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INFLUENCE OF EPINEPHRINE ON ALCOHOL DEHYDROGENASE ACTIVITY IN RAT HEPATOCYTE CULTURE

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Abstract—The effects of epinephrine on alcohol dehydrogenase activity and on rates of ethanol elimination were determined in rat hepatocyte culture. Continuous exposure of the hepatocytes to epinephrine (10 μM) in combination with dexamethasone (0.1 μM) enhanced alcohol dehydrogenase activity on days 4–7 of culture, whereas neither hormone alone had an effect. The increased alcohol dehydrogenase activity was associated with an increased rate of ethanol elimination. Acute addition of 10 μM epinephrine to hepatocytes maintained in culture with 0.1 μM dexamethasone did not change alcohol dehydrogenase activity, but resulted in an immediate marked, but transitory, increase in ethanol elimination within the first 5 min after the addition of the hormone. Prazosin, an α_1-adrenergic blocker, and antimycin, an inhibitor of mitochondrial respiration, were powerful inhibitors of the transient increase in ethanol elimination, whereas 4-methylpyrazole was only partially inhibitory. These observations indicate that epinephrine has a chronic effect in increasing alcohol dehydrogenase activity and ethanol elimination and, also, an acute transient effect of increasing ethanol elimination which is not limited by alcohol dehydrogenase activity.

Chronic administration of epinephrine increases the rate of ethanol elimination in rats [1], while acute administration has no effect on ethanol elimination in rats [1] or dogs [2]. However, epinephrine has been shown to stimulate ethanol oxidation in isolated hepatocytes [3] and in the perfused liver [4] of the rat. The increased rates of ethanol oxidation have been attributed to the well known effect of epinephrine of increasing hepatic oxygen consumption [1, 4, 5]. The effect of epinephrine on liver alcohol dehydrogenase has not been determined. Immobilization stress, which increases catecholamine levels, results in increases in both alcohol dehydrogenase and rates of ethanol elimination [6]. Acute and chronic ethanol administration also increase rates of ethanol oxidation [7, 8] and hepatic oxygen consumption [9, 10], but have no effect or decrease alcohol dehydrogenase activity [11]. It has been suggested that these effects of ethanol are mediated by epinephrine since epinephrine levels are elevated after ethanol consumption [12], and both the increases in ethanol oxidation and oxygen consumption are inhibited by adrenalectomy and alpha-adrenergic blockers [9, 13].

The purpose of this study was to determine the acute and chronic effects of epinephrine on alcohol dehydrogenase activity and on rates of ethanol elimination in rat hepatocyte culture.

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MATERIALS AND METHODS

Animals and materials. Male Sprague–Dawley rats were obtained from the Charles River Breeding Laboratories, Wilmington, MA. NAD^+ (grade I) was obtained from Boehringer-Mannheim, Indianapolis, IN. Collagenase (Type I), ethyleneglycol-bis-(β-aminoethyl ether)-N,N,N^1,N^1-tetraacetic acid (EGTA), tricine, trypan blue, 4-methylpyrazole, 2,4-dinitrophenol, oligomycin, antimycin A, yohimbine, bovine albumin and calf thymus DNA were purchased from the Sigma Chemical Co., St. Louis, MO. Prazosin was a gift of Pfizer, Inc., New York, NY. Insulin and penicillin were purchased from E. R. Squibb & Sons, Inc., Princeton, NJ. Streptomycin was obtained from Eli Lilly, Indianapolis, IN. Epinephrine and gentamicin were purchased from Elkins-Sinno, Inc., Cherry Hill, NJ. Dexamethasone was purchased from Lypho Med, Inc., Melrose Park, IL. Propranolol was obtained from Ayerst Laboratories Inc., New York, NY. Plastic culture dishes were obtained from Allied Fisher Scientific, Plainview, PA. Vitrogen 100 purified bovine dermal collagen (Type I) was obtained from the Collagen Corp., Palo Alto, CA. CEM serum-free culture medium and supplementary growth factor (SGF-7) were purchased from Scott Laboratories Inc., Fiskeville, RI.

Hepatocyte isolation and cell culture. Hepatocytes were isolated and cultured according to the methods of Bissell and Guzelian [14]. Rats weighing 150–200 g were anesthetized with ether. The livers were perfused *in situ* through the portal vein with Hanks' balanced salt solution containing 0.5 mM EGTA and 2.5 mM tricine, followed by the complete culture

medium containing 0.03% collagenase. The soft livers were removed and the hepatocytes separated from non-parenchymal cells by centrifugation at 60 *g* for 2 min, a process that was repeated three times, each time after washing and resuspending the cells in cool collagenase-free medium. Cell number and percent viability were assessed by counting an aliquot in the presence of 0.1% trypan blue. Only hepatocyte suspensions with a viability greater than 95% were cultured. The cells were cultured in 60 mm × 15 mm petri culture dishes previously coated with 50 μg of collagen. A total of 3.5×10^6 cells in 3.0 ml of culture medium was placed on each dish. The culture medium was serum-free CEM with the following supplemental growth factors (SGF-7): insulin (250 units/L), transferrin, selenium, epidermal growth factor, fetuin, and bovine serum albumin–oleic acid, and linoleic acid complexes. It also contained penicillin (1×10^5 units/L), streptomycin (0.13 mM), and gentamicin (0.18 mM). The medium was saturated with 95% O_2 and 5% CO_2. The cultures were incubated at 37° in a humidified atmosphere of 95% O_2 and 5% CO_2.

Alcohol dehydrogenase activity. The effect of epinephrine was tested by adding it to the culture medium used for one set of cultures, while omitting it from an equal number of control cultures. Both the hormone containing medium and the control medium were changed every 24 hr. Hepatocytes were harvested by replacing the medium with 2 ml of 0.5 M Tris–HCl, pH 7.2, containing 0.25% Triton X-100, followed by scraping with a rubber spatula. The isolated hepatocytes were homogenized and then sonicated in pulses for a total of 20 sec. Alcohol dehydrogenase was determined in the homogenate at 37° by the method of Crow *et al.* [15]. The reaction mixture was 1.0 ml and considered of 0.5 M Tris–HCl, pH 7.2, 18 mM ethanol, 2.8 mM NAD^+, and 0.01 to 0.10 ml of the liver homogenate. A blank reaction without ethanol was run in each case. The alcohol dehydrogenase activities were then calculated from the molar extinction coefficient of 6.22 mM^{-1} cm^{-1} for NADH. One unit of enzyme activity is defined as the formation of 1 μmol of NADH per min under these conditions. Michaelis–Menten constants for ethanol and NAD^+ were obtained from determinations of alcohol dehydrogenase activity at non-saturating concentrations of the substrate and the coenzyme. The kinetic constants were calculated using the computer program of Cleland [16]. Lactate dehydrogenase activity was determined by the method of Plagemann *et al.* [17]. The enzyme activities were expressed per mg of protein and per mg of DNA. Protein was determined by the method of Lowry *et al.* [18] with bovine serum albumin used as a standard. DNA was assayed by the method of Richards [19]. The viability of the cells was assessed by measurement of the appearance of lactate dehydrogenase in the medium in the 24-hr periods. Medium lactate dehydrogenase was expressed as a percent of total (cellular + medium) lactate dehydrogenase. The functional capability of the hepatocytes was monitored by measuring urea synthesis, by determining the appearance of urea [20] in the medium over periods of 24 hr. Urea concentration was determined by the method of Gutmann and Bergmeyer [21]. The morphological appearance of the monolayers was monitored by inverted phase contrast microscopy and was characteristic of adult hepatocytes.

In one experiment, epinephrine concentrations were determined in the medium on day 5 of culture. The medium at the end of the day were stored at −70°. Epinephrine was determined by Wolfgang Vogel from the Thomas Jefferson University in Philadelphia, PA, using the radiometric-enzymatic assay of Coyle and Henry [22]. After a 24-hr exposure of the medium to the hepatocytes in culture, an initial mean preincubation epinephrine concentration in the medium of 11.6 μM decreased by 40% to a mean concentration of 7.0 μM.

Ethanol elimination. The rate of ethanol elimination after chronic exposure of the hepatocytes to epinephrine was determined by incubation of the hepatocyte culture in fresh culture medium, which did not contain the hormones that produced the enzyme changes, and in the presence of 8 mM ethanol for 90 min at 37° as described by Crow *et al.* [15]. The acute effects of epinephrine on the rate of ethanol elimination were determined after addition of fresh culture medium containing 10 μM epinephrine and 8 mM ethanol to hepatocyte cultures not previously exposed to epinephrine. Samples of the incubation medium for the determination of ethanol by gas–liquid chromatography [6] were obtained at 0, 20, 40, 60, and 90 min in the chronic experiments, and in addition at 5, 10, and 15 min in the acute experiments. A set of culture plates, containing medium but no cells, was included as a control to monitor evaporation losses during the 90-min incubation. Ethanol elimination was calculated from the rate of decrease in ethanol concentration by the method of least squares. The hepatocytes were harvested at the end of the incubation for determinations of alcohol and lactate dehydrogenases, protein, and DNA using the methodology already described.

Pyridine dinucleotides. Hepatocyte culture plates were washed with Hanks' balanced salt solution, scraped, and frozen in liquid nitrogen. The entire process was done within 5 sec. The cells were extracted in acid for determination of NAD^+ and $NADP^+$ and in base for determinations of NADH and NADPH. The determinations were done by reverse phase high-performance liquid chromatography by the method of Jones [23] with modifications. A 15-cm Excellopac ODS B C18 5 micron mesh column (R. E. Gourley Co., Laurel, MD) was used. NAD^+ and $NADP^+$ were separated by isocratic elution with 0.1 M potassium phosphate buffer, pH 6.0, containing 3.75% methanol and detected spectrophotometrically at 254 nm. NADH and NADPH were eluted with the same buffer containing 3% methanol, but detected fluorometrically by activation at 254 nm and emission in the 400–700 nm range. The concentrations of the pyridine nucleotides per culture were determined with external standards also extracted in either acid or base.

Statistical analysis. Five to twelve plates were analyzed for each experimental condition each day. The values are expressed as means ± SE. The data were analyzed by Student's *t*-test when appropriate,

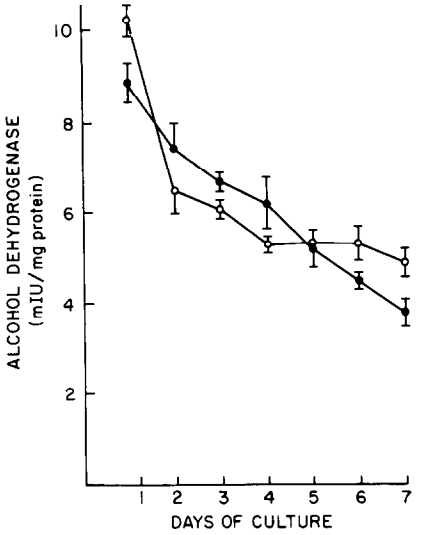

Fig. 1. Effect of epinephrine (10 μM) in combination with dexamethasone (0.1 nM) on alcohol dehydrogenase activity in hepatocyte culture. The hepatocytes were exposed to medium containing dexamethasone (●) or the combination of epinephrine and dexamethasone (○). The medium was changed every 24 hr. The enzyme activities are indicated as means ± SE of five culture plates.

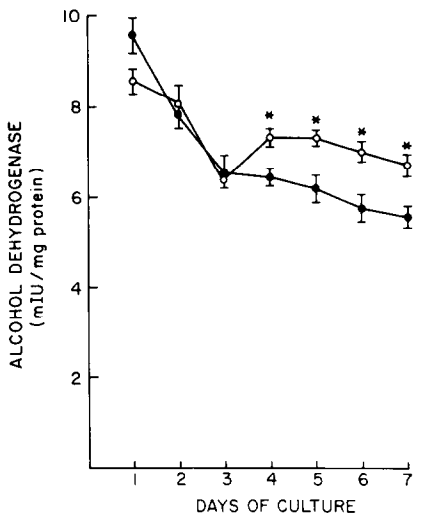

Fig. 2. Effect of epinephrine (10 μM) in combination with dexamethasone (0.1 μM) on alcohol dehydrogenase activity in hepatocyte culture. The hepatocytes were exposed to medium containing dexamethasone (●) or the combination of epinephrine and dexamethasone (○). The enzyme activities are indicated as means ± SE of five culture plates. An asterisk (*) indicates a statistically significant difference versus control, $P < 0.05$

or by analysis of variance when comparing means of more than two groups. The Duncan new multiple range test was used to estimate differences between means [24]. The Dunnett procedure was used to compare multiple treatment means with control.

RESULTS

Chronic exposure of hepatocytes to 10 μM epinephrine without dexamethasone (not shown) or in the presence of 0.1 nM dexamethasone (Fig. 1) did not result in any significant change in alcohol dehydrogenase activity. A higher concentration of dexamethasone (0.1 μM) prevented a steady decline in the enzyme activity, resulting in a relatively stable enzyme activity between days 3 and 7 of culture (Fig. 2). Epinephrine (10 μM) in the presence of the higher concentration of dexamethasone (0.1 μM) resulted in increased alcohol dehydrogenase activity on days 4–7 of culture (Fig. 2). The effects of epinephrine on the alcohol dehydrogenase activity were dependent on the concentration of epinephrine to which the hepatocytes were exposed (Fig. 3). Epinephrine in concentrations ranging from 1 to 100 μM combined with 0.1 μM dexamethasone resulted in higher enzyme activity than obtained with epinephrine alone ($P < 0.01$). An epinephrine concentration of 10 μM was required to increase alcohol dehydrogenase activity above that found with dexamethasone (0.1 μM) alone ($P < 0.01$).

The Michaelis–Menten constants (K_m) of alcohol dehydrogenase for ethanol and NAD^+ after 5 days in culture are shown in Table 1. The presence of epinephrine, dexamethasone or their combination had no effect on the K_m for ethanol. The K_m for NAD^+ was increased slightly by epinephrine alone, but was not affected by dexamethasone alone or the combination of epinephrine and dexamethasone.

The hormonal effects on alcohol dehydrogenase activity were the same when expressed per DNA concentration. The concentration of DNA per mg of protein was not changed significantly by epinephrine, dexamethasone, or their combination. Cell viability was increased by 0.1 μM dexamethasone. The

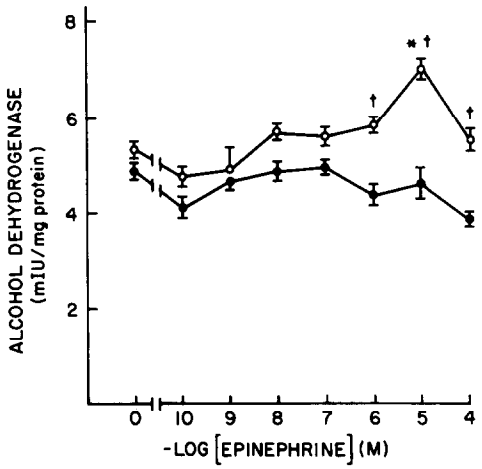

Fig. 3. Effect of increasing concentrations of epinephrine on alcohol dehydrogenase activity in hepatocyte culture. The hepatocytes were exposed to medium containing epinephrine alone (●) or epinephrine plus 0.1 μM dexamethasone (○) for 5 days. Statistical differences are indicated as: *$P < 0.01$ as compared to 0.1 μM dexamethasone alone, and †$P < 0.01$ as compared to exposure to the same concentration of epinephrine without dexamethasone.

Table 1. Effect of hormones on the Michaelis–Menten constant (K_m) of alcohol dehydrogenase in hepatocyte culture

Hormone in the culture medium	K_m Ethanol	K_m NAD^+
	(mM)	(μM)
Control	1.8	75
Epinephrine	2.3	114
Dexamethasone	1.9	78
Epinephrine + dexamethasone	2.0	60

The hepatocytes were in culture with or without the addition of epinephrine (10 μM) or dexamethasone (0.1 μM) for 5 days. The cells were harvested in 0.5 M Tris-HCl buffer, pH 7.2, containing 0.25% Triton X-100 devoid of hormones. The kinetic constants were calculated according to the computer program of Cleland [16].

release of lactate dehydrogenase into the medium on days 1–7 of culture was 4.51 ± 0.45 and 6.62 ± 0.50% in the presence of dexamethasone (0.1 μM) and in its absence respectively ($P < 0.01$). The release of lactate dehydrogenase in the presence of a low concentration of dexamethasone (0.1 nM) was not different from that in its absence. Epinephrine in concentrations ranging from 0.1 nM to 0.1 mM did not affect cell viability in either the presence or absence of 0.1 μM dexamethasone.

Epinephrine (10 μM) increased urea synthesis during the 7 days of culture from 20.9 ± 0.5 μg/mg protein/24 hr to 47.9 ± 3.3 μg/mg protein/24 hr ($P < 0.001$). Demaxethasone in concentrations of 0.1 nM and 0.1 μM decreased urea synthesis to levels of 7.7 ± 0.8 and 7.0 ± 0.6 μg/mg protein/24 hr respectively ($P < 0.001$), and prevented the enhancing effect of epinephrine. Urea synthesis in the presence of 10 μM epinephrine was 5.9 ± 0.53 and 6.1 ± 0.56 μg/ml protein/24 hr when combined with 0.1 nM and 0.1 μM dexamethasone respectively. The effect of epinephrine in increasing urea synthesis in the absence of dexamethasone was only demonstrated at concentrations of 10 μM or greater (Fig. 4). A 100 μM concentration of epinephrine partially reversed the depressant effect of dexamethasone on urea synthesis.

The rate of ethanol elimination was enhanced after chronic exposure of the hepatocytes to 10 μM epinephrine in combination with 0.1 μM dexamethasone. Both an initial more rapid ethanol elimination measured in the first 20 min and a slower rate from 20–90 min were enhanced by epinephrine. The total rate of ethanol elimination over 90 min was 10.15 ± 1.40 μmol/mg protein/min in the hepatocytes exposed to epinephrine and dexamethasone as compared to 6.32 ± 0.48 μmol/mg protein/min in hepatocytes exposed to dexamethasone alone ($P < 0.05$).

The acute exposure of hepatocytes on day 4 of culture to 10 μM epinephrine in the presence of 0.1 μM dexamethasone resulted in an immediate enhancement of ethanol disappearance which occurred during the first 5-min sampling period (Fig. 5). Thereafter, between 5 and 90 min the rate of ethanol elimination was not affected by epinephrine.

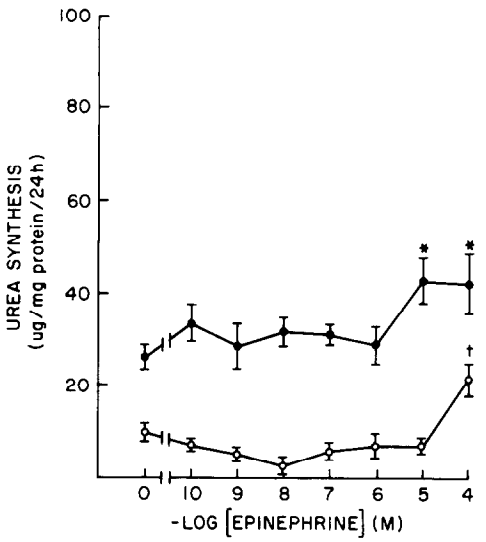

Fig. 4. Effect of increasing concentrations of epinephrine on the rate of urea formation in hepatocyte culture. The hepatocytes were exposed for 5 days to medium containing epinephrine (●) or epinephrine plus 0.1 μM dexamethasone (○). Urea formation is indicated as mean ± SE of five culture plates. Statistical differences are indicated as: *$P < 0.05$ as compared to control cultures lacking epinephrine and dexamethasone, †$P < 0.05$ as compared to 0.1 μM dexamethasone alone.

Prazosin, an α_1-adrenergic blocker, inhibited the initial rate of ethanol elimination in both control cultures and those exposed to 10 μM epinephrine (Table 2). The inhibition was essentially complete in the hepatocytes exposed to epinephrine. Yohimbine, an α_2-adrenergic blocker, had no effect on ethanol elimination (not shown). Propranolol, a β-adrenergic blocker, had no significant effect on ethanol elimination in control cultures, but decreased the enhancing effect of epinephrine on the initial rate of ethanol elimination. Antimycin, which inhibits both NAD^+-dependent and flavin-dependent oxygen uptake, was a powerful inhibitor of the initial rate of ethanol elimination in control and epinephrine-exposed hepatocyte cultures. Oligomycin, an inhibitor of ADP-dependent oxygen uptake, caused a moderate decrease in the initial rate of ethanol elimination in epinephrine-exposed hepatocytes, but not in controls. 4-Methylpyrazole decreased the initial phase of ethanol elimination in epinephrine-exposed hepatocytes, but not in controls. The subsequent rate of ethanol elimination between 5 and 90 min was not inhibited by either prazosin or propranolol. Antimycin continued to be inhibitory in both control and epinephrine-exposed cultures, while oligomycin continued to have an inhibitory effect in the cultures exposed to epinephrine. 4-Methylpyrazole resulted in 74.1 and 87.1% inhibition of the rate of ethanol elimination between 5 and 90 min in the control and epinephrine-exposed hepatocyte cultures respectively.

The changes in pyridine nucleotides during ethanol metabolism are shown in Table 3. NAD^+ decreased transiently at 10 min, while NADH decreased from 5 to 20 min after the addition of ethanol. These

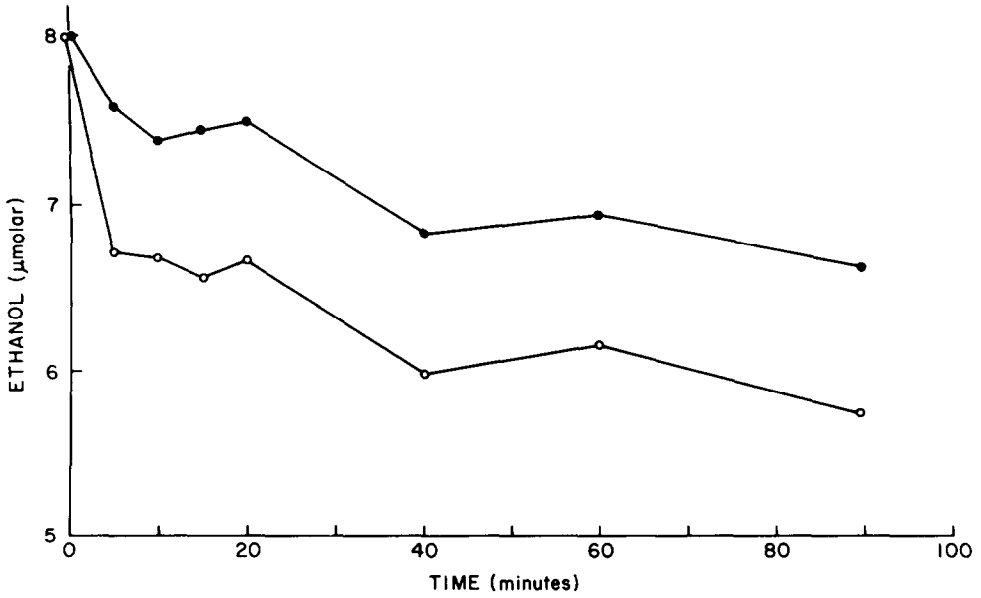

Fig. 5. Effect of acute epinephrine (10 μM) addition on the disappearance of ethanol from the medium of cultured hepatocytes. The hepatocytes were in culture with medium containing 0.1 μM dexamethasone alone. At the end of day 4 of culture, the medium was replaced by fresh medium containing 8 mM ethanol and either 0.1 μM dexamethasone (●) or the combination of 10 μM epinephrine plus 0.1 μM dexamethasone (○). The medium was sampled at the time intervals indicated for ethanol concentration. The values are expressed as means of five culture plates.

changes were associated with increases in both $NADP^+$ and NADPH. At 5 min after the addition of the ethanol, there were increases in both NAD^+/NADH and $NADP^+$/NADPH ratios from 3.0 to 5.80 and from 0.16 to 0.30 respectively. These ratios then decreased gradually toward control values. In the presence of epinephrine, the decrease in NAD^+ occurred at 5 min and was more marked and

Table 2. Effect of inhibitors on rates of ethanol elimination in cultured rat hepatocytes exposed acutely to epinephrine compared to controls

Type of compound added		Ethanol elimination (nmol/min/mg cell protein)	
		0–5 min	5–90 min
Control		40.30 ± 12.66	4.79 ± 1.10
+ Prazosin	10 μM	10.00 ± 9.04*	3.21 ± 0.67
+ Propranolol	10 μM	22.30 ± 16.97	6.13 ± 1.55
+ Antimycin	4 μM	6.91 ± 2.29*	2.00 ± 0.49*
+ Oligomycin	4 μM	43.91 ± 4.16	4.06 ± 0.69
+ 4-Methylpyrazole	4 mM	40.95 ± 3.66	1.24 ± 0.85*
Epinephrine		148.37 ± 11.09†	5.88 ± 0.63
+ Prazosin	10 μM	0.83 ± 0.74‡	4.63 ± 0.60
+ Propranolol	10 μM	32.91 ± 10.71‡	5.79 ± 1.59
+ Antimycin	4 μM	1.69 ± 0.78‡	1.23 ± 0.47§
+ Oligomycin	4 μM	69.61 ± 10.70‡	2.45 ± 1.09§
+ 4-Methylpyrazole	4 mM	57.20 ± 2.16‡	0.76 ± 0.64‡

The hepatocytes were in culture with the addition of dexamethasone (0.1 μM) for 4 days. The cultures were preincubated for 60 min with fresh medium containing no additives or one of the inhibitory compounds listed. Ethanol (8 mM) was then added to the culture medium together with 10 μM epinephrine or an isovolumetric amount of medium (control). Ethanol elimination was determined over 90 min. All values are expressed as means ± SE of five culture plates.

*, † Significantly different from control culture without additions: * $P < 0.05$, and † $P < 0.01$.

‡, § Significantly different from epinephrine culture without additions: ‡ $P < 0.01$, and § $P < 0.05$.

Table 3. Changes in pyridine nucleotides during ethanol elimination in cultured rat hepatocytes in the presence and absence of epinephrine

Minutes after ethanol addition	NAD^+	NADH	$NADP^+$	NADPH
		(nmol/culture)		
Control				
0	1.28 ± 0.06	0.46 ± 0.01	0.21 ± 0.04	1.33 ± 0.10
5	1.33 ± 0.07	0.23 ± 0.02*	0.76 ± 0.06*	1.52 ± 0.17
10	1.15 ± 0.03†	0.29 ± 0.01*	0.31 ± 0.03†	1.81 ± 0.11†
15	1.29 ± 0.08	0.29 ± 0.02*	0.38 ± 0.06*	1.80 ± 0.09†
20	1.31 ± 0.09	0.32 ± 0.03*	0.31 ± 0.03†	1.84 ± 0.09†
Epinephrine				
0	1.38 ± 0.06	0.46 ± 0.01	0.21 ± 0.04	1.23 ± 0.10
5	1.13 ± 0.02†‡	0.19 ± 0.02*	0.78 ± 0.04*	1.31 ± 0.16
10	1.13 ± 0.09	0.23 ± 0.02*§	0.64 ± 0.05*§	1.59 ± 0.18
15	1.07 ± 0.07†	0.28 ± 0.02*	0.50 ± 0.05*	1.64 ± 0.04†
20	1.25 ± 0.03†	0.33 ± 0.01*	0.61 ± 0.02*§	1.91 ± 0.09*

The experimental details are the same as those described in Table 2. All values are expressed as means ± SE of 3–7 culture plates.

*, † Significantly different from culture without ethanol (0 time): * $P < 0.01$, † $P < 0.05$.

‡, § Significantly different from control culture at same sampling time: ‡ $P < 0.01$, § $P < 0.05$.

prolonged, lasting up to 20 min. Epinephrine also caused a higher and more sustained increase in $NADP^+$ during ethanol elimination. The NAD^+/NADH ratio was not affected by epinephrine at the various times. By contrast, the $NADP^+$/NADPH ratio was higher at all time points after addition of ethanol in the presence of epinephrine.

Alcohol dehydrogenase activity was not affected by acute exposure of the hepatocytes in culture to 10 μM epinephrine in the presence of 0.1 μM dexamethasone. In addition, alcohol dehydrogenase activity was not altered by the presence of inhibitors of ethanol elimination tested in culture other than 4-methylpyrazole. After prolonged exposure to 4-methylpyrazole (4 mM), alcohol dehydrogenase activity was decreased by 95 and 93% in control and epinephrine-exposed hepatocyte cultures respectively.

DISCUSSION

This study shows that epinephrine increased alcohol dehydrogenase activity in hepatocytes in culture and that this change was dependent on the presence of dexamethasone. The increase in alcohol dehydrogenase activity caused by epinephrine in the presence of dexamethasone paralleled prior observations *in vivo* of an increase in alcohol dehydrogenase after stress induced by immobilization [6] in which levels of catecholamines and corticosteroids are increased markedly. The 10 μM concentration of epinephrine that increased alcohol dehydrogenase activity in hepatocyte culture was much higher than the mean plasma concentration of 3.9 nM found in non-stressed rats *in vivo* [25]. However, following immobilization stress, the plasma levels of epinephrine increase about 50-fold [25, 26]. Other investigators have noted that concentrations of epinephrine required to obtain significant changes in metabolic processes are much higher than plasma levels [27].

Corticosteroids are required for the optimal effect of epinephrine in enhancing gluconeogenesis, glycolysis, and lipolysis [28], and have a permissive or additive effect on the action of epinephrine in inducing a number of hepatic enzymes [29–31]. In this study corticosteroids had a permissive effect on the increase of alcohol dehydrogenase by epinephrine. In other studies, corticosteroids were found to have a permissive role in the increase of liver alcohol dehydrogenase by chronic ethanol feeding in mice [32], and by growth hormone in hepatocyte culture [33]. Adrenalectomy abolishes the effect of experimental uremia in increasing liver alcohol dehydrogenase activity [34].

An alteration in enzyme protein turnover is the most likely mechanism for the increase in liver alcohol dehydrogenase activity. Alcohol dehydrogenase has a half-life of 4–5 days [35]. Changes in the enzyme activity became apparent only after chronic exposure of the hepatocytes to epinephrine for a few days. No change or only a slight increase was found in the Michaelis–Menten constants for ethanol and NAD^+ respectively. Epinephrine inhibits non-specific hepatic protein synthesis [36], but increases the synthesis of a few specific enzymes such as tyrosine-α-ketoglutarate transaminase [30] and phosphoenolpyruvate carboxylase [37].

The effect of epinephrine in increasing urea synthesis in hepatocyte culture in this study agrees with prior observations of stimulation of urea synthesis by epinephrine in isolated hepatocytes [38]. Dexamethasone decreased urea synthesis and suppressed the enhancing effect of epinephrine in increasing urea synthesis. Corticosteroids stimulate non-specific protein synthesis [39] and have been described to increase survival of hepatocytes in culture [40]. On the other hand, corticosteroids enhance the action of epinephrine in increasing cyclic AMP [31] and gluconeogenesis [28]. In one study, isoproterenol, but not epinephrine, was more effective in increasing urea synthesis in hepatocytes from adrenalectomized rats than from normal rats [41].

The rate of ethanol oxidation is determined by the activity of alcohol dehydrogenase and the rate of

reoxidation of NADH. The increase in both the activity of alcohol dehydrogenase and the rate of ethanol elimination after chronic exposure of the hepatocytes to epinephrine and dexamethasone suggests that, in these circumstances, the activity of the enzyme is the major determinant of ethanol oxidation. Previously, increases in the rate of ethanol elimination after chronic administration of epinephrine to rats were attributed to increased hepatic oxygen respiration; however, alcohol dehydrogenase was not determined [1]. The increase in ethanol elimination after stress induced by immobilization in rats was also associated with an elevated alcohol dehydrogenase activity [6].

The marked but transient increase in the rate of ethanol elimination after the acute exposure of the hepatocytes to epinephrine was not associated with a change in alcohol dehydrogenase activity. This effect of epinephrine was principally mediated by α_1-adrenergic receptors and NAD^+-dependent oxygen uptake. Other investigators found that, in the perfused liver, epinephrine stimulates the rate of ethanol metabolism to 160% of control rates, with a half-life of less than 2 min and in association with an increased oxygen uptake [4]. Furthermore, the effect of epinephrine in increasing ethanol oxidation by isolated hepatocytes was also found to be mediated by α_1-adrenergic receptors [3]. The effect of epinephrine in increasing ethanol disappearance is most likely related to increased transfer and mitochondrial oxidation of NADH mediated by increased calcium mobilization via α_1-adrenergic receptors [42]. Of note is that the increase in free cytosolic calcium following stimulation of hepatocytes with epinephrine occurs in seconds but is transient, slowly returning toward basal levels by 5–10 min [43]. Catecholamines in the perfused liver increase oxygen uptake within seconds, reaching maximal effects in about 1 min [44]. The decrease in NAD^+ during ethanol metabolism is consistent with the utilization of this coenzyme during ethanol and acetaldehyde oxidation. NADH, however, did not accumulate but also decreased. These decreases in NAD^+ and NADH were associated with increases in $NADP^+$ and NADPH, suggesting conversion of NADH to NADPH by transhydrogenation. Part of the NADPH would then be oxidized to $NADP^+$ when utilized as a coenzyme in dehydrogenase or mixed-function oxidase reactions. Epinephrine may enhance these conversions since its presence resulted in higher levels of $NADP^+$ during ethanol elimination. Of note is that the acute administration of catecholamines has been found to increase the activity of the $NAD(P)^+$ transhydrogenase reaction in rat liver mitochondria [45].

It is uncertain whether most of the early phase of ethanol elimination occurs by oxidation via alcohol dehydrogenase. The early rate of ethanol disappearance was very high, and 4-methylpyrazole was not very effective in inhibiting this rate in control hepatocytes and only 61% effective in inhibiting the epinephrine-enhanced rates of ethanol elimination. The early phase of ethanol disappearance in the control cultures of 40.3 nmol/mg protein/min was six times higher than the measured alcohol dehydrogenase activity of 6.72 ± 0.23 nmol/mg protein/min on day 4 of culture. Furthermore, the rate after epinephrine exposure increased to 148.4 nmol/mg protein/min which is 22-fold higher than that of the measured enzyme activity. It is possible, however, that the assay of the enzyme *in vitro* does not reflect its activity in the hepatocytes in culture. This could occur because of the presence of an optimal milieu in culture which is not reproduced *in vitro*, or because of loss of enzyme or partial inactivation during harvesting and disruption of the cells prior to assay. Of note is that alcohol dehydrogenase activity of isolated hepatocytes prior to plating was 21.3 ± 1.6 μmol/mg protein/min [46]. Also, rat liver alcohol dehydrogenase activity is accelerated 50- to 100-fold using a coupled assay including lactaldehyde [47]. It is also possible that a different metabolic pathway not dependent on alcohol dehydrogenase contributes to the alterations in initial rates of ethanol disappearance. The microsomal ethanol-oxidizing system may participate in the elimination of ethanol and contribute to the postulated increased oxidation of NADPH to $NADP^+$ discussed previously. Another pathway to be considered is non-oxidative formation of fatty acid ethyl esters [48], particularly since the fatty acid ethyl ester synthase catalyzing this reaction is stimulated by calcium. This pathway, however, is unlikely to be a major contributor to the ethanol disappearance since the K_m for ethanol of the fatty acid ethyl ester synthase is several times higher than the 8 mM ethanol concentration used to measure the ethanol elimination, and the maximal rate of formation of the esters is relatively low, even at high ethanol concentrations [49, 50].

The rate of ethanol elimination following the initial rapid disappearance was not affected by epinephrine. This latter ethanol elimination was most certainly the result of ethanol oxidation by alcohol dehydrogenase since it was in the range of the enzyme activity and was strongly inhibited by 4-methylpyrazole.

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