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Ethanol Effect on Activation and Maintenance of Cold Stress Response in Immature Rats

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SPIERS, D. E., S. A. KELLEHER AND P. A. EICHEN. *Ethanol effect on activation and maintenance of cold stress response in immature rats.* PHARMACOL BIOCHEM BEHAV **54**(3) 555–564, 1996. – Rats at thermoneutral ambient temperature (T_a) exhibit change in thermoregulatory response to ethanol (EtOH) from 2 to 15 days of age. In the present study, rats at 2–3, 8–9, and 14–15 days of age were administered either saline or EtOH (4 g/kg b.wt.; IP) using two different routines to determine EtOH effect on specific cold defense mechanisms. Injection of EtOH in the first routine occurred after exposure to cold T_a, to determine effect on maintenance of cold thermogenesis. EtOH-induced metabolic depression increased from 3 to 8 days of age, with little change after this time. Injection of EtOH in the second routine was at thermoneutral T_a, followed at 20 min postinjection by rapid exposure to cold T_a to determine effect on activation of cold thermogenesis. EtOH-treatment delayed onset of cold thermogenesis at 2–3 and 14–15 days of age, and completely eliminated 8–9-day-old response to cold T_a. Rats exposed to cold T_a at 2–3 days of age exhibited a slower rate of EtOH absorption and lower blood EtOH concentration than rats in the older groups, to explain some age differences in EtOH response.

Thermoregulation Immature rat Cold stress Hypothermia Metabolic rate

TREATMENT of adult rats and mice with ethanol (EtOH) results in numerous short-term alterations in body function. An often cited change is the reduction in body temperature control, as determined from measurement of rectal or colonic temperature (T_{co}). The basic response is a monophasic hypothermia (2,8,17,26) of magnitude dependent on dose and thermal status of the animal (7,8,10,18,19,21,23,24,34) at the time of treatment. The primary reason for the reduction in T_{co} at thermoneutral and cold T_a is a rapid decrease in metabolic heat production (7,34), with little evidence of increased peripheral heat loss under these conditions (18,34).

Less information is available on the effect of EtOH on thermoregulatory ability of the immature rodent during the postnatal period, when there is rapid growth and development of the central nervous system (4). One difficulty associated with physiological studies of this period is the large change in homeothermic ability. A rat model that defines the ontogeny of homeothermic ability in the rat over the first 19 days following birth has been developed in our laboratory (30). Both rectal and tail skin temperatures at specific T_a increase in the rat during this period. Resting metabolic rate (RMR; $W \times m^{-2}$) is also higher in older rats, but the slope of increase in metabolic rate (MR) at cold T_a is reduced from 5 to 19 days of age due to less peripheral heat loss with improved thermal insulation (30). Likewise, EtOH effect on thermoregulatory ability changes during this period. Tests of EtOH responsiveness from 2 to 15 days of age at thermoneutral T_a show a dose-dependent decrease in MR with a concomitant reduction in T_{co} (32,33). The magnitude of this response increases with age to support the hypothesis of increased sensitivity to EtOH during this period.

There have been few studies to determine how EtOH alters specific age-related homeothermic mechanisms. Such studies would require testing animals under controlled thermal challenge conditions. Kruckeburg et al. (15) injected 6–16-day-old rats daily with 4 g EtOH/kg b.wt. at T_a of 24°C. This T_a constitutes a cold challenge for rats of this age (30), and would result in significant hypothermia with little additional change in body temperature. Therefore, it was not surprising that Kruckeburg et al. (15) concluded that the neonatal rat is unaf-

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fected by EtOH, with no significant difference in T_{co} of treated and nontreated animals. Our study examined specific mechanisms of the cold response, and tested the hypothesis that sensitivity to EtOH increases with age during early postnatal development. Rats received acute injection of saline or EtOH (4 g/kg b.wt.) at 2-3, 8-9, or 14-15 days of age, with measurement of oxygen consumption, colonic, and skin temperatures for 2 h postinjection. Different cold T_a were used in this study to produce the same thermoregulatory status for each age group (i.e., doubling of MR from thermoneutral level). It is not possible to compare rats of different age using absolute MR because this value is not the same across age for similar thermoregulatory condition [e.g., RMR at lower critical temperature in 5-7 and 13-15-day-old rats is 25.58 and 32.52 $W \times m^{-2}$, respectively; (30)]. In addition two exposure routines were used in the present study to test different physiological processes. We had determined in an earlier study using adult rats (34) that EtOH response at cold T_a was different if EtOH was injected before or after cold exposure. In the present study, EtOH effect on maintenance of heat production and heat conservation processes at cold T_a were tested by injecting animals after 1 h exposure to cold T_a. Also, EtOH effect on activation of these processes was determined by shifting animals from thermoneutral to cold T_a at 20 min postinjection of EtOH. Blood EtOH concentration (BEC) was measured in a third study to determine if age-related differences in EtOH response could be attributed to different BEC or rate of change in BEC.

METHOD

Drug-naive adult rats (albino *Rattus norvegicus*) of the Sprague-Dawley strain were used to establish a breeding colony. Environmental conditions for the animal room included a 12 L : 12 D cycle (0700 lights on) and air temperature maintained at 25 ± 2°C for the duration of the study. All animals had ad lib access to standard laboratory chow and tap water, and were housed in solid-bottomed plastic cages with nesting material. Pups in each litter were culled to nine within 2 days of birth and maintained with the dam at all times except during the test period.

Physiological Test Procedures

All rats were individually tested at 2-3, 8-9, or 14-15 days of age, with day 1 designated as the day of birth. Sample size for each group was set at seven to nine, with no animal tested more than once. Tests were only conducted from 1100-1600 hours to minimize known circadian differences in thermoregulatory ability (29).

Each animal was weighed to the nearest 0.1 g when removed from the litter for testing. Temperature probes and an injection tube were attached prior to testing. This allowed measurement of body temperature at several sites and injection of EtOH without animal handling. Such activity at the time of injection is known to enhance EtOH-induced hypothermia in rats (3,22). Temperature probes consisted of copper-constantan thermocouples (40-gauge), with reference junctions in a bath of melting ice, for measurement of colonic temperature (T_{co}; 10-20 mm beyond anal sphincter) and skin temperatures at tail (T_{tail}; middorsal at base), back (T_{back}; interscapular), and (T_{abdo}) abdominal sites. Thermocouples were attached to shaved skin sites with flexible collodion, which was previously determined to not impair heat loss (30). Injection of EtOH was through a polyethylene PE-10 tube (inside diameter, 0.28 mm; o.d., 0.61 mm) inserted into the peritoneal cavity using a 20-gauge stainless steel needle as a sleeve passing through the abdominal wall. Immediately following tube insertion, the steel sleeve was withdrawn, but the tube left in place. Each animal was then inserted into a horizontal, cylindrical test compartment within a water-jacketed, clear-plastic metabolic chamber (30). Air and wall temperatures of the cylinders were independently controlled to establish minimal temperature differences (0.1°C) within the test environment. These temperatures were determined using thermocouples and averaged for a reliable estimate of T_a. Each animal rested in a holder (polyethylene mesh; 39.2% open area) located within each cylinder to ensure adequate air movement around the body.

Two different cold exposure routines were used to test animals in the three age groups. In each case, rats remained at the initial T_a for a minimum of 1 h or until MR reached a steady-state level (i.e., 10 min of stable values) to ensure adjustment to the test environment. Rats in the first routine were exposed to cold T_a prior to injection of EtOH or saline and maintained at this level for 120 min postinjection. Cold T_a was set for each age group to produce the same physiological response prior to injection (i.e., 100% increase in MR above thermoneutral level). These temperatures for rats at 2-3, 8-9, and 14-15 days of age were 31, 28, and 25°C, respectively. Cold T_a for control and experimental animals in each age group were nearly identical and constant for the duration of the postinjection period. Rats tested using the second routine were first exposed to T_a within the thermoneutral zone for each age group [i.e., 36, 35, and 33°C at 2-3, 8-9, and 14-15 days of age, respectively; (30)]. All animals remained at thermoneutral T_a for 20 min postinjection before decreasing T_a to the same level used in the first routine. It was determined in an earlier study (32), using rats from the same age groups, that maximum metabolic response to EtOH at thermoneutral T_a was achieved at 20 min postinjection. Decrease in T_a from thermoneutral to cold levels occurred in less than 5 min, with maintenance of constant T_a for both saline and EtOH groups for the 120 min.

Calculation of MR was achieved using a measure of oxygen consumption within an open-flow system. Dried room air passed through the containment cylinder (338 cc/min) to a Beckman model 755 paramagnetic oxygen analyzer for determination of oxygen content. All values of oxygen consumption (STPD) were expressed as $W \times m^{-2}$ based upon a standard respiratory quotient of 0.83, with whole-body surface area calculated using a previously derived equation (31).

All measurements were single-point determinations made at 5-min intervals for 20 min prior to injection and 120 min postinjection. Influent air was tested between 60 and 70 min postinjection to identify baseline level, and this resulted in a break in the record for this period. After this time, measurements were only made at 10-min intervals. An Acro Systems 900 computerized data acquisition system was used to record and initially analyze all data collected during the test period.

Blood Analysis

A separate study was conducted to determine BEC of animals in the three age groups at 20, 60, and 120 min postinjection of EtOH (IP; 4 g/kg) within the two cold exposure routines. Rats in both routines were removed from the temperature-controlled environment, injected, and immediately returned to either cold or thermoneutral T_a. In routine two, T_a was shifted from thermoneutral to cold levels at 20 min postinjection. Each rat, at the preassigned sample time, was decapitated and free-flowing blood collected using heparin-

ized Natelson tubes. Samples were then stored for up to 25 h at 4°C. Blood EtOH content of a 0.05–0.10 ml sample was measured using an enzymatic determination procedure as described by Kelly et al. (13).

Statistical Analysis

Initial analysis was the calculation of mean values for body temperature, MR, and T_a as functions of age, postinjection time, and treatment group. Repeated-measure ANOVA (27) of each age group was used to determine overall treatment- and time-related differences, plus any treatment × time interactions. Contrasts analysis was used to determine specific treatment and time differences. Comparisons of BEC was performed using three-way ANOVA to include age, cold exposure routine, and postinjection time (27). Relationship of skin temperatures to colonic temperature was determined using linear and quadratic regressions, with correlation coefficient used to quantify the strength of association between these variables. A minimum significance level for all statistical tests was preset at $p \leq 0.05$.

RESULTS

Ethanol Effect on Maintenance of Cold Defense

Thermoregulatory status of 2–3-day-old rats treated with EtOH (4 g/kg) or saline at cold T_a are presented in Fig. 1. A treatment difference in T_{co} was found for this age group, $F(1, 13) = 5.15$, $p = 0.04$, with overall T_{co} of EtOH-treated rats being 0.85°C below saline-treated rats. There was, however, no time effect, $F(17, 219) = 1.27$, $p = 0.21$, or time × treatment interaction, $F(17, 219) = 1.20$, $p = 0.26$. Metabolic responses of 2–3-day-old rats to EtOH was different from the T_{co} response (Fig. 1). There was no overall treatment difference between groups, $F(1, 13) = 0.695$, $p = 0.42$. In contrast to the T_{co} response, there was a time effect, $F(17, 219) = 1.92$, $p = 0.02$, and time × treatment interaction, $F(17, 219) = 2.16$, $p = 0.006$. Animals injected with saline exhibited no significant change in MR from 0 min. Injection of EtOH produced a significant decrease in MR at 15 min postinjection ($p = 0.003$), with slow recovery from 35 to 120 min. No significant change in T_{back} occurred with EtOH from 0 (34.62°C) to 120 min (34.21°C) postinjection. In contrast, T_{abdo} displayed significant treatment, $F(1, 14) = 9.40$, $p = 0.008$, time, $F(17, 236) = 2.03$, $p < 0.01$, and time × treatment effects, $F(17, 236) = 2.30$, $p = 0.003$. Change in this site temperature from 0 to 120 min was 33.99 to 33.33°C for EtOH-treated rats and 34.47 to 34.19°C for saline-treated rats. Magnitude of decrease in T_{abdo} following EtOH treatment (i.e., 0.95°C) was greater than the other measured sites. T_{tail} changed over the 120-min test period from 32.34 to 32.16°C for EtOH-treated rats and 32.66 to 32.50°C for saline-treated rats. This site temperature exhibited significant treatment effect, $F(1, 13) = 6.13$, $p = 0.028$, with a 0.5°C overall difference between groups, but no time or time × treatment effects.

Both T_{co} and MR of 8–9-day-old rats were significantly reduced below control level following injection of EtOH at cold T_a (Fig. 2). Treatment, $F(1, 14) = 47.81$, time, $F(17, 238) = 13.90$, and time × treatment, $F(17, 238) = 17.43$, effects of EtOH on T_{co} were at $p < 0.001$. Overall differences in T_{co} of saline- and EtOH-treated rats was 1.9°C, which was double the difference seen in 2–3-day-old rats. Significant decrease in T_{co} of EtOH-treated rats below initial level began at 5 min postinjection ($p < 0.001$). Metabolic depression also occurred with EtOH injection ($p < 0.01$), as demonstrated by treatment, $F(1, 14) = 12.84$, time, $F(17, 237) = 4.77$, and time × treatment, $F(17, 237) = 3.60$, effects. EtOH-induced reduction in MR below saline level was 10.9 W/m^2 for the entire treatment period, and the response pattern was biphasic. Rapid decrease in MR occurred within 5 min of EtOH treatment, followed by partial recovery to 15 min postinjec-

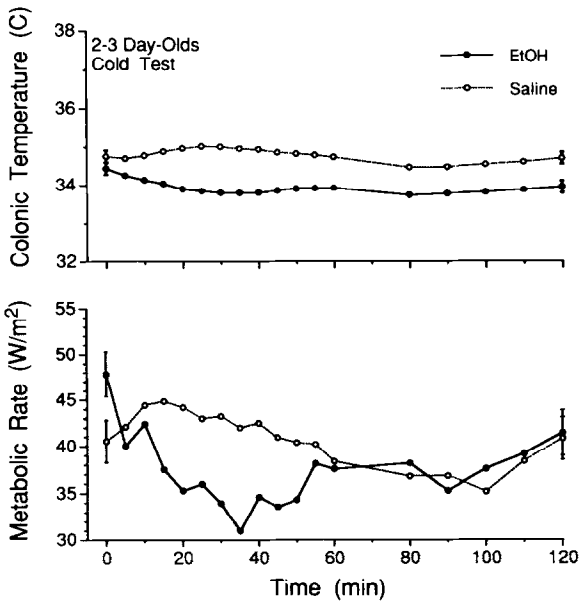

FIG. 1. Colonic temperature and metabolic rate of 2–3 day-old rats following injection of ethanol (4 g/kg) after one hour exposure to cold T_a. All points are group mean values, with vertical lines at 0 and 120 minutes indicating ± 1 SEM.

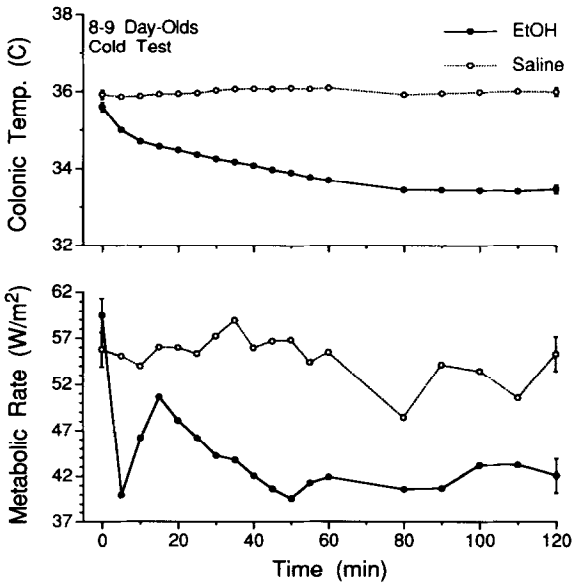

FIG. 2. Colonic temperature and metabolic rate of 8–9 day-old rats following injection of ethanol (4 g/kg) after one hour exposure to cold T_a. All points are group mean values, with vertical lines at 0 and 120 minutes indicating ± 1 SEM.

tion, and then a slower decrease (Fig. 2). MR remained depressed from 50 to 120 min postinjection. Skin temperature at all measured sites was reduced by EtOH injection ($p < 0.05$), with treatment, $F(1, 14) = 22.48$-49.20, time, $F(17, 238) = 5.62$-14.27, and time × treatment, $F(17, 238) = 5.42$-21.36, effects. Change in skin temperature during the 120-min test period was 35.72 to 35.80°C (saline) vs. 35.29 to 33.32°C (EtOH) for T_{back}, 35.31 to 35.41°C (saline) vs. 34.98 to 32.90°C (EtOH) for T_{abdo}, and 32.10 to 31.93°C (saline) vs. 31.73 to 30.46°C (EtOH) for T_{tail}.

Metabolic and T_{co} responses of 14-15-day-old rats to saline and EtOH at cold T_a are shown in Fig. 3. Once again, there were treatment, $F(1, 14) = 87.11$, time, $F(17, 238) = 26.31$, and time × treatment, $F(17, 238) = 40.01$, effects on T_{co} ($p < 0.001$). Treatment groups exhibited an overall T_{co} difference of 1.6°C, which was similar to that found in 8-9-day-old rats. By 5 min postinjection, T_{co} of EtOH-treated rats was below initial level ($p < 0.001$). Depression of MR ($p < 0.003$) by EtOH exhibited treatment, $F(1, 14) = 34.77$, time, $F(17, 238) = 2.25$, and treatment × time, $F(17, 238) = 3.56$, effects. As noted for 8-9-day-olds, this response was biphasic. Initial EtOH-induced reduction in MR was at 5 min, followed by partial recovery, and then a slow, second decline from 40 to 120 min postinjection. Comparison of skin temperature responses to EtOH and saline treatments showed ($p < 0.001$) treatment, $F(1, 14) = 19.79$-95.77, time, $F(17, 238) = 12.51$-29.33, and time × treatment, $F(17, 238) = 15.33$-41.15, effects. Change in skin temperature during the test period was 35.61 to 35.52°C (saline) vs. 35.62 to 34.14°C (EtOH) for T_{back}, 35.29 to 35.32°C (saline) vs. 35.13 to 33.22°C (EtOH) for T_{abdo}, and 30.11 to 30.32°C (saline) vs. 30.79 to 28.78°C (EtOH) for T_{tail}.

Skin temperatures of EtOH-treated rats were plotted as functions of T_{co} for all age groups (Fig. 4A-C) to determine if the change in site temperature was correlated with T_{co}. All sample times for each animal [i.e., (18)] were used for this determination. At 2-3 days of age, T_{back} and T_{abdo} was linearly related to T_{co} [$r(125) = 0.98$, $p < 0.001$, and $r(125) = 0.97$, $p < 0.001$, respectively]. In contrast, T_{tail} exhibited no relationship to T_{co} ($df = 107$) following EtOH injection. Similar correlations for the relationships between T_{back} and T_{abdo} [$r(143) = 0.96$, $p < 0.001$] with T_{co} were found for 8-9-day-old rats (Fig. 4B). In addition, T_{tail} was linearly related to T_{co} [$r(143) = 0.80$, $p < 0.001$], but the magnitude of the change in the temperature of this site with T_{co} was less than for trunk skin sites (slope = 0.61 vs. 0.90-0.95). The relationships for 14-15-day-old rats were very similar to 8-9-day-olds (Fig. 4C), with significant correlation coefficients ($p < 0.001$) for T_{back} [$r(143) = 0.90$)] T_{abdo} [$r(143) = 0.98$], and T_{tail} [$r(143) = 0.75$]. These comparisons show that a large portion of the change in skin temperature following injection of EtOH at cold T_a was related to T_{co}.

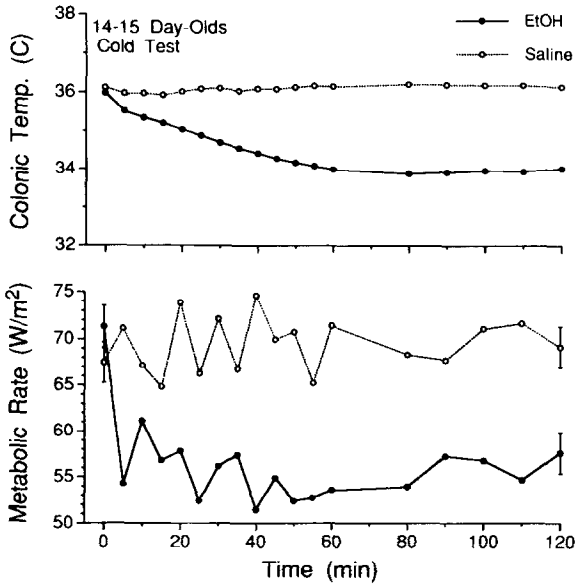

FIG. 3. Colonic temperature and metabolic rate of 14-15 day-old rats following injection of ethanol (4 g/kg) after one hour exposure to cold T_a. All points are group mean values, with vertical lines at 0 and 120 minutes indicating ± 1 SEM.

Ethanol Effect on Activation of Cold Response

Both T_{co} and MR are shown in Fig. 5 for 2-3-day-old rats injected at thermoneutral T_a, followed by exposure to cold T_a. A time effect ($p < 0.001$) was found for all values over the test period, due to the large shift in T_a and activation of cold-defense mechanisms. Although there was no treatment effect on T_{co}, $F(1, 14) = 1.14$, $p = 0.30$, there was time × treatment interaction, $F(21, 294) = 1.81$, $p < 0.017$. T_{co} of rats in saline and EtOH groups at 120 min of cold exposure had decreased 3.5 and 3.8°C, respectively. This decrease was below thermoneutral value at 5 min of cold exposure ($p < 0.001$). Separation in T_{co} response for treatment groups occurred only at 20 to 60 min of cold exposure ($p = 0.04$), with maximum reduction in T_{co} of EtOH-treated animals being 0.7°C below saline level at 45 min of cold exposure. There was no effect of treatment, $F(1, 14) = 2.61$, $p = 0.129$, on MR, or time × treatment interaction, $F(21, 293) = 1.162$, $p = 0.284$. The major difference between treatment groups was the 5-10-min delayed activation of thermogenic response in EtOH-treated rats. Skin temperature responses to cold exposure was very similar for both saline- and EtOH-treated groups. Change in skin temperature for the different sites from 0 min (preinjection) to 20 min (precold exposure) to 140 min (test end) was 37.45 to 37.47 to 34.16°C (saline) vs. 37.21 to 37.43 to 34.04°C (EtOH) for T_{back}, 37.52 to 37.58 to 33.84°C (saline) vs. 37.46 to 37.54 to 33.43°C (EtOH) for T_{abdo}, 36.48 to 36.67 to 32.12°C (saline) vs. 36.71 to 36.72 to 32.11°C (EtOH) for T_{tail}. No treatment differences or time × treatment interactions were noted for any skin temperature site at this age.

Response of 8-9-day-old rats in both treatment groups to a shift from thermoneutral to cold T_a is presented in Fig. 6. Saline and EtOH values for T_{co} showed treatment, $F(1, 14) = 90.30$, and time, $F(21, 294) = 509.77$, effects, and time × treatment interaction, $F(21, 294) = 31.46$, at $p < 0.001$ level. T_{co} of rats in both groups decreased rapidly with exposure to cold T_a, and was below thermoneutral value after 10 min at cold T_a ($p < 0.001$). Unexpectedly, the magnitude of T_{co} decrease at cold T_a was greater for saline-injected 8-9-day-old rats (i.e., 4.2°C) than for 2-3-day-old rats (i.e., 3.8°C). Also, 8-9-day-old rats injected with EtOH exhibited a larger overall reduction in T_{co} (i.e., 6.4°C) than 2-3-day-old rats, with no recovery at 140 min postinjection. MR response to cold exposure for the treatment groups showed treatment, $F(1, 14) = 69.35$, and time effects, $F(21, 289) = 8.18$, at $p < 0.001$ level. Saline-treated rats at 8-9 days of age exhibited an in-

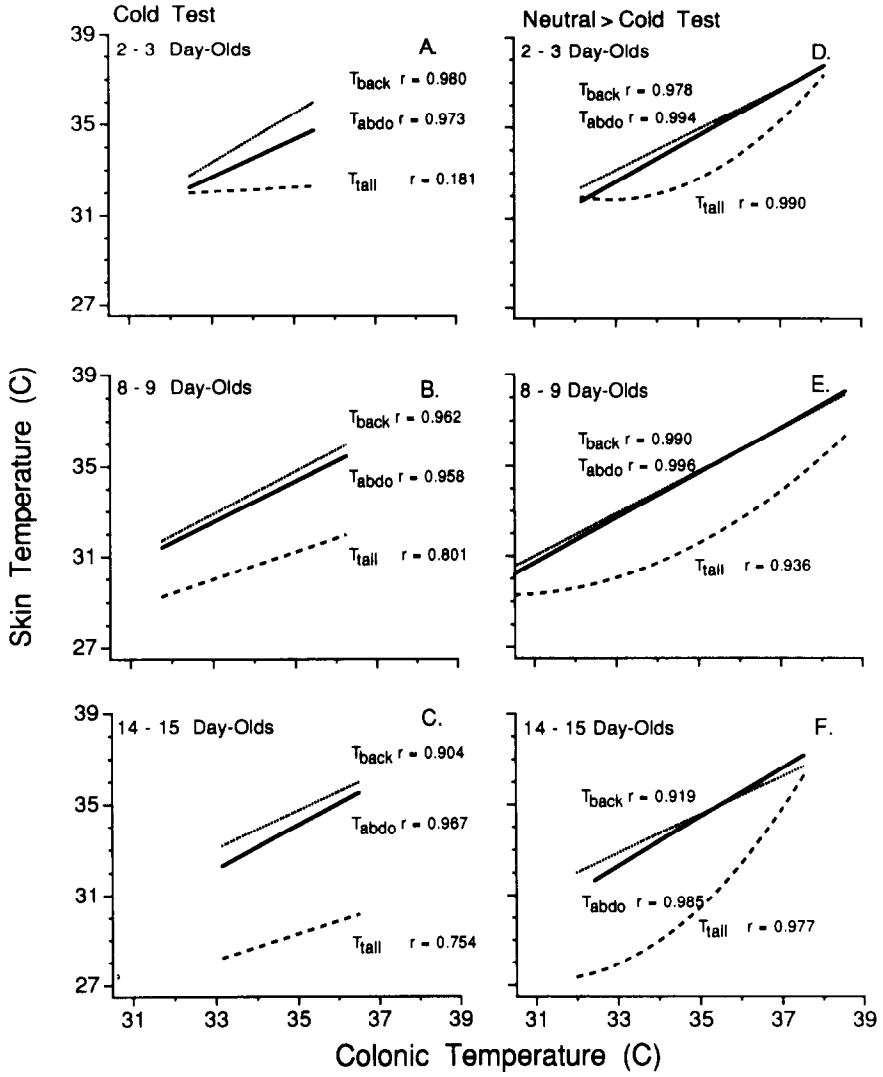

FIG. 4. Skin temperatures of ethanol-treated rats are shown as linear regression functions of colonic temperature. Sites include back (T_{back}), abdominal (T_{abdo}), and tail (T_{tail}) regions. Graphs A-C present relationships for rats in the three age groups administered ethanol following one hour cold exposure. Relationships in graphs D-F are for rats in the same age groups injected with ethanol prior to cold exposure. Correlation coefficient (r) is shown for each relationship.

crease in MR above thermoneutral value (34.82 W/m^2) at 35 min exposure to cold T_a ($p = 0.004$). Injection with EtOH completely eliminated the thermogenic response to cold T_a, and reduced MR below thermoneutral value at 20–60 min ($p < 0.04$). Because this response was unexpected, a second animal group was tested under the same conditions to verify this metabolic-depressive effect of EtOH at 8–9 days of age. Both T_{co} and MR responses of saline- and EtOH-treated rats to cold T_a are shown in Fig. 6. Again, there were treatment, $F(1, 14) = 9.08$, and time effects, $F(21, 294) = 180.84$, on T_{co}, and time × treatment interaction, $F(21, 294) = 5.33$, at $p < 0.009$. Cold T_a-induced decrease in T_{co} of saline-treated rats below thermoneutral level was similar to the first study (i.e., 3.6°C). EtOH at cold T_a lowered T_{co} by 6.3°C, which also agreed with the first study. MR of rats in the repeat study was affected ($p < 0.001$) by treatment, $F(1, 14) = 17.93$, and time, $F(21, 294) = 9.07$, with time × treatment interaction, $F(21, 294) = 5.84$, $p < 0.001$. Saline-treated rats exhibited an increase in MR ($p < 0.01$) above thermoneutral level after 25 min at cold T_a. Once again, EtOH treatment eliminated cold-induced increase in MR to support its metabolic-depressive effect on this age group.

Treatment of 8–9-day-old rats with EtOH prior to cold exposure decreased temperature of each skin site below saline value. The magnitude of this decrease was 0.53°C for back ($p < 0.01$), 0.74°C for abdomen ($p < 0.001$), and 1.52°C for tail ($p < 0.001$) sites. A further decrease occurred following cold exposure. Change in skin temperature for the different sites from 20 min (precold exposure) to 140 minutes (test end) was 38.03 to 34.46°C (saline) vs. 37.50 to 31.54°C (EtOH) for

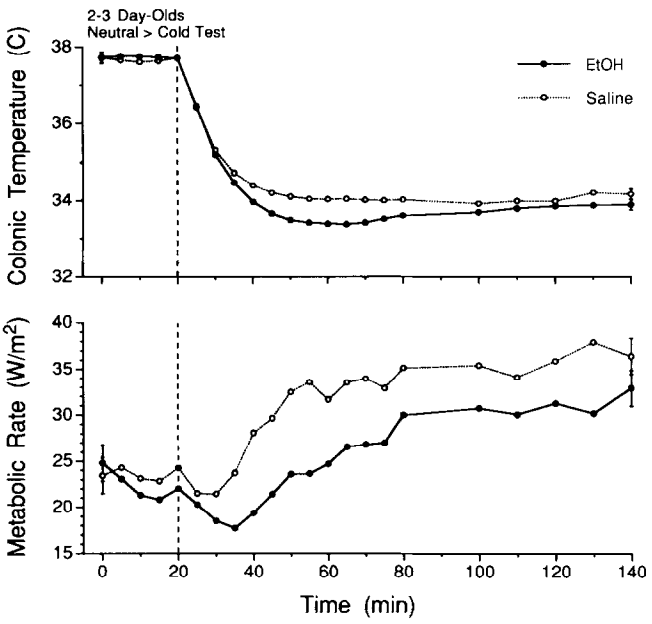

FIG. 5. Colonic temperature and metabolic rate of 2-3 day-old rats following injection of ethanol (4 g/kg) at thermoneutral T_a and exposure to cold T_a at 20 minutes postinjection. The vertical line at 20 minutes indicates the point at which T_a is shifted downward. All points are group mean values, with vertical lines at 0 and 140 minutes indicating ± 1 SEM.

T_{back}, 38.19 to 34.16°C (saline) vs. 37.46 to 31.14°C (EtOH) for T_{abdo}, 37.13 to 30.96°C (saline) vs. 35.62 to 29.44°C (EtOH) for T_{tail}. Comparisons of treatment groups for the three skin sites showed treatment, $F(1, 14) = 57.47\text{-}87.30$, $p < 0.001$, and time effects, $F(21, 293) = 404.92\text{-}576.07$, $p < 0.001$, and time × treatment interactions, $F(21, 293) = 3.50\text{-}28.71$, $p < 0.001$.

Responses of 14-15-day-old rats to cold T_a following treatment with saline and EtOH at thermoneutral T_a are shown in Fig. 7. Comparison of T_{co} showed effects of treatment, $F(1, 14) = 65.13$, and time, $F(21, 294) = 256.97$, and time × treatment interaction, $F(21, 294) = 39.16$, at $p < 0.001$. Saline-treated rats exhibited a reduction in T_{co} at 10 min exposure to cold T_a ($p < 0.001$), that stabilized at 1.9°C below thermoneutral level after 45 min. EtOH-treated rats decreased T_{co} at 15-20 min postinjection ($p < 0.007$), with additional reduction after 5 min ($p < 0.05$) at cold T_a to 4.1°C below thermoneutral level at 110 min ($p < 0.001$). Cold exposure following EtOH treatment also produced metabolic changes ($p < 0.05$) with treatment, $F(1, 14) = 65.69$, and time effects, $F(21, 292) = 50.58$, and treatment × time interaction, $F(21, 292) = 4.17$. No change in MR of saline-treated rats occurred prior to cold exposure, but EtOH-treated rats experienced an 18% reduction ($p < 0.05$) in MR at 20 min postinjection. Both treatment groups exhibited an increase in MR ($p < 0.001$) with cold exposure, which was delayed in EtOH-treated rats.

Skin temperatures of 14-15-day-old rats responded to both EtOH treatment and cold exposure. Change in skin temperature for the different sites from 0 min (preinjection) to 20 min (precold exposure) to 140 min (test end) was 36.82 to 36.79 to 34.82°C (saline) vs. 36.87 to 36.45 to 32.81°C (EtOH) for T_{back}, 37.10 to 37.03 to 34.95°C (saline) vs. 37.09 to 36.72 to 32.10°C (EtOH) for T_{abdo}, 35.83 to 35.78 to 29.86°C (saline) vs. 35.71 to 35.04 to 28.07°C (EtOH) for T_{tail}. Comparisons of skin temperatures for the three sites showed treatment, $F(1, 14) = 21.91\text{-}114.63$, and time effects, $F(21, 285) = 96.51\text{-}1042.01$, as well as, time × treatment interaction, $F(21, 285) = 9.96\text{-}30.97$. At 120 min of cold exposure, temperature differences between the two treatment groups were 2.0°C for back ($p < 0.001$), 2.9°C for abdomen ($p < 0.001$), and 1.8°C for tail sites ($p < 0.001$).

The relationship between skin temperature and T_{co} for EtOH-treated rats in the second exposure routine is shown in Fig. 4D-F. All sample times were used to derive this correlation. Trunk skin temperature at back ($df = 175$) and abdominal ($df = 151\text{-}175$) sites exhibited significant linear correlation ($p < 0.001$) with T_{co} for rats in the three age groups. In every case, there was overlap between lines for the same age group generated by the two exposure routines. The best relationship in the second routine between T_{tail} and T_{co} for the three age groups was quadratic ($df = 175$). The steeper slope for the decrease in T_{tail} with the initial reduction in T_{co} indicated vasoconstriction of this region at cold T_a, followed by a slower decrease in T_{tail} once constriction was complete. Again

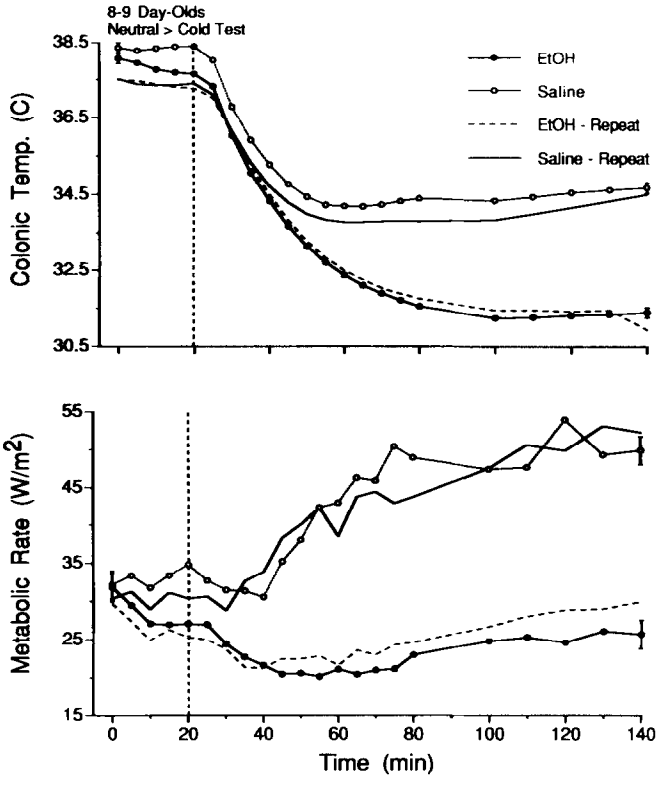

FIG. 6. Colonic temperature and metabolic rate of 8-9 day-old rats following injection of ethanol (4 g/kg) at thermoneutral T_a and exposure to cold T_a at 20 minutes postinjection. The vertical line at 20 minutes indicates the point at which T_a is shifted downward. All points are group mean values, with vertical lines at 0 and 140 minutes indicating ± 1 SEM.

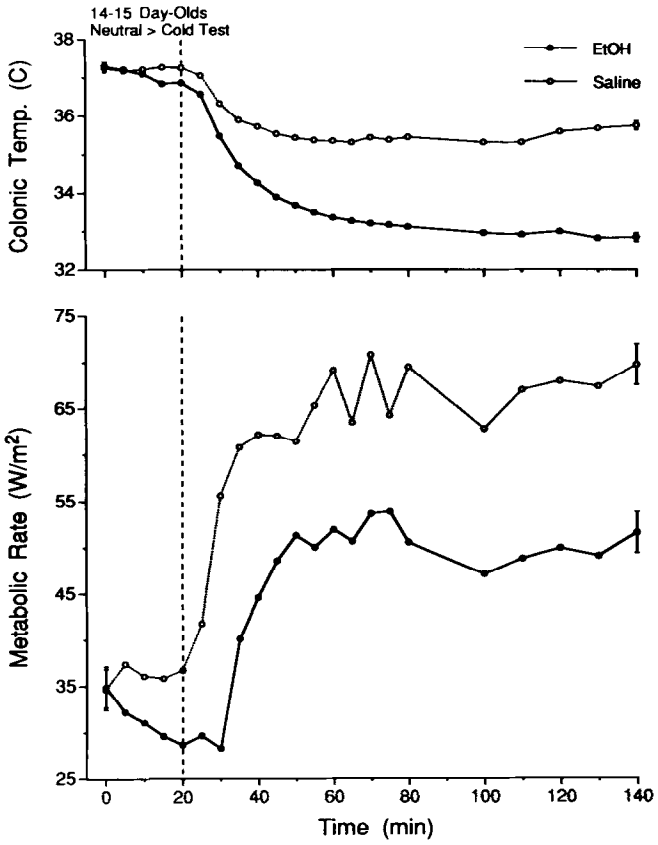

FIG. 7. Colonic temperature and metabolic rate of 14–15 day-old rats following injection of ethanol (4 g/kg) at thermoneutral T_a and exposure to cold T_a at 20 minutes postinjection. The vertical line at 20 minutes indicates the point at which T_a is shifted downward. All points are group mean values, with vertical lines at 0 and 140 minutes indicating ±1 SEM.

there was a high correlation between T_{tail} and T_{co} ($p < 0.001$), and overlap of the two exposure routines at the three ages.

Blood Ethanol Concentrations

Time-related BEC is presented in Fig. 8 for the age groups exposed to cold T_a before (Fig. 8A) and after (Fig. 8B) EtOH treatment. A three-way ANOVA was performed to determine effects of T_a exposure routine, sample time, and age on BEC. There was no significant effect of T_a exposure routine on BEC, $F(1, 105) = 1.64$, $p = 0.20$. There was, however, an age effect, $F(1, 105) = 42.1$, $p < 0.001$, with overall BEC of 2–3-day-old rats (336 mg/dl) being significantly less ($p < 0.001$) than either 8–9 (463 mg/dl)- or 14–15 (489 mg/dl)-day-old rats. In addition, there was a T_a exposure routine × age interaction, $F(2, 105) = 9.31$, $p < 0.001$. The effect of T_a exposure routine on BEC was not consistent across age groups, with both 2–3- and 8–9-day-old rats exhibiting an increase in BEC from continuous cold to transient cold conditions (i.e., 299 to 372 mg/dl and 440 to 490 mg/dl for 2–3- and 8–9-day-old rats, respectively) and 14–15-day-old rats displaying the opposite response (i.e., 523 to 455 mg/dl). Although the interactive effect of sample time and age was significant, $F(4, 105) = 3.28$, $p < 0.01$, it was only 2–3-day-old rats that exhibited a significant increase in BEC from 20 to 60 ($p = 0.04$) and 60 to 120 min ($p = 0.04$). There was no three-way interactive effect of exposure routine, age, and sample time on BEC, $F(4, 105) = 0.75$, $p = 0.56$.

DISCUSSION

Cold T_a used in the present study produced similar levels of challenge for animals in the three age groups, based on the fact that MR was twice the thermoneutral value (29,32). Rats as young as 2–3 days of age are able to increase thermogenesis at cold T_a (29,30). In spite of this ability to adjust heat production when cold-challenged, steady-state T_{co} was below thermoneutral level for each age group. The magnitude of this difference was less with advancing age, from 3.1°C at 2–3 days of age to 1.7 and 1.2°C at 8–9 and 14–15 days of age, respectively. Exposure of adult rats to 17°C cold challenge prior to EtOH treatment results in a 1.1°C reduction in T_{co} (34). Although 2–3-day-old rats were more hypothermic than older rats at cold T_a, the response to EtOH was less than for older animals. Over the 2-h test period, the maximum EtOH-induced reduction in T_{co} at 2–3 days of age was 0.7°C, com-

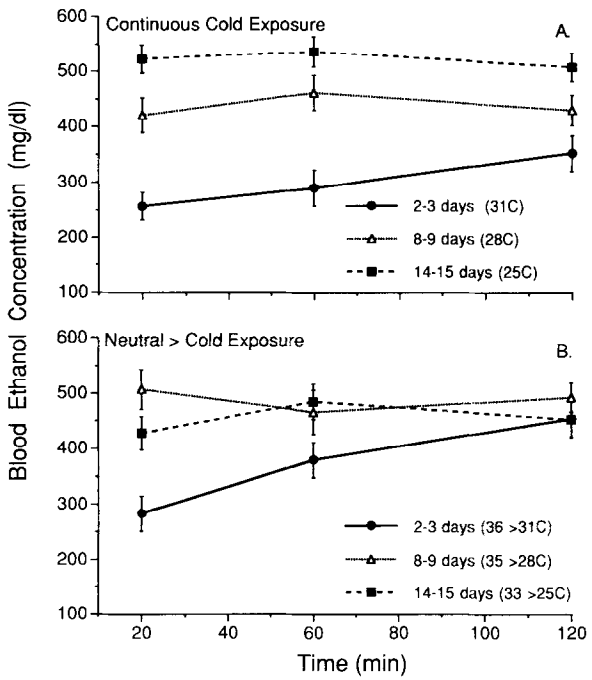

FIG. 8. Mean blood ethanol concentration (±1 SEM) for rats in the three age groups from 0 to 120 minutes postinjection of ethanol (4 g/kg) at cold T_a (A) and at thermoneutral T_a followed by exposure to cold T_a (B).

pared to 2.2°C and 2.1°C at 8–9 and 14–15 days of age, respectively. Even injection of 3 g EtOH/kg into adult rats at cold T_a results in 3.2°C decrease in T_{co} at 120 min postinjection. These results suggest that sensitivity to EtOH increases with age, even beyond 14–15 days of age.

Body temperature alone cannot be used to evaluate age differences in EtOH sensitivity at cold T_a when there is limited indication of active maintenance of homeothermy. A more suitable indicator is MR, which is elevated above the thermoneutral value at cold T_a. MR of adult (34) and immature rats (32) is depressed by 2–4 g EtOH/kg at thermoneutrality. Injection of EtOH at cold T_a produced 35% reduction in MR of 2–3-day-old rats, compared to 33 and 28% for 8–9- and 14–15-day-old rats, respectively. Although the magnitude of metabolic depression did not change with age, the pattern of response was very different for the three age groups. Recovery from this depression began in 2–3-day-old rats after 40 min. In older animals, there was a rapid initial decrease in MR followed by partial recovery and then a slow continued reduction in MR. It appears that at this age there is an early attempt to regain body temperature control that is not possible once EtOH reaches full effectiveness. In adult rats at cold T_a, the magnitude of EtOH-induced reduction in MR is approximately 24%, with no biphasic response and no recovery during the 120-min test period (34). One possible explanation for age differences in the pattern of response is that the young rat relies primarily on nonshivering thermogenesis for cold defense (6,20,28), with dependence on shivering activity after this time. Differences in metabolic pattern of response to EtOH at cold T_a may reflect age differences in tissue sites for thermogenesis (i.e., brown adipose tissue and liver vs. skeletal muscle). These results show that the magnitude of EtOH-induced depression of MR is similar from 2 to 15 days of age, but the pattern of response is very different during this period.

Exposure of immature rats to cold T_a 20 minutes following injection of EtOH and saline resulted in significant age-related differences in activation of thermogenesis. Thermoregulatory responses at 2–3 and 14–15 days of age were similar. At 2–3 days of age, MR of saline- and EtOH-treated rats increased 56 and 49%, respectively, with exposure to cold T_a. EtOH delayed MR increase by approximately 5 min, with a resulting decrease in T_{co} below saline value at 40–80 min postinjection. At 14–15 days of age, cold exposure produced a 93 and 88% increase in MR above thermoneutral level for saline- and EtOH-treated rats, respectively. As for the younger rats, the primary effect was delayed activation of thermogenesis by approximately 5–10 min. EtOH also depressed MR prior to cold exposure. With both the initial depression and delayed cold response, these rats were unable to raise MR to control value. This resulted in a larger decrease in T_{co} at 14–15 days of age than at 2–3 days of age. The adult response to EtOH is similar to that of 2–3- and 14–15-day-old rats (32). Administration of 3 g EtOH/kg BW prior to cold exposure (17°C) delays cold-induced increase in MR by 40 min, to result in reduced T_{co} below control value. In general, EtOH treatment prior to cold exposure in immature and adult rats delays activation of cold-induced thermogenesis.

The response of 8–9-day-old rats to EtOH followed by cold exposure was unexpected. Saline-treated animals exhibited the standard cold-induced increase in MR, which plateaued after 110 min postinjection. The 70% increase in MR above thermoneutral value was similar to the other age groups. In contrast, MR of EtOH-treated rats never increased with cold exposure and, in fact, decreased from thermoneutral value by 16–26%. These results show that EtOH treatment of rats at this stage of development reduces responsiveness to a cold stressor. As a result of this inability to activate MR at cold T_a, there was a larger decrease in T_{co} compared to the other age groups. There is no specific explanation as to why rats at this age would exhibit this level of sensitivity to EtOH only under these conditions. Brain growth spurt in this species does peak at 9–10 days of age (4), and, of course, the central nervous system is a primary site of EtOH action (5,14,16). In addition, it is known that rats pass through a stress-nonresponsive period from 2–3 to 14 days of age (36), during which time there is reduced ACTH response to different stressors (37). It is possible that the unusual response of 8–9-day-old rats to EtOH/cold stress combination was related to the status of the hypothalamic-pituitary axis at this stage of development.

Skin temperatures were measured at three separate sites to evaluate EtOH effect on several functions associated with thermoregulatory ability. The tail of the rat is extremely important for short-term dissipation of body heat (25). The interscapular or back skin site overlies a major deposit of brown adipose tissue (11), and a region of nonshivering thermogenesis. T_{abdo} was measured as an inactive vasomotor or thermogenic skin site for comparison with the other regions. At cold T_a, T_{tail} was lower than the other sites. This was expected with increased distance from the trunk of the animal. T_{back} was slightly higher than T_{abdo}, possibly due to its location above brown adipose tissue. An earlier study showed a slight elevation of T_{back} above T_{abdo} for immature rats exposed to cold T_a (30). Nearly all measured skin sites, except the tail of 2–3-day-old rats, exhibited a strong correlation with T_{co} following EtOH treatment. This would suggest that a large portion of EtOH-induced change in skin temperature was due to change

in T_{co}. It was expected that T_{tail} would follow T_{co} because it was likely that blood vessels of the tail were vasoconstricted at cold T_a, and would not exhibit further constriction under these conditions. The adult rat exhibits intense vasoconstriction of tail blood vessels at cold T_a (38), with minimal blood flow below 25°C (25). There was no indication of additional activation of brown fat thermogenesis with the reduction in T_{co} of EtOH-treated rats. Such activation would have been displayed as a departure of T_{back} from the linear relationship. Exposure to cold T_a following EtOH injection resulted in decreases in T_{abdo} and T_{back} for the three age groups that were highly correlated with T_{co}. In fact, these responses overlapped skin temperature responses of rats administered EtOH at cold T_a. Change in T_{tail} with a decrease in T_{co} was curvilinear for rats in the three age groups. This was likely due to the onset of vasoconstriction at T_a below thermoneutrality. Once vasoconstriction was in effect, there was an overlap of T_{tail} for the two cold exposure routines. In general, EtOH-induced reduction in skin temperature of the rat at cold T_a was primarily related to the reduction in T_{co}, with no indication that EtOH had a direct effect on vasomotor activity of the three tested sites.

Other studies have shown an age-related increase in EtOH toxicity (1) and behavioral responsiveness (16). In the present study, differences in thermoregulatory response to EtOH at cold T_a between 2-3- and 8-15-day-old rats could be attributed to slower rate of EtOH absorption in the younger group. A reduced rate of EtOH absorption in 2-3-day-old rats has also been found at thermoneutral T_a (32). In the adult rat, a rapid rise in BEC increases the level of intoxication and magnitude of hypothermia (9,35). Lower BEC in 2-3-day-old rats might also contribute to the reduced EtOH response at this age. One reason for the lower level in 2-3-day-old rats is possibly a greater dilution of EtOH in younger animals. Total body water decreases with age (5,12) and water space is the primary distribution site for EtOH within the body. Although BEC and rate of EtOH absorption could explain differences in thermoregulatory response of 2-3-day-old rats and older animals, there was no evidence that such factors were responsible for 8-9- and 14-15 day-old differences.

The present study shows that there are age differences in thermoregulatory response to EtOH at cold T_a that are primarily related to the effect on metabolic heat production. Maintenance of cold-induced thermogenesis is reduced with EtOH treatment, and the magnitude of this effect increases from 3-14 days of age. Activation of cold-induced thermogenesis is delayed by EtOH at 2-3 and 14-15 days of age. However, peak sensitivity of this activity to EtOH over this two week period is at 8-9 days of age, when EtOH treatment prevents an increase in thermogenesis above thermoneutral level. Additional studies are needed to determine why there are specific age-related differences in the response to EtOH.

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