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Effects of Intravenous Ethanol and of 4-Methylpyrazole on Alcohol Drinking in Alcohol-Preferring Rats[1]

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WALLER, M. B., W. J. McBRIDE, L. LUMENG AND T.-K. LI. *Effects of intravenous ethanol and of 4-methylpyrazole on alcohol drinking in alcohol-preferring rats.* PHARMAC. BIOCHEM. BEHAV. 17(4) 763–768, 1982.—Studies were undertaken to determine if elevated blood alcohol concentrations (BAC), produced by intravenous (IV) infusion of ethanol or by intraperitoneal (IP) administration of 4-methylpyrazole (4-MP), could reduce the free-choice oral alcohol consumption of adult male alcohol-preferring rats (P-rats). The IV infusion of ethanol either on a 24 or 12 (dark) hourly dose schedule reduced the amount of ethanol voluntarily ingested. There was a significant ($p<0.05$) inverse correlation between the amount of ethanol consumed orally and the amount of ethanol infused. Daily fluid and caloric intakes were not compromised. When the amount of ethanol infused was 85% or more of the control oral intake, there was a significant correlation between ethanol intake and tail-blood alcohol levels, taken at 5 min ($r=0.98$; $p<0.05$) and 55 min ($r=0.93$, $p<0.05$) after the last dark cycle infusion. Below the preinfusion level of 85%, the BAC were variable and did not correlate well with total ethanol intake. After a single IP injection of 4-MP, 90 mg/kg body wt, BAC increased from 10 mg% to 50–65 mg% for 2–3 days. Concomitant with the rise in BAC, these animals decreased their drinking of 10% ethanol and proportionately increased their water intake. The present studies suggest that pharmacological factors, distinct from orosensory cues, are important in regulating voluntary ethanol drinking behavior in the P-rats.

Alcohol-preferring rats Alcohol-drinking behavior Intravenous ethanol infusion 4-Methylpyrazole administration

TWO lines of rats, one with a natural preference for alcohol, the P line, and the other with an aversion to drinking alcohol, the NP line, have been selectively bred in our laboratories [9,12]. When food, water and a 10% (v/v) ethanol solution are freely available, the amount of alcohol consumed by the male P and NP rats (S-12 generation) are 6.3±0.3 (mean±SEM) and 1.0±0.3 g of absolute ethanol/kg body weight/day, respectively. The daily consumption of alcohol by the P rats approaches their alcohol metabolic rate and consistently elevates their blood ethanol concentrations during the dark cycle [8]. The amount of alcohol consumed daily also remains constant when the concentration of the alcohol drinking solution is increased from 10 to 30% [12]. Additionally, in an operant situation, the P rats work to obtain 10% ethanol from a dipper when water and food are freely available [17]. These findings collectively indicate that the P rats have a natural consummatory drive for alcohol. Furthermore, it has been shown recently that the P rats develop physical dependence and the alcohol withdrawal syndrome, after chronic voluntary consumption of 10% ethanol for 15 to 20 weeks [20].

As an oral self-administration animal model, the P rats are uniquely suited for studies of the factors that govern alcohol drinking behavior. Previously, we have demonstrated that free-choice alcohol ingestion by the P rats can be increased by caloric deprivation or by the addition of a sweetener to the alcohol drinking solution [11]. The latter findings suggest that orosensory cues are important factors that limit and promote drinking. On the other hand, post-ingestional factors, such as the CNS pharmacologic effects of ethanol, may also play a role. In the studies reported here, we have attempted to dissociate orosensory cues from the pharmacological actions of ethanol in governing free-choice alcohol drinking by use of two experimental approaches. The first utilizes intravenous infusion of ethanol to minimize orosensory cues and examines its effect on the daily free-

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choice drinking of 10% ethanol and on blood ethanol concentrations. The second approach uses 4-methylpyrazole, an alcohol dehydrogenase inhibitor [2,7], to raise blood alcohol concentrations and determines its effect on the free-choice consumption of ethanol.

METHOD

Procedure for Intravenous Infusion of Ethanol

Experiment 1. Four adult male P rats (384–470 g) of the S-12 generation were individually housed in a temperature- and humidity-controlled environment with 12 hour day-night cycles. Powdered food (Wayne Lab-Blox; Allied Mills, Inc., Chicago, IL), presented in spill-resistant feeders, water and a solution of 10% (v/v) ethanol were freely available throughout the experiment. For 5–6 days, the amount of food, water and 10% ethanol consumed was monitored to establish baseline values. After this preinfusion control period, a polyethylene catheter was implanted in the external jugular vein for chronic intravenous infusions [18]. The catheter was held by a harness-swivel assembly [5] that allowed the rat to move freely about the cage. Following the operation, 0.9% NaCl containing heparin was infused hourly (0.1 ml delivered over 10 sec) to prevent clogging of the catheter and the ingestive behavior was again monitored until baseline values were reached.

When baseline drinking was reestablished, hourly intravenous infusions of ethanol were started via the indwelled catheter. The amount of ethanol infused was calculated for each animal based on its mean daily ingestion (g of ethanol/kg body weight/day) during the preinfusion period and was expressed as percent of its control oral ethanol intake. Daily infusions in 24 doses totalling 20, 35, 45, 65, 85, 110 and 130% of the preinfusion daily voluntary oral intake of ethanol were presented in ascending order. Ethanol solutions were prepared in 0.9% NaCl at concentrations that delivered the hourly required amount in 0.1–0.4 ml infused over 10–40 sec. Thus the total fluid infused ranged from 2.4–9.6 ml/day. Each solution was infused for three days, whereupon the ethanol concentration was adjusted to deliver the next higher dose of infusion. Immediately following the three day infusion of the highest ethanol concentration, saline was again infused in these animals. Food, water and ethanol consumptions were monitored throughout the experiment and for the 4–7 days of a post-alcohol infusion control period.

The calories derived from ethanol (oral and intravenous) and food were calculated using the values 7.0 kcal/g for ethanol and 3.0 kcal/g for chow (personal communication, Allied Mills, Inc.), respectively.

A second group of six adult male P rats (340–400 g) was also studied with the above regimen. However, the daily infusions of ethanol were extended to 150 and 175% of the preinfusion voluntary oral intake and the post-alcohol infusion period was deleted. In addition, tail blood samples for ethanol determinations were collected at 5 and 55 min after the last infusion of each dose of ethanol during the dark cycle.

Experiment 2. The procedural details were the same as those described for Experiment 1 except that four adult male P rats were given 12 hourly infusions of ethanol delivered only during the dark period of the light-dark cycle to total 75, 100, 125 or 150% of the average preinfusion daily ethanol consumption.

Similarly, a second group of three adult male P rats was used for determining blood alcohol levels at 5 and 55 min after the last infusion of doses of ethanol totalling 100, 125 and 150% of the daily preinfusion oral consumption.

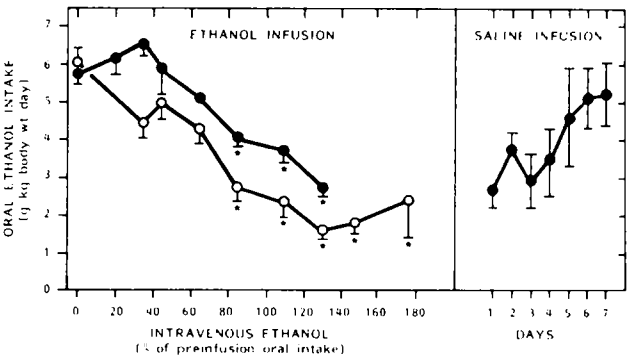

FIG. 1. Effects of hourly intravenous ethanol infusions (24 doses/day) on voluntary oral ethanol (10% v/v) intake by P-rats. The amount of ethanol infused intravenously is expressed on the abscissa as a percent of the mean oral intake determined during a preinfusion control period. Two separate groups of P-rats were used for this experiment. The first group (N=4) is indicated by the dark circles and the second group (N=6) by the open circles. Animals from the first group (dark circles) received the post-alcohol saline infusion immediately following infusion of the last ethanol concentration. $^*p<0.05$ with respect to preinfusion value.

Procedure for Administration of 4-Methylpyrazole

Experiment 3. Three male P rats (475–525 g) were individually caged as described earlier. They had been given free access to food, water and 10% ethanol for two weeks before the experiment. In the first three days of the experiment, tail blood ethanol concentrations and food and fluid intake were measured to establish baseline values. On the fourth day, a single intraperitoneal dose of 4-methylpyrazole (4-MP), 90 mg/kg body weight, or saline was injected. This dose, as determined from dose-response curves performed in other animals, was found to inhibit voluntary intake of alcohol solutions without compromising food and fluid intake. Data were then collected for an additional six days to assess the effect of the single injection of the alcohol dehydrogenase inhibitor.

Blood Ethanol Concentration Measurements

Tail blood samples were collected in heparinized capillary tubes. After centrifugation, the plasma fractions were sampled for ethanol determination by direct injection into a Hewlett-Packard 5730A gas chromatograph supplied with a flame ionization detector and a 3380A integrator. The glass columns were packed with 50% Porapak Q and 50% Porapak R (100/120 mesh) and the oven temperature was 105°. n-Propanol was used as the internal standard.

Statistical Analysis

Analysis of variance and Newman-Keuls tests were used for multiple comparisons, while the Student's *t*-test (2-tailed) was applied when two groups were compared. A linear regression equation was used to determine the correlation coefficients (r). The statistical significance of r was tested with the *t* distribution. The results are expressed as mean values ± SEM.

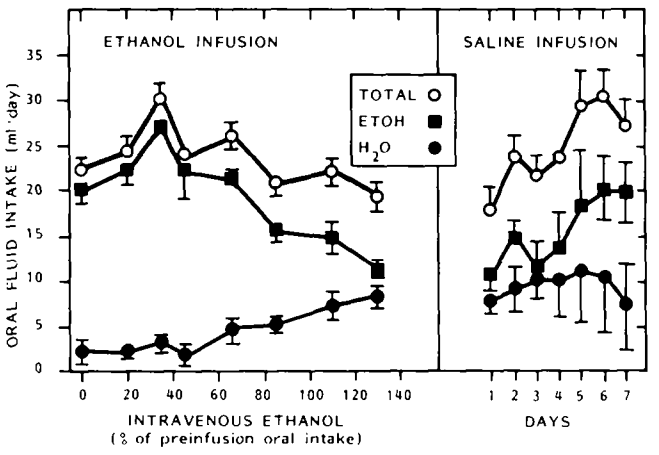

FIG. 2. Effect of hourly intravenous ethanol infusions (24 doses/day) on the voluntary oral consumption of 10% (v/v) ethanol, water and total fluid intake by P-rats from the first group (see Fig. 1 for details). The rats received the post-alcohol saline infusion immediately following infusion of the last ethanol concentration.

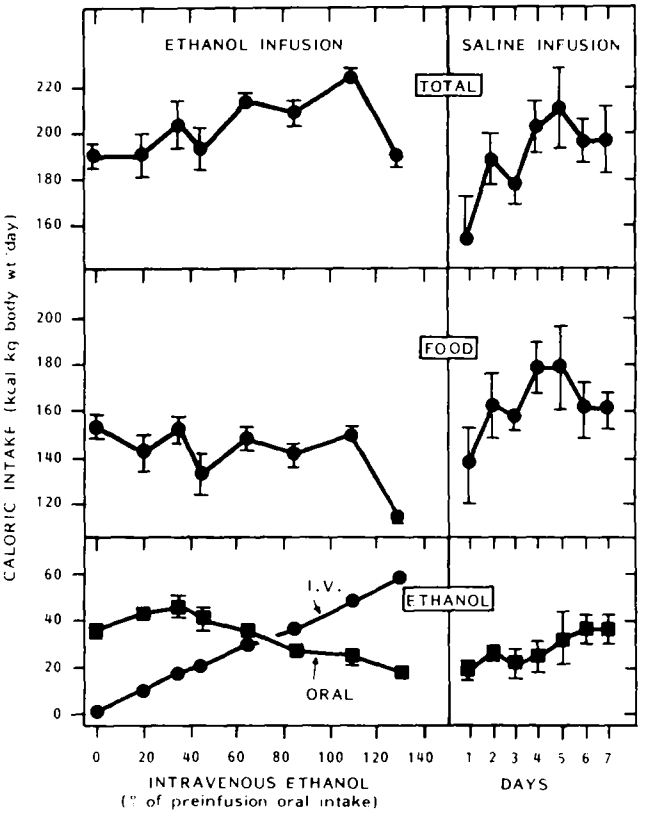

FIG. 3. Effect of 24 hourly intravenous of ethanol on the caloric intake of the P-rats from the first group (see Fig. 1 for details).

RESULTS

Effects of Intravenous Ethanol Infusion on Drinking Behavior

Experiment 1. Figure 1 shows the effect of intravenously infused ethanol given by the 24 hourly dose schedule upon the oral consumption of ethanol by P animals. For the first group (solid circles), voluntary drinking during the preinfusion control period averaged 5.8±0.3 g of ethanol/kg body weight/day, and individual alcohol consumptions ranged from 5.3±0.3 to 6.3±0.8 g/kg/day. The intravenous infusion of ethanol at 20, 35, 45 and 65% of the preinfusion daily oral intake did not significantly alter oral ethanol consumption. However, when the amount of ethanol infused increased to 85% or more of the control oral intake, the amount of ethanol voluntarily ingested decreased significantly ($p<0.05$) and eventually declined to 45% of the control level. There was a significant inverse correlation ($r=-0.70$; $p<0.005$) between the amount of ethanol infused intravenously and the amount of ethanol consumed orally. During the 7-day post-alcohol saline infusion period, the free-choice consumption of ethanol returned to 5.3±0.8 g/kg/day or 90% of the control value. On the last day of this period, individual drinking scores ranged between 4.6 to 6.7 g/kg/day.

Concurrent with the decrease in oral alcohol intake that accompanied ethanol infusion, the volume of water consumed increased, producing no net change in the total volume of fluids ingested (Fig. 2). Following the alcohol infusion period (saline infusion), the average amount of water consumed tended to remain elevated even though ethanol consumption returned to the control level. However, this period was characterized by such a wide degree of variability in the amounts of water consumed that the water intake was not significantly different from that before ethanol infusion (Fig. 2).

As the number of calories derived from the infused ethanol increased, that portion provided by alcohol ingestion decreased (Fig. 3). Solid food consumption remained stable except at the highest dose of ethanol infused. While this could reflect an aversive effect of the infused alcohol, it seems unlikely since the infusion of 125% and 150% of the control oral intake in Experiment 2 did not significantly alter food consumption. Additionally, at all other doses of ethanol infused, the total daily caloric intake was not adversely affected. After the alcohol infusion period, calories from both ethanol and food returned to control levels. There was, however, an initial depression of total caloric intake that may reflect the sudden loss of calories from the intravenously infused ethanol.

Similar results were obtained for the second group of P animals on the 24 hourly dose schedule (Fig. 1, open circles). The intravenous infusion of ethanol did not significantly alter the free-choice consumption of alcohol until the amount of ethanol infused was increased to 85% or more of the preinfusion oral intake. Thereafter, the amount of ethanol voluntarily ingested decreased significantly ($p<0.05$) and declined to 30–45% of the preinfusion level. The total daily intake (oral plus infusion) of alcohol was not significantly altered by the infusion of ethanol, except at the highest infusion levels, i.e., 150 and 175% (Fig. 4, top panel). Tail-blood alcohol levels were determined at 5 and 55 minutes after the last of the 24 hourly intravenous of ethanol for each of the different doses of ethanol (Fig. 4, bottom panel). Blood alcohol levels were highly variable and most values were below 50 mg% except at the highest infusion levels. Since 10% ethanol was freely available at all times, the alcohol in blood derives from both oral intake and intravenous infusion. When the amount

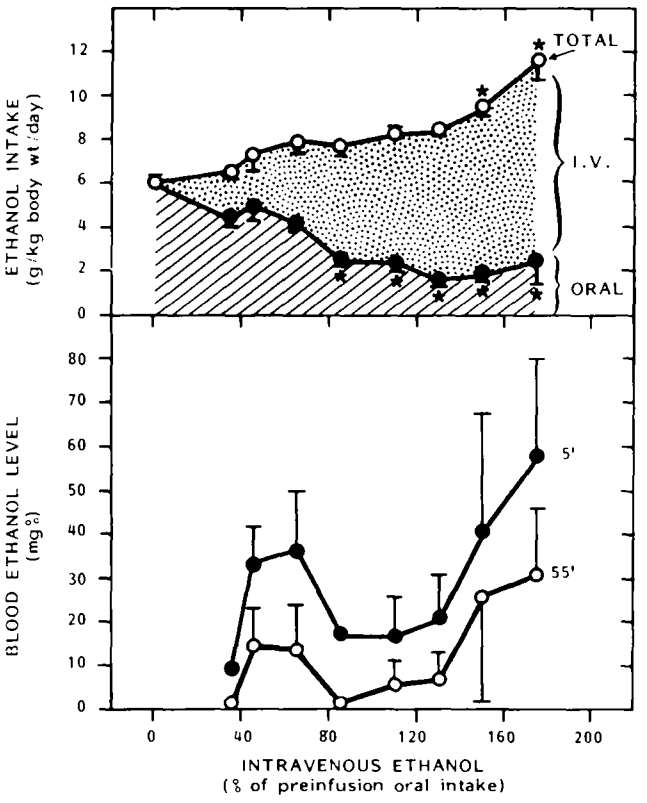

FIG. 4. Effect of 24 hourly infusions of ethanol on the total and oral intake of ethanol (top panel) and on the tail-blood alcohol levels (bottom panel) taken 5 and 55 minutes after the last dark-cycle infusion of ethanol into the second group of P-rats (see Fig. 1 for details). *$p<0.05$ with respect to preinfusion value.

of ethanol infused was less than 85% of the preinfusion oral intake and free-choice drinking of alcohol was unimpaired, the mean blood alcohol levels at 5 minutes post-infusion varied from 10 to 35 mg% (Fig. 4). When the amount of ethanol infused was greater than 85% of the preinfusion oral intake, there was a significant correlation between the total ethanol intake and the blood alcohol levels at both 5 min ($r=0.98$; $p<0.05$) and 55 min ($r=0.93$; $p<0.05$). However, the Newman-Keuls test revealed no statistically significant differences in blood alcohol concentrations across infusion levels at either 5 or 55 min after infusion. Most likely this reflects the large within and between animal variation arising from the uncontrolled, voluntary oral ethanol consumption of the animals.

Experiment 2. The P rats drink approximately 75% of their alcohol during the dark period [20]. Hence, the effect of intravenous ethanol given only during the dark hours was examined. In this experiment (data not shown but pattern similar to 24 hour schedule), the mean ethanol consumption of the four P animals prior to the ethanol infusion was 6.7±0.3 g/kg/day, and individual free-choice ingestion ranged from 6.1±0.7 to 7.2±0.5 g/kg/day. The intravenous infusion of ethanol during the 12 hr dark period totalling 75% or more of the amount ingested daily by each of the animals during the preinfusion period resulted in a significant ($p<0.05$) decline in oral consumption of ethanol. At the highest infusion level (150% of preinfusion oral intake), oral intake was decreased 63% from 6.7±0.3 to 2.5±0.4 g/kg/day. Ethanol ingestion declined significantly ($r=-0.67$, $p<0.005$) with increasing doses of intravenous ethanol, and the ingestion of ethanol returned to near the control level during the 4-day post-alcohol saline infusion period (6.2±0.8 g/kg/day on the third day). The patterns of caloric and fluid intakes were similar to those observed with the 24 hourly infusion groups in Experiment 1, and the total daily intakes were not significantly changed by the infusion of ethanol during only the dark period.

TABLE 1

BLOOD ALCOHOL CONCENTRATIONS IN P RATS AFTER THE LAST OF THE 12 HOURLY INFUSIONS OF SALINE OR ETHANOL GIVEN ONLY DURING THE DARK PERIOD

	Blood Ethanol Conc. (mg%) (mean ± S.E.M.; N=3)	
	5'	55'
Saline	8 ± 3	9 ± 4
100% Preinfusion Level*	42 ± 5	38 ± 14
125% Preinfusion Level	85 ± 38	83 ± 41
150% Preinfusion Level	73 ± 17	69 ± 25

*See Method section for description of percent of preinfusion oral intake.

Blood alcohol concentrations after the last of the 12 hourly infusions of ethanol totalling 100, 125 and 150% of the daily oral intake are shown in Table 1. The alcohol levels in the blood were higher than those produced by the 24 hourly infusion protocol (Fig. 4) and most values were below 100 mg%. This is because the amount of ethanol infused with each dose in this experiment is approximately twice that employed in Experiment 1.

Effect of 4-Methylpyrazole Administration on Drinking Behavior

Experiment 3. Experiments were also performed with the P rats (given free access to 10% ethanol and water) using 4-methylpyrazole (4-MP) administration to elevate blood ethanol levels. Before the administration of 4-MP, the P rats drank predominantly the 10% ethanol solution and their daily consumption of absolute ethanol averaged 5.1 g/kg/day. A typical animal maintained its blood ethanol concentrations in the range of 3 to 76 mg% and the mean concentration for this group of P rats was 10±3 mg%. After a single injection of 4-MP, the blood ethanol concentrations increased and this effect lasted variably for up to three days (Table 2). Concomitant with the rise in blood ethanol concentrations, these animals decreased their drinking of the 10% alcohol solution and increased their water intake such that their total fluid intake was not reduced. The effects of 4-MP were transient as both blood alcohol concentration and alcohol drinking behavior returned to pre-drug levels within five days after drug administration. In these experiments, injection of saline did not alter alcohol drinking scores and blood ethanol concentrations (data not shown).

DISCUSSION

The present studies indicate that the level of blood alco-

TABLE 2

EFFECT OF 4-METHYLPYRAZOLE ADMINISTRATION ON FREE-CHOICE ALCOHOL DRINKING BEHAVIOR AND BLOOD ETHANOL CONCENTRATIONS IN THE P RATS

Day		Ethanol Solution Consumed (ml)	Blood Ethanol Concentration† (mg%)
Pre-Drug		29 ± 3	10 ± 3
Post-Drug	1	23 ± 2	15 ± 11
	2	12 ± 1*	65 ± 21
	3	18 ± 3*	51 ± 20
	4	24 ± 3	18 ± 2
	5	26 ± 3	10 ± 4
	6	32 ± 1	10 ± 4

*Significantly different from control values, $p<0.01$.

†Tail blood alcohol levels were assayed every six hours and the values averaged for each animal for each day.

hol may be a factor that limits the free-choice alcohol drinking of the P rats. By either intravenous infusion of ethanol or administration of 4-MP, blood ethanol concentrations in excess of 50 mg% produced significant curtailment of voluntary alcohol drinking (Fig. 4, Table 2). Hourly intravenous infusions of ethanol either throughout the 24-hour day/night cycle (Figs. 1–4) or only during a 12-hour night cycle resulted in decreased oral consumption of alcohol. As the dose of ethanol administered intravenously was increased, the amount consumed orally decreased proportionately (Fig. 3). The decreased oral ethanol consumption was apparently not due to any deleterious effect of the ethanol infusions since neither total fluid intake nor daily caloric intake was seriously impaired (Figs. 2, 3). These results indicate that the P animals compensated for the amount of ethanol infused intravenously by drinking less and are similar to those previously reported for mice [13], rats [19], and monkeys [21]. If orosensory cues were the only determinant of ethanol ingestive behavior in the P rats, the infusion of ethanol might have been expected to have little or no effect on oral ethanol consumption.

It is uncertain whether ethanol *per se* or its product, acetaldehyde, is directly responsible for regulating alcohol ingestion. At the present, the dose-response relationship of acetaldehyde to its aversive and reinforcing properties in ethanol-drinking behavior is poorly defined. There is no question that high levels of acetaldehyde, e.g., in the alcohol-disulfiram reaction and in many Orientals who lack an isozyme of aldehyde dehydrogenase [4], can produce unpleasant symptoms and even untoward physical effects. On the other hand, a body of evidence also suggests that lower levels of acetaldehyde may play a role in promoting alcohol-drinking. Intraventricular injection of acetaldehyde and its condensation products has been reported to reinforce ethanol drinking in rats [1, 3, 14–16]. In agreement with previous reports, administration of 4-MP led to marked elevations of blood ethanol concentrations [2, 6, 10] and to a dramatic decrement in voluntary alcohol ingestion by the P rats (Table 2). However, since 4-MP is a potent inhibitor of alcohol dehydrogenase [7,10], this pharmacologic agent should decrease significantly blood acetaldehyde levels. The results, therefore, do not support the notion that acetaldehyde is the aversive factor that regulates alcohol drinking in these animals.

From the experiments reported here, it would appear that elevated blood levels of ethanol, *per se*, and not acetaldehyde, may be responsible for limiting ethanol-drinking in the P-line of rats. However, it is difficult to ascertain with accuracy what this upper limit of blood ethanol concentration may be. Highly variable within- and between-animal ethanol drinking patterns and delay in absorption of ethanol from gut into blood may be factors contributing to the problem. Although the results do not quantify the relative contributions of orosensory cues and the pharmacological effects of ethanol, they do show that pharmacological factors, distinct from orosensory cues, are important in regulating the voluntary ethanol drinking behavior of the P-rats.

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