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EFFECTS OF ETHANOL AND ACETALDEHYDE ON THE BIOSYNTHESIS OF TESTOSTERONE IN THE RODENT TESTES

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SUMMARY

The effects of ethanol and acetaldehyde on testicular steroidogenesis were examined in enzymatically dispersed cells of the rodent testes. Both drugs significantly inhibited gonadotropin-stimulated steroidogenesis, but acetaldehyde was considerably more potent (>1000 times) than ethanol. To determine the step in testosterone's biosynthetic pathway which was inhibited by the two drugs, cells were incubated in the presence of [^{3}H]pregnenolone and [^{3}H]progesterone, and the amount of label incorporated into testosterone and its precursors was determined. Ethanol and acetaldehyde inhibited only the conversion of androstenedione to testosterone; none of the other precursors of testosterone was affected.

INTRODUCTION

Ethanol decreases serum testosterone levels after its administration in the male (1-4). Ethanol has also been found to suppress testicular steroidogenesis *in vitro*, but substantial inhibitions occurred only at nonphysiological concentrations (5-8). This suggests that the metabolism of ethanol to acetaldehyde, which occurs readily *in vivo* but only minimally *in vitro* (9), is the mechanism responsible for ethanol's inhibition of testosterone production. In support of this conclusion acetaldehyde has been found to be a potent inhibitor of testicular steroidogenesis *in vitro* at physiological concentrations (6,9). In the present studies we examined the effects of ethanol and acetaldehyde *in vitro* on the formation of testosterone from labeled precursors in an effort to isolate the step in the biosynthesis of testosterone affected by the two drugs.

MATERIALS AND METHODS

Enzymatically Dispersed Cell Preparations: Enzymatically dispersed cells were prepared as described previously (10). Studies of drug effects on gonadotropin-stimulated testosterone production *in vitro* were carried out as described previously (9). Testosterone levels and drug concentrations in the media were determined as described elsewhere (9,10).

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Incubation with [^{3}H]Progesterone and [^{3}H]Pregnenolone: The isotopic purity of the [^{3}H]progesterone (56.5 Ci/mM) and [^{3}H]pregnenolone (17.2 Ci/mM) was determined by TLC (11). Incubations were carried out as described above, but in these studies incubations contained [^{3}H]pregnenolone or [^{3}H]progesterone and ethanol or acetaldehyde. The concentrations of acetaldehyde and ethanol were selected on the basis of initial studies (see Figure 1) to provide a 30-50% inhibition of testicular steroidogenesis. This range was selected to provide significant decreases in testicular steroidogenesis without the possibility of nonspecific cellular toxicity which might occur at higher concentrations. Aliquots of the media were extracted with ethyl acetate and dried under a stream of N_2. The residues were dissolved in methanol, containing carrier steroids, and were spotted on silica gel GF TLC plates. The plates were developed as described previously (11); the steroids were visualized with ultraviolet light or 5% phosphomolybdic acid. The spots were scraped from the plates and counted. The identity of the various steroids was determined by recrystallization to constant specific activity (12). Recoveries were found to be within 85-90% in all studies, and results are expressed as CPM incorporated into each product.

Generation of Interfering Compounds: To determine whether acetaldehyde or ethanol might spontaneously react with testosterone under our assay or extraction conditions to form a compound which would comigrate with one of the steroids examined, 50 nCi testosterone was incubated with 100 μM acetaldehyde or 200 mM ethanol under the incubation conditions described above, except that no cells were included. The media were extracted and subjected to TLC as described above.

RESULTS

Effects of Ethanol and Acetaldehyde on Testicular Steroidogenesis: As shown in Figure 1, ethanol and acetaldehyde inhibited gonadotropin-stimulated testicular steroidogenesis; however, acetaldehyde was effective at μMolar, whereas ethanol was effective at mMolar concentrations.

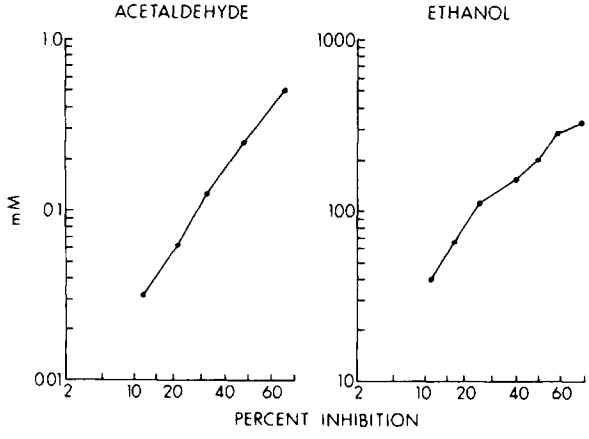

Figure 1. The effects of acetaldehyde and ethanol, expressed as percent inhibition, on human menopausal gonadotropin-stimulated (5 mIU) testosterone production in intact enzymatically dispersed cells of the rodent testes. Values are the means of three experiments carried out in triplicate. Standard errors averaged less than 5% of the mean at all points.

TABLE 1
Effects of acetaldehyde on the incorporation of [^{3}H]pregnenolone

Precursor	Control	%[a]	Acetaldehyde	%[a]
Pregnenolone	2,858.0 (±114.8)	5.0	2,810.0 (±183.4)	4.7
Progesterone	5,802.9 (±308.4)	10.3	6,531.1 (±656.0)	10.9
17-α-hydroxyprogesterone	1,260.8 (±137.5)	2.2	1,379.5 (±194.8)	2.3
Androstenedione	21,775.1 (±568.2)	38.4	30,368.3 (±877.8)	51.1[b]
Testosterone	24,886.6 (±921.4)	43.9	18,303.5 (±472.3)	30.8[c]
Total	56,583.4	100	59,392.4	100

The effects of acetaldehyde (100 μM) on the incorporation of [^{3}H]pregnenolone into the precursors of testosterone via the preferred route of metabolism. Dispersed cells were prepared as described in the Methods section and were incubated with 1.25 nCi [^{3}H]pregnenolone with or without 100 μM acetaldehyde. Values are means (±SEM) CPM representing three experiments carried out in triplicate. [a]Percent of total CPM (botton line); [b]significantly higher ($p<0.01$) when compared to control; [c]significantly lower ($p<0.01$) when compared to control.

Effects of Acetaldehyde on the Incorporation of [^{3}H]Pregnenolone into Testosterone: The effects of acetaldehyde on the incorporation of [^{3}H]pregnenolone into testosterone and its precursors are shown in Table 1 for the "preferred" route of testosterone's biosynthesis in the rodent testes. The only step in the biosynthesis of testosterone significantly affected was the conversion of androstenedione to testosterone; there was a significant accumulation of CPM in androstenedione in acetaldehyde-treated cells, relative to controls, and a proportional drop in the CPM in testosterone. The effects of acetaldehyde on the nonpreferred route of testosterone's biosynthesis and on the incorporation of [^{3}H]progesterone into testosterone and its precursors were also examined with identical results.

Effects of Ethanol on the Incorporation of [^{3}H]Pregnenolone and [^{3}H]Progesterone into Testosterone: The effects of ethanol on the incorporation of [^{3}H]pregnenolone into testosterone and its precursors are shown in Table 2 (the preferred route of biosynthesis). Ethanol selectively caused a significant increase in CPM in androstenedione, when compared to controls and a corresponding drop in CPM in testosterone. Similar results were obtained when [^{3}H]progester-

TABLE 2
Effects of ethanol on the incorporation of [^{3}H]pregnenolone

Precursor	Control	%[a]	Ethanol	%[a]
Pregnenolone	4,829.7 (± 701.5)	11.1	4,782.4 (± 240.1)	10.5
Progesterone	6,853.2 (± 640.4)	15.8	6,982.6 (± 920.2)	15.3
17-α-hydroxyprogesterone	2,579.2 (± 171.4)	5.9	2,392.6 (± 404.5)	5.3
Androstenedione	18,156.0 (±1440.6)	41.9	23,779.8 (±1765.2)	52.3[b]
Testosterone	10,862.2 (± 998.3)	25.0	7,492.2 (± 531.9)	16.4[c]
Total	43,280.3	100	45,429.6	100

The effects of ethanol (200 mM) on the incorporation of [^{3}H]pregnenolone into the precursors of testosterone. Values are means (±SEM) CPM of three experiments carried out in triplicate. (See legend to Table 1 for further details). [a]Percent of the total CPM (bottom line); [b]significantly higher ($p<0.01$) when compared to control; [c]significantly lower ($p<0.01$) when compared to control.

one was employed as the precursor and when either the preferred or nonpreferred route of testosterone biosynthesis was examined.

Spontaneous Generation of Interfering Compounds: Neither ethanol nor acetaldehyde affected the CPM in testosterone, androstenedione, or any other precursor of testosterone.

DISCUSSION

The present results indicate that ethanol and acetaldehyde block testicular steroidogenesis by inhibiting the conversion of androstenedione to testosterone. Both drugs caused an accumulation of CPM in androstenedione and a corresponding fall in CPM incorporated into testosterone; none of the other precursors of testosterone was affected. Since the percent reduction in CPM incorporated into testosterone corresponded to the reduction in gonadotropin-stimulated testosterone production, as measured by radioimmunoassay (Figure 1), and the increase in CPM in androstenedione completely offset the reduction in CPM in testosterone, an inhibition of the conversion of androstenedione to testosterone can completely account for ethanol and acetaldehyde's effects on testicular steroidogenesis - at least *in vitro*.

There has been only one other study of the effects of ethanol on the biosynthetic pathway for testosterone. Gordon et al.(13) reported that rats,

chronically maintained on ethanol, had a marked deficiency in Δ^5-3β-dehydrogenase activity. They found, however, that NAD^+ completely reversed this deficit, leading them to conclude that the effect of ethanol on testicular steroidogenesis *in vivo* was due to a change in the NAD^+/NADH ratio, occurring as a result of its metabolism. Unfortunately, Gordon et al.(13) did not examine whether the enzyme involved in the conversion of androstenedione to testosterone, 17-β-hydroxysteroid oxidoreductase, was affected by chronic ethanol administration so no conclusions can be drawn at the present time about the *in vivo* significance of the effects we have observed. It should be noted, however, that in preliminary studies we have found that the activity of this enzyme was competitively inhibited by acetaldehyde. Moreover, this enzyme does not require NAD^+ as a cofactor (14); thus, it seems improbable that a change in the redox state could explain ethanol's effects on its activity. In fact, we have shown that NAD^+ did not reverse the effects of ethanol or acetaldehyde on testicular steroidogenesis *in vitro* (unpublished observations). Thus, although a change in the NAD/NADH ratio may contribute to the effects of ethanol and acetaldehyde *in vivo*, such an alteration is clearly not required for the drugs to significantly block testicular steroidogenesis.

The possibility that ethanol and acetaldehyde favor the reverse reaction of testosterone to androstenedione, rather than blocking the forward reaction, cannot be ruled out completely in our studies. However, since the same enzyme is involved in both reactions and it appears to be competitively inhibited by acetaldehyde, the likelihood that acetaldehyde or ethanol enhances the conversion of testosterone to androstenedione seems remote. Thus, our data seem to be best explained simply by a drug-induced inhibition of 17-β-hydroxysteroid oxidoreductase.

In the present studies we found that actaldehyde was considerably more potent than ethanol in inhibiting testicular steroidogenesis. These results, and much earlier work (5,6,9), suggest that under *in vivo* and *in vitro* conditions both ethanol and acetaldehyde may be involved in inhibiting testicular

steroidogenesis and that acetaldehyde may be the primary agent responsible for these effects.

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