critique of animal analogues of alcoholism. In: Majchrowicz E, Noble EP, editors. Biochemistry and pharmacology of ethanol vol. 2. New York: Plenum, 1979. pp. 533–60.

Czachowski CL, Samson HH, Denning CE. Blood ethanol concentrations in rats drinking sucrose + ethanol solutions. Alcohol: Clin Exp Res 1999;23:1331–5.

Devaud LL, Purdy RH, Morrow AL. The neurosteriod, 3α-hydroxy-5α-pregnane-20-one, protects against bicuculline seizures during ethanol withdrawal in rats. Alcohol: Clin Exp Res 1995;19:350–6.

Files FF, Denning CE, Hyytia P, Kiianmaa K, Samson HH. Ethanol-reinforced responding by AA and ANA rats following the sucrose-substitution initiation procedure. Alcohol: Clin Exp Res 1997;21:749–53.

Gatto GJ, Murphy JM, McBride WJ, Lumeng L, Li TK. Effects of fluoxetine and desipramine on palatability-induced ethanol consumption in the alcohol-nonpreferring (NP) line of rats. Alcohol 1990;7:531–6.

Gauvin DV. Blood alcohol concentrations in rats drinking or intubated with ethanol. Alcohol: Clin Exp Res 1999;23:1945–7.

Halsted CH, Robles EA, Mezey E. Distribution of ethanol in the human gastrointestinal tract. Am J Clin Nutr 1973;26:831–4.

Hodge CW, Samson HH, Lewis RS, Erickson HL. Specific decreases in ethanol- but not water-reinforced responding produced by the 5-HT_3 antagonist ICS 205–930. Alcohol 1993;10:191–6.

Kalant H. Absorption, diffusion, distribution, and elimination of ethanol. In: Kissin I, Begleiter H, editors. The biology of alcoholism: vol. 1. Biochemistry. New York: Plenum, 1971. pp. 1–24.

Kampov-Polevoy AB, Matthews DB, Gause L, Morrow AL, Overstreet DH. P rats develop physical dependence on alcohol via voluntary drinking: changes in seizure thresholds, anxiety, and patterns of alcohol drinking. Alcohol: Clin Exp Res 2000;24:278–84.

Israel Y, Valenzuela JE, Salazar I, Ugarte G. Alcohol and amino acid transport in the human small intestine. J Nutr 1969;98:222–4.

Mascord D, Smith J, Starmer GA, Whitfield JB. Effect of oral glucose on the rate of metabolism of ethanol in humans. Alcohol Alcohol. 1988;23:365–70.

Miller DS, Stirling JL, Yudkin J. Effect of ingestion of milk on concentrations of blood alcohol. Nature 1966;212:1051.

Morrow AL, Herbert JS, Montpied P. Differential effects of chronic ethanol administration on $GABA_A$ receptor α_1 and α_6 subunit mRNA in rat cerebellum. J Mol Cell Neurosci 1992;3:251–8.

Pawan GLS. Vitamins, sugars and ethanol metabolism in man. Nature 1968;220:374–6.

Rawat AK. The effects of fructose and other substances on acetaldehyde and ethanol metabolism in the rat. Res Commun Chem Pathol Pharmacol 1977;18:665–76.

Rezvani AH, Grady DR. Suppression of alcohol consumption by fenfluramine in Fawn–Hooded rats with serotonin dysfunction. Pharmacol, Biochem Behav 1994;48:105–10.

Roberts AJ, Heyser CJ, Koob GF. Operant self-administration of sweetened versus unsweetened ethanol: effects on blood alcohol levels. Alcohol: Clin Exp Res 1999;23:1151–7.

Samson HH. Initiation of ethanol reinforcement using a sucrose-substitution procedure in food- and water-sated rats. Alcohol: Clin Exp Res 1986;10:436–42.

Samson HH, Tolliver GA, Lumeng L, Li TK. Ethanol reinforcement in the alcohol nonpreferring rat: initiation using behavioral techniques without food restriction. Alcohol: Clin Exp Res 1989;13:378–85.

Samson HH, Files FJ, Denning C, Marvin S. The use of sucrose + ethanol solutions in operant continuous-access situations to model high ethanol consumption. Alcohol: Clin Exp Res 1997;21:108A.

Schwarz-Stevens K, Samson HH, Tolliver GA, Lumeng L, Li TK. The effects of ethanol initiation procedures on ethanol reinforced behavior in the alcohol-preferring rat. Alcohol: Clin Exp Res 1991;15:277–85.

Sedman AJ, Wilkinson PK, Sakmar E, Weidler DJ, Wagner JG. Food effects on absorption and metabolism of alcohol. J Stud Alcohol 1976;37:1197–214.

Sellers EM, Kalant H. Alcohol intoxication and withdrawal. N Eng J Med 1976;294:757–62.

Slawecki CJ, Samson HH, Hodge CW. Differential changes in sucrose + ethanol and sucrose maintained responding by independently altering ethanol or sucrose concentrations. Alcohol: Clin Exp Res 1997;21:250–60.

Waller MB, McBride WJ, Lumeng L, Li TK. Induction of dependence on ethanol by free-choice drinking in alcohol-preferring rats. Pharmacol, Biochem Behav 1982;16:501–7.

Wedel M, Pieters JE, Pikaar NA, Ockhuizen T. Application of a three-compartment model to a study of the effects of sex, alcohol dose and concentration, exercise and food consumption on the pharmacokinetics of ethanol in healthy volunteers. Alcohol Alcohol. 1991;26:329–36.