Prolonged Ethanol Consumption Increases Testosterone Metabolism in the Liver

Abstract. *Male alcoholics often suffer from features of hypogonadism related to abnormal metabolism of sex steroids. Since the activity of testosterone reductases is rate limiting for testosterone metabolism in the liver, the effect of prolonged ethanol consumption by rats and human volunteers on the activities of these microsomal and cytosolic enzymes was studied. In rats, long-term ethanol ingestion doubled microsomal 5α-testosterone reductase activity, a major pathway for testosterone metabolism, while in human volunteers the activity was increased two- to fivefold. These changes may play a role in the altered androgenic activity of the chronic alcoholic.*

Male alcoholics, with and without overt liver disease, exhibit certain hypogonadal features, such as testicular atrophy (*1*), impaired spermatogenesis (*2*), impotence and decreased libido (*2*), as well as overt abnormalities in the metabolism of testosterone (*2–4*), androstenedione (*4*), and the estrogenic steroids estrone and estradiol (*5*). The irreversible metabolism of circulating testosterone is initiated in the liver by microsomal Δ^4-3-ketosteroid reductases. The microsomal enzyme is a steroid 5α-reductase, while the cytosolic enzyme is a 5β-reductase (*6*).

We have shown that long-term ethanol intoxication leads to increased activities in the liver of microsomal enzymes that metabolize drugs in the liver (*7*); the activity of Δ^4-3-ketosteroid reductase in the liver can be induced by drug administration (*8–10*). We now report the effect of prolonged administration of ethanol on hepatic testosterone reductase activity in rats and human volunteers.

Sixteen male Sprague-Dawley rats, weighing approximately 150 g, were fed a liquid, nutritionally adequate diet (*11*). Each animal was paired with another fed a similar diet, in which ethanol was isocalorically substituted for carbohydrate, accounting for 36 percent of total calories. After 24 days the animals were killed, and the microsomal and soluble fractions of the livers were obtained by standard methods (*10*).

The assay mixture for the measurement of testosterone-reductase activity contained 0.01*M* potassium acetate buffer (*p*H 5.6), reduced nicotinamide adenine dinucleotide phosphate ($7.2 \times 10^{-4}M$), testosterone ($6 \times 10^{-4}M$), containing [4-^{14}C]- or [7α-^{3}H]testosterone, 10^6 count/min), and liver enzyme preparation (microsomes or cytosol) in a total volume of 1 ml. The mixture was incubated at 37°C and 0.2-ml portions were taken at 10-minute intervals. The reaction was terminated by placing each portion in 4 ml of dichloromethane. The testosterone-reductase activity was determined by two methods; either by the rate of formation of reduced metabolites of ^{14}C- or ^{3}H-labeled testosterone after isolation and identification, or by the rate of disappearance of the Δ^4-3-ketosteroid substrate, as measured by the decrease in absorbance at 240 nm (*10*).

Table 1 shows the effect of long-term administration of ethanol on the rate of testosterone reduction in rats. The mean activity of testosterone reductase in the microsomal fraction was more than doubled, compared with that of pair-fed controls. By contrast, reductase activity in the soluble fraction was essentially unchanged.

To extend the findings of animal experiments to man we studied five male human volunteers ranging in age from 21 to 38 years. After giving informed consent, they were maintained at the Clinical Research Center of the Mount Sinai Hospital. Three were normal, nonalcoholic individuals, whose weekly consumption of ethanol did not exceed an average of 70 g. Two were chronic alcoholics who had abstained from drinking alcoholic beverages for at least 7 to 10 days prior to the study. The volunteers were given a routine hospital diet supplemented with vitamins, including folic acid, for 4 to 5 days. At that time results of all routine clinical laboratory tests (including serum bilirubin, the activities of glutamic oxalacetic and pyruvic transaminases and that of alkaline phosphatase) were within normal limits. An aspiration liver biopsy was then performed. The volunteers were subsequently given the same dose of ethanol as a 15 percent solution (weight to volume) in a flavored beverage every 3 hours, and a diet that contained excess protein, vitamins (including folic acid), and minerals (*12*). The total caloric intake was 3600 to 3800 calories per day, distributed as follows: ethanol, 42 percent; protein, 14 percent; fat, 32 percent; and carbohydrate, 12 percent. The total daily amount of ethanol consumed was usually 220 g or about 3 g per kilogram of body weight. After 4 weeks, ethanol was withdrawn; 18 hours later another liver biopsy specimen was obtained. The weight of the volunteers, which ranged from 64 to 79 kg, did not change significantly during the study.

Table 1. Effect of long-term ethanol consumption in rats on the activities of hepatic microsomal 5α- and cytosolic 5β-steroid reductase activity (nanomoles of testosterone reduced per 10 minutes per milligram of protein). Each group consisted of 16 rats. N.S., not significant.

Subject	Microsomal 5α-reductase activity*	Cytosolic 5β-reductase activity*
Control	22.3 ± 1.9	5.4 ± 0.2
Ethanol-fed	43.4 ± 3.1	5.0 ± 0.2
Percent change†	115 ± 22.7	−7.6 ± 3.7
P	< .005	N.S.

*Mean ± standard error of the mean. †Means of individual pairs.

Table 2. Effect of chronic ethanol consumption on total hepatic testosterone reductase activity (nanomoles of testosterone reduced per 10 minutes per milligram of protein of liver homogenate).

Subject	Testosterone reductase activity		Percent change
	Before ethanol	After ethanol	
D.M.	13.9	70.3	+ 406
W.K.*	8.9	19.9	+ 124
T.A.*	5.6	15.9	+ 184
J.S.	6.1	12.0	+ 97
J.H.	6.3	25.2	+ 300
Mean ± standard error	8.2 ± 1.5	28.7 ± 10.6	+ 222

*Alcoholic.

Table 2 shows the testosterone-reductase activity in the human liver before and after ethanol consumption. Owing to the small amount of tissue available in an aspiration biopsy specimen, the enzyme activity was assayed only in whole homogenate (*9*). The reductase activity was augmented between two- and fivefold after ethanol administration.

The data indicate that long-term administration of ethanol to male rats and humans leads to a striking increase in the activity of microsomal 5α-reductase activity in the liver. However, the 5β-reductase activity, which is found in the cytosol, is not affected. Proliferation of smooth endoplasmic reticulum and increased activities of some microsomal enzymes, including a reductase, are produced by long-term ethanol ingestion (*7*). The activities of other cytosolic enzymes, for example, alcohol dehydrogenase and glutamic oxalacetic transaminase, are not altered by long-term ethanol consumption (*13*). An increase in the activity of testosterone reductase, the rate-limiting hepatic enzyme for testosterone metabolism, may enhance the removal of testosterone and other Δ^4-3-ketosteroids in the liver, which, in turn, may play a role in the altered androgenic activity in chronic alcoholics. Our preliminary

data indicate that, in man, there is no compensatory increase in the biosynthesis of testosterone under these conditions.

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Experience Modifies the Plastic Properties of Identified Neurons

Abstract. *Crickets (*Acheta domesticus*) were reared in the presence of continuously repeating tone pulses. The responses of large abdominal interneurons to similar tone pulses were then compared. The giant interneurons of treated specimens are more resistant to habituation than those of control specimens.*

Exposure to sensory input during postnatal development can influence the nature of the vertebrate nervous system (*1*). If sensory input could be demonstrated to influence the development of the numerically simpler nervous system of an invertebrate, we might be able to determine, at the level of identified neurons, the mechanisms by which environmental modification of a nervous system occurs. We report here a successful modification of the response properties of identifiable interneurons by manipulation of sensory input during maturation. We found that interneurons exposed to continuous stimulation during postembryonic development habituate to that stimulus more slowly than do interneurons of unexposed specimens.

Our experiments were carried out on the abdominal giant interneurons of the cricket *Acheta domesticus*. These interneurons, which are the largest in the cricket nervous system, are excited by substrate vibrations detected by receptors located in the body wall and by sound and wind currents detected by mechanoreceptors located on paired abdominal sensory appendages, the cerci. The sensitivity to tones, which will be our major interest here, results from a monosynaptic input from sensory cells coupled to receptive hairs located on the cerci. The largest interneurons are sensitive to tones below 1000 hertz with peaks at approximately 100 and 500 hertz (*2*).

We reared crickets from hatching to adulthood in the presence of continually produced tone pulses of 500 hertz and 100 to 105 db (*3*). Tone pulses were 300 msec long and occurred at a rate of 1 to 2 per second. Control specimens were reared in identical cages in identical incubators but never heard the sound pulses. As the background noise level in these incubators was 80 db, we used a relatively high-intensity stimulus for treatment. In the first two experiments we reared 50 to 60 specimens randomly selected at hatching from two (and in the second experiment, four) females and tested 20 male specimens from these groups. In a third experiment we reared identical numbers of treated and control larvae from a single female and, in addition, exchanged the incubators weekly. All specimens were tested between 2 and 5 weeks after the adult molt. The treatment effects were identical in each experiment, although variability was reduced in the experiment where all specimens were siblings.

Table 1. Statistical analyses of habituation rates. The data for each specimen were fitted to a power curve of the form $Y = bX^m$. This curve was transformed to a straight line of the form $\ln Y = \ln b + (m) \ln X$. A *t*-test was then used to determine whether the slopes of control and treated groups were different. All the specimens in this experiment were male siblings. There were 14 specimens in the control group and 15 in the treated group. Abbreviations: c, control group; t, treated group; N.S., not significant.

Pulses per second	Constants		*t*	d.f.	*P*
	m	*b*			
0.5					
c	−.127	5.77	0.02	27	N.S.
t	−.097	5.64			
1.0					
c	−.334	6.78	−3.18	27	.01
t	−.176	6.16			
1.5					
c	−.422	6.47	−3.22	27	.01
t	−.231	5.36			
2.0					
c	−.794	7.12	−6.50	27	.001
t	−.303	5.17			
2.5					
c	−.944	6.97	−4.16	27	.001
t	−.433	4.37			

A phasic response consisting of six to eight action potentials was recorded at the onset of a tone pulse (Fig. 1A, upper trace). This extracellularly recorded activity was fed to a spike height discriminator. The threshold of the discriminator was set so that only the phasically driven large amplitude action potentials were counted (Fig. 1A, upper trace, dotted line). The action potentials above threshold were displayed as a series of dots on the face of a storage oscilloscope, which displayed the response decrement visually (Fig. 1B). Spike counts were obtained directly from this display.

The activity in a single interneuron accounts for most of the extracellular action potentials. Extracellular recording electrodes identical to those described above were placed in the usual position just anterior to the terminal ganglion and surrounded with Vaseline. A recording micropipette was then inserted in the soma of the medial giant interneuron (MGI) using methods described elsewhere (*4*). The neuron was anatomically identified by injecting the soma with Procion yellow or co-