Pharmacology Biochemistry & Behavior, Vol. 24, pp. 33–38, 1986. © Ankho International Inc. Printed in the U.S.A. 0091-3057/86 $3.00 + .00

Ethanol-Induced Cardiac Hypertrophy: Correlation Between Development and the Excretion of Adrenal Catecholamines

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Received 29 March 1985

ADAMS, M. A. AND M. HIRST. *Ethanol-induced cardiac hypertrophy: Correlation between development and the excretion of adrenal catecholamines.* PHARMACOL BIOCHEM BEHAV **24**(1) 33–38, 1986.—The progression of cardiac hypertrophy and the effects on adrenal medullary catecholamine content and release were determined in rats given ethanol, in conjunction with a supplementary diet, by gavage, every eight hours for periods up to 96 hours. Ethanol caused a marked depletion of adrenal medullary adrenaline content which fell to about 25 percent of control after the full time course. During this time, there were two- to fourfold increases in urinary noradrenaline content and five- to tenfold increases in urinary adrenaline. Cardiac hypertrophy, with increases in protein content and tissue weight of both ventricles, was evident at 24 hours of intoxication. By 96 hours of treatment these markers had increased more than 20% over control values. Proportional heart weight increases per day correlated significantly with daily urinary catecholamine excretion, providing support for the hypothesis that ethanol induced cardiac hypertrophy in the rat results from catecholaminergic stimulation, primarily of adrenal medullary origin.

Ethanol Cardiac hypertrophy Catecholamines Adrenal medulla

SINGLE intoxicating doses of ethanol stimulate the secretion of adrenal medullary catecholamines in man [8,22] and in a number of animal species [16, 23, 24]. The release of catecholamines is proportional to the rate of increase in blood ethanol levels [16,21]. Chronic ethanol exposure produces activation of adrenal catecholamine synthesis and release when measured after several weeks of treatment [13,25]. Activation of the adrenal medulla and the peripheral sympathetic nervous system has been associated previously with hypertrophy of the heart [29,30]. Some studies employing chronic ethanol treatments have demonstrated moderate cardiac enlargement [27,28]. The results of many of these experiments, however, have been confounded by incomplete data on the severity of the experimental intoxication. Adams and Hirst [3] reported that severe ethanol intoxication over four days rapidly promoted cardiac hypertrophy. There was, in parallel, a marked increase in secretion of adrenal medullary catecholamines, although the content of the gland was much reduced. There was regression of cardiomegaly during a subsequent abstinence period, an interval during which adrenal medullary activity returned towards normal levels.

In chronic studies with ethanol, Rossi *et al.* [27] determined that dietary deficits, in conjunction with the effects of ethanol ingestion, combine to induce enlargement of the heart. In the four day alcohol exposure studies noted above, ethanol was administered as the sole source of nutrient [3,4]. In the latter investigation there was an elevation in catecholamine excretion with resumption in ad lib feeding in the post-intoxication period, suggesting that there had been a deficiency of precursors for catecholamine biosynthesis during the treatment phase. In the present investigation, intubations of ethanol were supplemented with a balanced diet. The impact of the additional dietary component on adrenal medullary and urinary catecholamines was assessed. In addition, the time course of ethanol-induced alterations in the heart and its major sections were determined.

METHOD

Male, Sprague-Dawley rats weighing between 290 and 350 grams were housed individually in wire-mesh cages provided with food and water ad lib. During a two day period prior to the start of the study the rats were given free access to a purified diet developed by the American Institute of Nutrition in 1976 [7]. On the third day, rats were randomly assigned to one of two treatment groups: Group E (n=24) then received a solution of ethanol in water (10% w/v) containing Liquidiet (Bio-Serv Inc., 7.5%), and Group C (n=24), the control animals pair-matched to Group E rats, received maltose-dextrin in water (17.5% w/v), isocaloric with the ethanol solution, similarly containing Liquidiet (7.5%). Gastric intubations of these solutions were administered by premature infant feeding tube (size 8 Fr.) every eight hours for up to 96 hours. The initial dose of ethanol to Group E animals was 5 g/kg. The quantity of ethanol given to a Group E rat at each subsequent dosing period was based on the level of intoxication it displayed at that time, according to criteria described by Majchrowicz [18].

Body weights and urine volumes were recorded daily for each rat. Urine was collected into glass tubes containing

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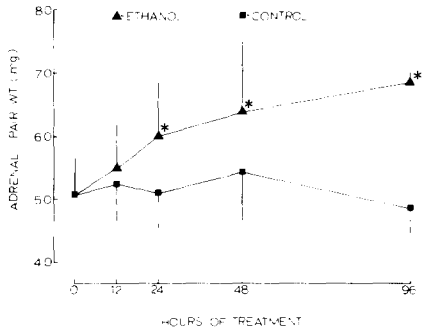

FIG. 1. Effect of severe ethanol intoxication, up to 96 hours, on adrenal gland pair weight. The asterisks denote a significant difference between ethanol and control group weights at the same time period, $p<0.05$.

hydrochloric acid (0.5 ml, 10 N) and a portion (5.0 ml) of the 24 hour sample stored at −80° until analyzed. Groups of ethanol-treated rats (n=6) and their paired controls (n=6) were killed by decapitation, under sodium pentobarbital anesthesia (Somnotol, 75 mg/kg), at 12, 24, 48, or 96 hours. In addition, a separate group of rats (n=6), housed in an identical manner for five days, were killed without receiving an intubation to serve as a zero time control. Blood samples were taken from the tails of all Group E rats, two hours after their final intubation, for determinations of blood ethanol levels by a gas chromatographic procedure [6].

Adrenal glands and the heart were rapidly removed and cleaned of extraneous tissues. The hearts were quickly rinsed in cold water, blotted dry, and then dissected into three sections: atria (A), right ventricular free wall (RV) and left ventricle plus septum (LVS), prior to weighing. Heart sections and adrenals were weighed and then frozen (−80°).

Catecholamine contents of urine samples, adrenal glands and heart tissues were determined by a procedure involving liquid chromatography with electrochemical detection. The liquid chromatographic system consisted of a Varian 5060 pump equipped with an autosampler, and a Beckman Ultrasphere-ODS column (5 μm; 0.46×15 cm). A model LC-2A electrochemical controller (Bioanalytical Systems) was used with a TL-5A, glassy carbon, electrochemical cell. The potential was set at 0.8 volts against a silver-silver chloride reference electrode. Chromatographic responses were recorded on a Varian CDS 401 data system, which identified and quantified the chromatographic peaks based on relative retention times and peak area ratios to the internal standard. The mobile phase consisted of potassium dihydrogen phosphate (1.0%), octyl sodium sulphate (100 mg%) and disodium-EDTA (10 mg%), dissolved in methanol/water (10% v/v). The pH of the prepared solution was adjusted to 4.5.

The heart sections were homogenized (Polytron homogenizer, 30 sec), over ice, in perchloric acid (0.4 N) containing the internal standard, 3,4-dihydroxybenzylamine (DHBA). The A and RV sections were homogenized in 1 ml of acid containing 0.2 μg of internal standard, whereas the LVS was digested in 2 ml of acid containing 0.5 μg of DHBA. The mixture was centrifuged (30,000 g, 10 minutes, 1–5°) and the resulting clear supernatant was filtered (Millipore 0.45 μm, type HA) prior to chromatographic analysis. Protein content in the remaining pellet fraction of the homogenized heart sections was determined by the method of Lowry *et al.* [17].

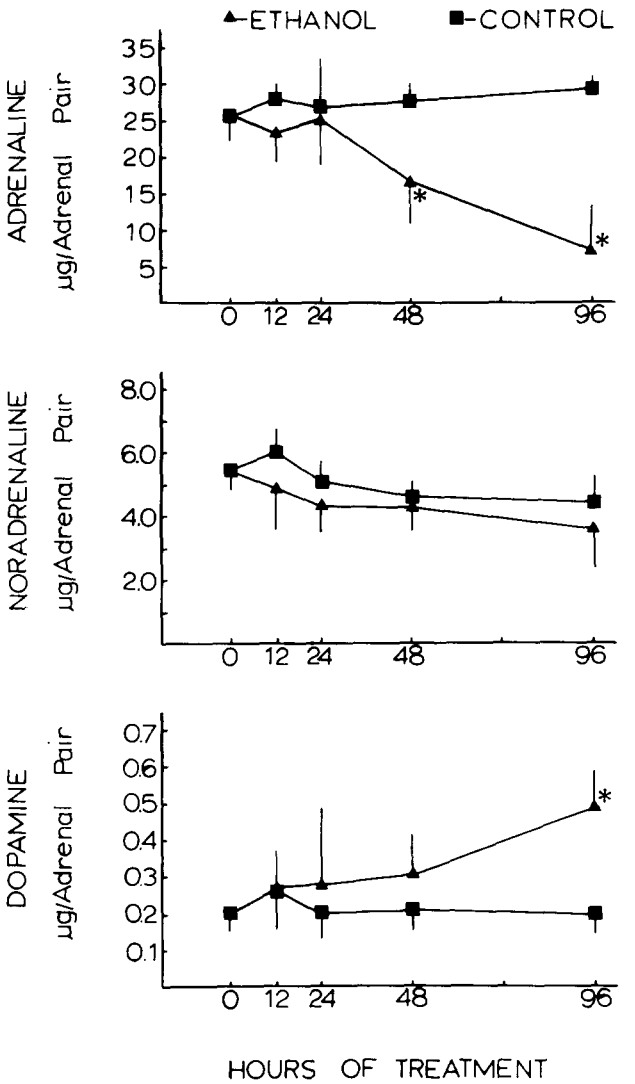

FIG. 2. Adrenal gland catecholamine content changes after various periods of severe ethanol intoxication or control treatment. The asterisks denote a significant difference between the adrenal content of the ethanol-treated group and the control group at the same time period, $p<0.05$.

Urinary catecholamines were extracted for chromatographic analysis using an alumina extraction method based on that of Anton and Sayre [9]. The chromatographic internal standard (1.0 μg, 3,4-dihydroxybenzylamine) was added to each acidified urine sample (5.0 ml) prior to hydrolysis to decompose conjugates (85°, 45 min). A sample of the hydrolyzed urine (2.0 ml) was placed into Tris/sodium hydroxide buffer (10 ml, 1.0 M Tris, pH 8.0) immediately prior to the addition of aluminum oxide (300 mg). The resulting mixture was shaken (10 min) and then centrifuged (5 min, 3500 g), before aspirating and discarding the clear supernatant. The remaining alumina was washed (2×10 ml) with water and centrifuged, as above. The final wash was discarded prior to addition of hydrochloric acid (2.0 ml, 0.1 N) to elute the catecholamines. This mix was shaken (10 min) and centrifuged (5.0 min, 3500 g), and the supernatant collected and analyzed for catecholamines, as described above. The ad-

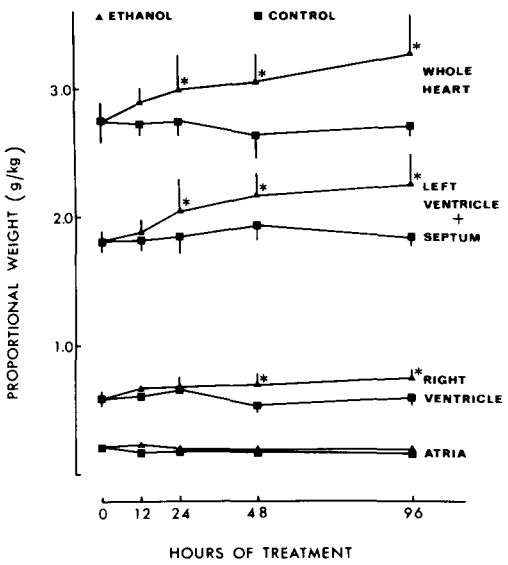

FIG. 3. Changes in the proportional weight, tissue weight per body weight, of the whole heart, left ventricle plus septum, right ventricle and atria of ethanol and control treated rats. The asterisks denote a significant difference between weight of tissues in the ethanol treated and control groups at the same time period, $p<0.05$.

renal glands were prepared for catecholamine analysis by a method described previously [2].

Numerical data are presented as mean plus and minus standard deviation in all figures, tables and text. The data were evaluated statistically using a computer program, GANOVA (M. L. Brecht and J. A. Woodward, Version 8/83, Dept. Psychology, UCLA, 1983), which performed a multiple analysis of variance and comparisons of means using the Student-Newman-Keuls multiple range test. Linear regressions and stepwise solutions of regressions were determined using a multiple regression program (Regress II, Human Systems Dynamics, CA, 1983).

RESULTS

Ethanol-treated rats generally remained ataxic throughout the period of intubation. The daily amount of ethanol administered ranged from 5.5±1.54 g/kg/day to 10.7±0.95 g/kg/day over the four treatment days. Blood ethanol levels, measured two hours after the final gavage in the 12, 24, 48 and 96 hour treated rats, were 308±81.7 mg%, 387±91.7 mg%, 384±65.4 mg% and 353±34.2 mg%, respectively. Rats from both groups lost about ten percent of their body weights over the course of the four day treatments: falling from 325±9.8 g (Group E) and 324±15.9 g (Group C) to 287±9.7 g and 288±12.3 g, respectively, over 96 hours.

As shown in Fig. 1, adrenal pair weights in the control group did not change during the treatment period. Analyses of the adrenal weights showed a significant main effect of the ethanol treatment, $F(1,62)=18.4$, $p<0.001$; time, $F(4,62)=3.0$, $p<0.05$; and the interaction between the two, $F(4,62)=3.2$, $p<0.05$. Adrenal glands in the ethanol-treated group were significantly enlarged after 24 hours of intoxication. There were further increases in the size of the adrenal glands at 48 and 96 hours of ethanol exposure, resulting in a 1.4-fold enlargement above controls over the complete time course.

TABLE 1

CHANGES IN CARDIAC NORADRENALINE CONCENTRATION DURING SEVERE ETHANOL INTOXICATION

Concentration (ng/gram)	Hours of Treatment 0	12	24	48	96
Group C	828.9 ±187.03	659.5 ±139.19	696.4 ±111.47	816.6 ±151.66	682.7 ±131.56
Group E		692.3 ± 76.79	733.4 ±115.7	578.4* ±114.85	557.5* ±193.74

*Denotes significant difference compared to contemporary Group C value, $p<0.05$.

The adrenal gland contents of adrenaline, noradrenaline and dopamine are presented in Fig. 2. There were no changes in the adrenal content of these catecholamines in Group C rats over time. In contrast, adrenaline content was significantly affected by the ethanol treatment, $F(1,50)=34.4$, $p<0.001$; time, $F(4,50)=7.4$, $p<0.001$; and the interaction of these variables, $F(4,50)=14.3$, $p<0.001$; falling to about 60% of control at 48 hours of ethanol intoxication, and declining further, to about 25%, by the end of the treatment. Noradrenaline levels showed no main effect of treatment. There was, however, a significant effect of time, $F(4,50)=3.3$, $p<0.05$; Group E rats having lower content at 48 and 96 hours. Analyses of dopamine content showed main effects of treatment, $F(1,50)=10.2$, $p<0.005$; time, $F(4,50)=3.0$, $p<0.05$; and treatment by time, $F(4,50)=3.4$, $p<0.05$. At 96 hours of treatment, dopamine content had risen over twofold to 0.48±0.138 μg/adrenal pair.

The changes produced in the proportional weight (g/kg body weight) of the heart and sections are presented in Fig. 3. Analysis of the data yielded a significant treatment: RV, $F(1,50)=21.3$, $p<0.001$; LVS, $F(1,50)=29.8$, $p<0.001$; whole heart, $F(1,50)=36.6$, $p<0.001$; time: RV, $F(4,50)=4.7$, $p<0.005$; LVS, $F(4,50)=9.1$, $p<0.001$; whole heart, $F(4,50)=3.5$, $p<0.05$; and treatment by time effect: RV, $F(4,50)=6.5$, $p<0.001$; LVS, $F(4,50)=4.7$, $p<0.005$; whole heart, $F(4,50)=4.7$, $p<0.005$. A significant level of hypertrophy was evident at 24 hours of ethanol treatment and progressed further to about 20% after 96 hours of intoxication. The increases in proportional left ventricular plus septal weight, which incorporates about 70% of total heart mass, paralleled the changes in the whole heart, enlarging to over 22% of control weight at 96 hours. The right ventricular free wall, representing about 23% of the total heart weight, similarly hypertrophied to an extent which correlated with the whole heart changes. The atria did not show any significant effects of the treatments.

Protein contents of the A, RV and the LVS of hearts, from rats in both treatment groups at 24 and 96 hours, were determined. Analysis of this data from the Group E and Group C rats showed that there were no significant changes in the percent protein contents of the sections at the two treatment times. The cardiac protein contents of control- and ethanol-treated rats at 96 hours were 6.2±1.16 mg and 8.1±1.08 mg for A, 19.6±1.17 mg and 22.0±1.57 mg for the RV and 100.6±11.84 mg and 120.3±11.36 mg for the LVS, respectively. There was then a parallel increase in protein content and total tissue weight gain.

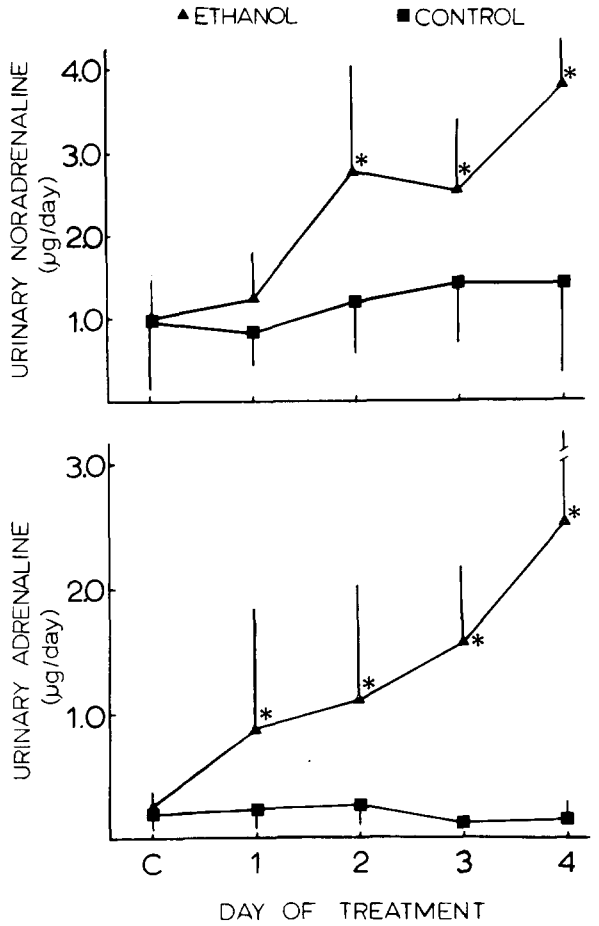

FIG. 4. Twenty-four hour urinary catecholamine excretion in ethanol and control treated rats. The asterisks denote differences between quantities excreted by ethanol and control treated groups on each day, $p<0.05$.

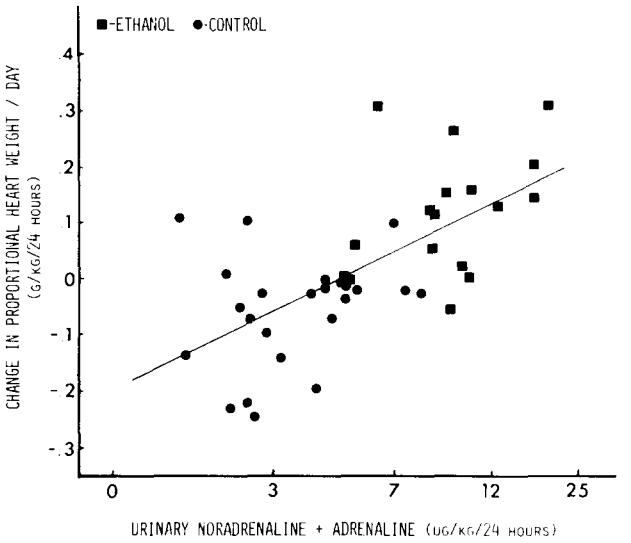

FIG. 5. Relationship between the average twenty-four hour urinary excretion of noradrenaline plus adrenaline per body weight and the change (from zero-time controls) in proportional whole heart weight per twenty-four hour period in ethanol and control treated rats. $r=.6445$, $F(1,39)=27.72$, $p<0.001$.

Cardiac noradrenaline concentration is presented in Table 1. Statistical analysis of treated groups showed that there was a significant treatment by time interaction, $F(3,40)=3.1$, $p<0.05$. Comparison of means of Group E and Group C values shows a significant reduction of noradrenaline concentration at 48 hours. The concentrations in A, RV and LVS, although not presented, demonstrated changes similar to those occurring in the whole heart.

Twenty-four hour urinary adrenaline (Adr) and noradrenaline (Nor) measures are presented in Fig. 4. Evaluation of both sets of catecholamine data shows a significant effect of treatment: Adr, $F(1,110)=74.5$, $p<0.001$; Nor, $F(1,110)=47.3$, $p<0.001$; time: Adr, $F(4,110)=9.0$, $p<0.001$; Nor, $F(4,110)=17.4$, $p<0.001$; and of the time by treatment interaction: Adr, $F(4,110)=9.4$, $p<0.001$; Nor, $F(4,110)=8.0$, $p<0.001$. The results show that the excretion of catecholamines by control animals remained relatively stable throughout the treatment. The ethanol treatment, however, markedly altered their excretion. Nor levels were increased severalfold over control, a change that persisted for the duration of the experiment. The alteration in Adr excretion was much more pronounced, rising from pretreatment levels of about 0.2 µg per day to more than 2.0 µg per day on the final day of intoxication. Overall, this elevation represented a sixfold increase in adrenaline levels averaged over the four day ethanol-treatment period. The total urinary excretion of Adr correlated with the magnitude of the changes in adrenal gland Adr content, $r=.833$, $F(1,39)=89.1$, $p<0.001$.

Figure 5 demonstrates the relationship of 24 hour urinary Adr plus Nor quantities to calculated changes in proportional whole heart weight per day in rats of both groups. The change in heart size was estimated from the difference in weight of individual hearts and the mean heart weight of the rats killed prior to the start of intubations, divided by the number of days of treatment. The urinary catecholamine data are presented, on a log scale, as the average excretion per day as a proportion of the final body weight. Analysis of this relationship by linear regression demonstrates a significant correlation. Stepwise solution of the three predictor variables, Adr, Nor and the sum of these, demonstrated that the latter was the best indicator of proportional heart weight changes per day, $r=.644$, $F(1,39)=27.7$, $p<0.001$; although the correlation of Adr alone, $r=.642$, $F(1,39)=27.4$, $p<0.001$, was similar to that of the sum of catecholamines, whereas the correlation of Nor alone, $r=.559$, $F(1,39)=17.7$, $p<0.001$, was the least predictive variable.

DISCUSSION

Majchrowicz [18] induced physical dependence to ethanol in rats using three to five administrations per day, by gavage, over a four day interval. In the present study, three intubations per day maintained the ethanol-treated rats at levels of intoxication comparable to those in the Majchrowicz study. Rats treated with ethanol, and their pair-matched controls, lost similar amounts of body weight over the 96 hour experimental period. This weight lost was less than half of that reported in previous investigations, which did not incorporate the supplementary diet [4,6]. Accordingly, the lesser reduction probably resulted from additional calories provided by the liquid diet supplement to the intubation solution.

Adrenal gland hypertrophy, induced by severe ethanol intoxication, was demonstrated previously to be due to cortical enlargement [4]. That study suggested that the change in the cortex resulted from elevated activity, primarily of ACTH release, within the hypothalamic-pituitary-adrenal axis. This is consistent with the results of Ellis [14] who demonstrated that hypophysectomy totally inhibited the increase in adrenal corticosterone release, evoked by ethanol, in the rat. Mendelson *et al.* [19] showed that chronic ethanol ingestion in humans activated the adrenal cortex.

Associated with the cortical activation, Adams *et al.* [4,6] demonstrated that severe ethanol intoxication produced a marked stimulation of the adrenal medulla. This was manifested by severe depletion of both adrenaline and noradrenaline [4], but not of dopamine content [1], although increased levels of the catecholamines appeared in the urine. In those studies, ethanol was the primary source of calories, as intoxicated animals consumed only minimal quantities of food. A supply of dietary amino acids, required for the synthesis of catecholamines, was, thereby, absent. The profound depletion of the adrenal catecholamines may, therefore, have been enhanced by a deficiency in this precursor pool. In the present experiments, rats were supplemented with a diet containing the appropriate precursor amino acids. The increase in adrenal medullary dopamine content and the persistence of noradrenaline content, observed in this study, supports the suggestion that reduced precursor availability contributed to the pattern of catecholamine depletion observed earlier [4]. In the present study, it seems that noradrenaline synthesis kept pace with release. In contrast, the medullary adrenaline content, as observed previously [4], declined progressively over the course of the ethanol exposure. This depletion, in conjunction with elevated urinary levels of adrenaline, indicates greatly enhanced stimulation of release of adrenaline without adequate compensatory synthesis and storage.

As mentioned, it was found in the earlier study that ethanol-induced adrenal medullary catecholamine release was reflected in elevated urinary adrenaline and noradrenaline levels [4]. The present report confirmed those findings and further demonstrated that ethanol-evoked medullary stimulation, in conjunction with dietary supplementation, produced a persistent elevation in urinary catecholamine excretion for the duration of treatment. These results indicate that the degree of adrenal stimulation does not diminish over four days of severe intoxication. Ethanol treatment promoted a greater increase in excretion of adrenaline than of noradrenaline, a result which may be indicative of more selective activation of the adrenal medulla over the peripheral noradrenergic nervous system.

In a previous study, cardiac noradrenaline concentration was decreased after prolonged ethanol intoxication and 16 to 18 hours after cessation of ethanol treatment [3]. The present effects on noradrenaline concentration showed similar trends, which may follow the development of the hypertrophy. As these changes in noradrenaline levels in the hypertrophied hearts are comparable to previous findings [3,10], then compensatory increases in the sympathetic nervous system may not accompany developing cardiomegaly. This could result in a decrease in the density of innervation. The role that cardiac catecholamine changes play in the function of the hypertrophied heart remains to be evaluated.

In the normal rat, it has been well established that heart weight is tightly correlated with body weight [11,12]. The ratio of heart weight to body weight (proportional heart weight) is maintained during periods of short-term body weight reduction resulting from food deprivation [11,12]. In the present study, rats from Group C, throughout the course of treatment, had proportional heart weights consistent with results of others. On the other hand, the proportional heart weights of ethanol-treated rats were increased at 24 hours and at later times.

Cardiomegaly has been produced using various experimental manipulations including increasing cardiac afterload [20, 29, 30], cardiac nerve stimulation [10], or elevating circulating catecholamine levels of endogenous [3, 29, 30], or exogenous origin [15]. Most of these investigations suggested that catecholamines have a primary causative role in the development of cardiomegaly [3, 10, 15, 29, 30]. Some of these studies have suggested that hypertrophy developed as a consequence of high circulating catecholamines of adrenal medullary origin [3,30]. This concept is further supported by results which have shown that adrenal denervation [30], adrenalectomy [20,26], or β-receptor blockade [29] may retard or totally inhibit experimentally-induced cardiomegaly.

Adams and Hirst [3] suggested that ethanol-induced cardiac hypertrophy was concurrent with adrenal medullary activation. The present results demonstrate that cardiomegaly, determined from both tissue weight and protein content changes, was evident at 24 hours of intoxication, and developed further until the end of treatment. This progression paralleled the medullary adrenaline depletion and the elevation in urinary catecholamine excretion. The coincident enlargement of the ventricular heart sections suggests that hypertrophy resulted from a generalized stimulation. Womble *et al.* [29,30] implicated circulating adrenaline as the primary hormone regulating cardiac hypertrophy, resulting from increased cardiac afterload. In the present experiment, the correlation of urinary catecholamines to the increase in heart weight lends further support to the concept of a causative role for catecholamines in ethanol-induced cardiomegaly. It is worthy of note that the ethanol-induced changes in urinary catecholamines were greater for adrenaline. Accordingly, these results, and the correlative analysis between heart weight and adrenaline excretion, emphasize the contribution that catecholamines from the adrenals may make to the development of cardiomegaly.

In preliminary studies, Adams *et al.* [5] found that adrenal medullary catecholamine turnover, determined using α-methyl-para-tyrosine to inhibit synthesis, was stimulated several times above control after two days of ethanol intoxication. In other tissues with a high degree of sympathetic innervation, including heart, kidney and intestine, there were no differences in turnover between the ethanol-treated and control groups. These findings suggest that release of adrenal medullary catecholamines, evoked by ethanol intoxication, represents the greatest contribution to increased peripheral sympathetic activity. The integration of these findings with the present study lends credence to the hypothesis that ethanol-induced cardiomegaly results primarily from catecholaminergic stimulation of adrenal medullary origin. Further investigations incorporating adrenal medullectomy and peripheral chemical sympathectomy are in progress to test this hypothesis.

ACKNOWLEDGEMENTS

The authors would like to thank Randell Walker, Julie Gudelis and Barbara Zapolski for their technical assistance, and Marilyn Potter for typing the manuscript. This research was supported by the Ontario Heart Foundation (Grant 3-32).

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