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EFFECT OF ALCOHOL ON RENAL VITAMIN D METABOLISM IN CHICKENS

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SUMMARY

Intraperitoneal administration of ethanol to young chickens (both vitamin D-replete and vitamin D-deficient) produced a significant impairment of renal 25 hydroxyvitamin D_3 1α-hydroxylase (EC 1.14.13.13) activity with no significant change in serum calcium or phosphorus. In ethanol treated D-replete chicks the renal 25 hydroxyvitamin D_3 24-hydroxylase activity was enhanced, and serum 25 hydroxyvitamin D_3 was significantly increased. The alkaline phosphatase levels in the D-deficient ethanol treated chicks were significantly less than the controls. Our data suggest that the impairment of the metabolic effects of vitamin D due to ethanol occurs chiefly via a renal, rather than a hepatic mechanism. Furthermore, 1α -hydroxylated metabolites of vitamin D would appear to be the logical treatment of choice for the bone disease of alcoholism.

INTRODUCTION

Alcoholics have been shown to have a reduced bone mass compared to controls (1,2,3). Decreased blood levels of $25OHD_3$ have been reported in alcoholics (4,5,6) but the degree of decrease and the proportion of alcoholics affected are disputed as are the factors responsible (4,5,6,7), although concurrent malnutrition is commonly implicated. Osteomalacia is prevalent in alcoholics with and without cirrhosis. Verbanck et al (4) found histological osteomalacia in 80% of 24 female and 65% of 36 male alcoholics without cirrhosis, and they found no correlation between plasma $25OHD_3$ levels and the degree of osteomalacia. Similar findings have been reported by others (8).

Hypocalcaemia has been observed after an oral dose of ethanol in chicks (9), dogs (10) and in intact and parathyroidectomized rats (11).

Abbreviations: $25OHD_3$, 25 hydroxyvitamin D_3; $1,25(OH)_2D_3$, 1,25 dihydroxyvitamin D_3; 24-OHase, 25 hydroxyvitamin D_3 24-hydroxylase; 1α-OHase, 25 hydroxyvitamin D_3 1α-hydroxylase (EC 1.14.13.13); $25,26(OH)_2D_3$, 25,26 dihydroxyvitamin D_3; PTH, parathyroid hormone; CT, calcitonin.

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In rats ethanol elicited an increase in PTH, but this did not prevent or correct the concomitant hypocalcaemia (12); indeed administration of PTH to ethanol treated parathyroidectomized rats was also ineffective (11). In man Williams et al (13) demonstrated that ingestion of ethanol in amounts often consumed by social drinkers (0.8g/kg in one hour) caused significant increases in both serum PTH and plasma CT without change in serum calcium. PTH is usually a positive tropic modulator for the formation of $1,25(OH)_2D_3$ in the kidney (14), and one might, therefore, anticipate an increase in the renal 1α-OHase on alcohol treatment. Furthermore, ethanol is known to inhibit energy dependent mitochondrial calcium uptake in the liver (15). Inhibition of calcium uptake has been shown to inhibit the renal 1α-OHase (16). We have, therefore, investigated the effect of ethanol on the activity of the renal 1α-OHase using *in vitro* incubations of chicken renal mitochondria from chickens chronically dosed with ethanol intraperitoneally.

MATERIALS AND METHODS

Day old first cross Leghorn - Australorp cockerels were commenced on either a vitamin D-deficient diet (17) or an identical diet containing 1 mg vitamin D_3 per kg food. From day 7 to day 21, the chickens were dosed intraperitoneally with 50% ethanol in a volume of 2ml/kg body weight, and compared with undosed chickens from the same batch kept under the same conditions. On day 21 the chickens were killed by decapitation; serum was collected for measurement of calcium (by atomic absorption spectrometry) alkaline phosphatase (18) and serum inorganic phosphate (19). Serum $25OHD_3$ measurements were made using the competitive protein binding assay of Haddad and Chyu (20) with the following modifications:- 1) In the extraction procedure serum (100µl) was diluted with water (500µl) and then extracted twice with 1.8 ml ether, and 2) in the competitive protein binding assay, incubation was at 4°C for three hours.

Kidneys were removed for measurement of 24-OHase and 1α-OHase activity. Kidney mitochondria were prepared and incubations were performed as described by Gray et al (21) but with 40ng of {26,27 - ^{3}H} $25OHD_3$ (1.1Ci/mmol) as substrate. Chromatography was according to Henry and Norman (22).

RESULTS AND DISCUSSION

Ethanol administered intraperitoneally for 14 days had no effect on calcium or phosphorus levels in serum taken 24 hours after the last dose of ethanol in both vitamin D-replete and vitamin D-deficient chicks (table 1). Although all D-deficient chicks had significantly elevated alkaline phosphatase levels compared with D-replete chicks, the ethanol dosed D-deficient chicks had significantly reduced alkaline phosphatase levels compared to the D-deficient controls. In D-replete chicks ethanol dosing produced a significant increase in serum $25OHD_3$ levels. Vitamin D-deficient chicks had undetectable serum levels of $25OHD_3$. The renal 24-OHase activity of vitamin D-replete chicks was enhanced and the 1α-OHase activity of both vitamin

Table 1

Effect of Chronic I.p. Administration of Ethanol

TREATMENT	SERUM				KIDNEY	
	Total Calcium	Phosphate	Alkaline Phosphatase	25-OH-D3	24-Hydroxylase	1-Hydroxylase
	mM	mM	IU/l ($x10^{-3}$)	mM	percent§	percent§
VITAMIN D-REPLETE						
Control	2.31±0.06	2.22±0.24	1.99±0.01	138.5±23.3	6.9±1.6	3.8±0.6
Ethanol (2ml 50%/ kg b.w./day	2.40±0.04	2.30±0.12	1.67±0.12	226.0± 8.0*	15.0±2.5*	1.0±0.4**
VITAMIN D-DEFICIENT						
Control	1.45±0.11	2.38±0.35	17.67±1.12	< 2.5$	<1.0$	22.7±2.47
Ethanol (2 ml 50%/ kg b.w./day	1.64±0.09	1.58±0.33	7.59±1.01**	< 2.5$	<1.0$	13.3±1.90**

Animals were dosed for 14 days prior to sacrifice. All values are means ± S.E.M. Each group consisted of 5-8 animals. §, 24-hydroxylase and 1α-hydroxylase activities are expressed as the percent of recovered radioactivity occurring as the metabolite per 10 mg mitochondrial protein; $, Below assay detection limit; *, $p < 0.05$ } Significantly different from; **, $p < 0.01$ } control.

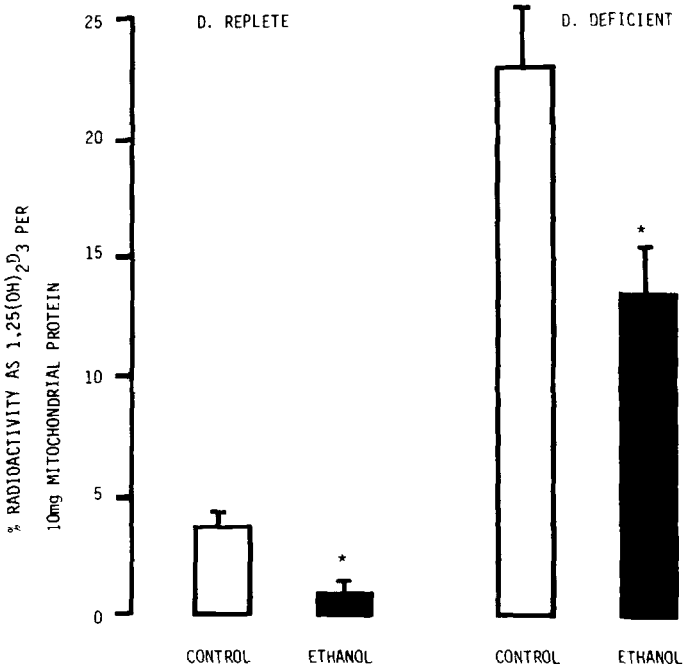

Figure 1 - Effect of chronic i.p. administration of ethanol on renal 1α-hydroxylase.
Bars show means ± S.E.M. Each group consisted of 5-8 chickens.
* $p < 0.01$ significantly different from control.

D-replete and vitamin D-deficient chicks was inhibited by ethanol (table 1, fig 1). The above effects were observed without a significant change in serum calcium concentration compared to controls. It should be noted that the chromatography system of Henry and Norman (22) results in $25,26(OH)_2D_3$ and $1,25(OH)_2D_3$ co-migrating (23). D-deficient chicks do not produce $25,26(OH)_2D_3$ (23), but in the D-replete chicks ethanol could have affected the level of $25,26(OH)_2D_3$ rather than the level of $1,25(OH)_2D_3$. However, chromatography of extractions of kidney incubations from control and ethanol treated D-replete animals using the system of Tanaka et al (23) showed no difference in the amount of $25,26(OH)_2D_3$ present.

The increase in serum $25OHD_3$ levels in ethanol dosed D-replete chicks is in line with findings of Mezey et al (24) who reported no change in serum $25OHD_3$ levels in rats fed ethanol as 36% of their caloric intake. Also, Lund et al (25) reported significant increases in serum $25OHD_3$ following peroral administration of vitamin D_3 to alcoholics; the decreases

reported by others (4,5,6) could thus be due to malnutrition or lack of sunshine (4,5,25) or possibly inhibition of the enterohepatic circulation of $25OHD_3$ (26).

The relatively decreased levels of serum alkaline phosphatase found in the ethanol dosed D-deficient chicks compared to the controls suggests a relative decrease in bone formation compared to controls. Summerskill et al (27) in fact found decreased bone formation and increased bone resorption on quantitative micro-radiography in two alcoholics with cirrhosis and osteoporosis compared to 5 normal controls. Mezey et al (24) have also found that young rats on a D-replete diet and fed ethanol (36% of caloric intake) had significantly lower serum alkaline phosphatase levels than controls.

The inhibition of the 1α-OHase activity in the ethanol dosed D-replete and D-deficient chicks without a significant change in serum calcium may represent a direct effect of ethanol on renal mitochondria (15,16).

Alternatively, ethanol may be affecting the 1α-OHase via some indirect agent. Ethanol has been shown to increase serum levels of other hormones involved in calcium metabolism, namely PTH (12,13) and CT (13). It is known to interfere with the expected action of PTH on renal calcium excretion (28) and on serum calcium (11,12,13). Our results suggest that ethanol may also interfere with the positive tropic effect of PTH on the renal 1α-OHase (14). Calcitonin is apparently without effect on the 1α-OHase (29).

Oral administration of ethanol to rats has been shown to interfere with duodenal calcium transport assessed by the in vitro gut sac technique (3) and calcium absorption in these animals is purported to be unresponsive to treatment with $1,25(OH)_2D_3$ (31); however, the measurements were performed at a time (24 hours after injection) when the effect of the exogenously administered hormone would have been minimal (32). Furthermore, the dosage of ethanol used (20% ethanol in water as the sole source of fluid for 12 days) was excessive.

A similar study to that reported here using orally administered ethanol is in progress, and should our findings be confirmed, it seems likely that the effect of ethanol on vitamin D metabolism is primarily of renal origin rather than hepatic. This suggests that in humans, apart from abstinence from alcohol, the use of 1α-hydroxylated metabolites of vitamin D may prove to be effective in the management of alcoholics with bone disease. Our studies

reinforce the opinion of Shah et al (12) that ethanol is an inappropriate solvent to use in any studies on calcium metabolism.

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