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Gonadal Alcohol and Aldehyde Dehydrogenase: In Vivo and In Vitro Effects of Psychoactive and Endocrine Agents

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MESSIHA, F. S. *Gonadal alcohol and aldehyde dehydrogenase: In vivo and in vitro effects of psychoactive and endocrine agents.* PHARMACOL BIOCHEM BEHAV **35**(1) 29–33, 1990.—The in vivo effect of amantadine, chlorpromazine and reserpine on testicular aldehyde dehydrogenase (T-ALDH) was studied as a function of mouse strain. The effect of Leu-enkephaline and tetrahydropapaverine on rodent T-ALDH was also studied in vivo. The in vitro effect of chlorpromazine, papaverine and scopolamine on rodent subcellular T-ALDH and testicular alcohol dehydrogenase (T-ADH) were evaluated. A strain-linked difference in endogenous T-ALDH among the three mouse strains studied was determined. Individual injection of chlorpromazine or reserpine inhibited only albino ICR T-ALDH which was alleviated by pretreatment with amantadine and, thereby, suggesting antagonism between amantadine and these agents. The Leu-enkephaline administration induced T-ALDH from saline control. Tetrahydropapaverine did not influence the enzymes studied in vivo compared to an insignificant in vitro induction of T-ADH by the O-methylated analogue papaverine. Chlorpromazine noncompetitively inhibited T-ADH in vitro. The results indicate the modulation of the enzymes studied, which are involved in both detoxification of ethanol and biogenic amine-derived aldehyde intermediates, by agents affecting the endocrine system. This suggests the potential of these testicular enzymes in the evaluation of alcohol- and drug-induced endocrine adverse reactions.

Amantadine Chlorpromazine Mouse strains Papaverine Testicular enzymes

CHRONIC use of psychoactive agents such as antipsychotic medications, reserpine, tricyclic antidepressants (2, 3, 10–12, 28, 29, 38) and alcohol (25–27, 33, 36, 37) often produce endocrine abnormalities in the susceptible subject. These agents change functional activity of certain biogenic amines (BA), alter the output of certain sex hormones and/or both which may underly some of the drug-induced endocrine adverse reactions. In addition, testicular alcohol dehydrogenase (T-ADH), the enzyme responsible for the primary metabolic detoxification of ethanol (ET), has been implicated in ET produced sterility in alcoholic men (33) and has been also shown to be altered by ET in rodents (19,25). The ADH and aldehyde dehydrogenase (ALDH), which metabolizes acetaldehyde (AC), also catalyze the reductive and oxidative metabolic pathway of BA-derived aldehyde intermediates, respectively. Accordingly, a relationship between such psychoactive agents and gonadal ADH and ALDH is likely.

The present study evaluated the in vivo effect of the antipsychotic medication chlorpromazine (CPZ), a BA receptor blocker, reserpine (RES), a BA depletor, and their interaction with amantadine (AMN), a dopaminergic agonist, on these gonadal enzymes in rats and mice. The effect of Leu-enkephaline (LNK) and tetrahydropapaverine (THP) on T-ALDH was also studied in vivo. In addition, specific activities of gonadal rat T-ADH and T-ALDH were determined in vitro in the presence and absence of papaverine (PAP), an opium alkaloid, which has found application in the diagnosis and therapy of erectile impotence in man (32,34) and of scopolamine (SCP), an anticholinergic agent with an abuse potential, which could also adversely affect endocrine function.

METHOD

Adult male Sprague-Dawley rats (Holtzman Farm Co., Madison, WI), 90–110 days old, and male mice 9 to 12 weeks old, were housed in a laboratory with 12-hr light-dark cycle and had access to Purina food and water ad lib until the time of sacrifice.

In the first in vivo study, three mouse strains were used. These were the Sprague-Dawley albino ICR and the black inbred C_{57}BL/6 (Sprague-Dawley Co., Madison, WI) and the albino BALB/C (Charles River Breeding Laboratories, Wilmington, MA) strains. They were housed in animal facility for 10 to 14 days

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before initiation of the experiment. All drugs were dissolved in saline and were injected intraperitoneally (IP). Each mouse strain was divided into six groups of 15 to 18 mice each. The initial four groups received saline followed 15 min later by either saline, ANM, 100 mg/kg, CPZ, 0.2 mg/kg or RES, 0.2 mg/kg. The two remaining groups were injected AMN, 100 mg/kg, 15 min prior to either CPZ, 0.2 mg/kg (designated AMN/CPZ group) or RES, 0.2 mg/kg (designated AMN/RES group). These dose regimens were repeated six times over 30 consecutive hours. The animals were then sacrificed 30 min post the terminal injection.

In the second in vivo study, Sprague-Dawley rats were administered THP, 50 mg/kg, IP/day for 6 consecutive days, and the control received the vehicle saline before they were sacrificed 30 min post the final treatment.

In the third in vivo study, albino ICR mice were injected LNK, 100 mg/kg, IP, twice over 48 hr and the animals were decapitated 24 hr later. The controls received saline.

Animals were killed by decapitation and the testis was removed and immediately homogenized in ice-cold 0.1 M KCl buffer pH 6.8 by sonication. The supernatant was subjected to differential centrifugation to obtain the nuclear (NC), mitochondrial (MT) and cytoplasmic (CT) testicular subcellular components as described in detail elsewhere (18). Aliquots of these subcellular fractions were used for the protein determination, by the biuret method, and the assays of T-ADH and T-ALDH which were determined spectrophotometrically at 25°C (17,24).

The compounds tested in vitro were PAP, SCP and CPZ. They were dissolved in distilled water and were added to the reaction mixture in a volume not greater than 0.1 ml. The final concentration of these compounds in the 3.0 ml reaction mixture was 5×10^{-5} M for CPZ, 10^{-4} M for PAP and 10^{-3} M for SCP. The stock solutions were freshly prepared daily. The enzymatic assays were performed in duplicates in the presence and absence of the substrate (control sample) or the testing substance (drug sample).

The lineweaver and Burk plots (16) were performed in the absence and presence of 5×10^{-5} M CPZ for the determination of the relationship between substrate concentration and velocity of the reaction (V_{max}). These plots were used to calculate the V_{max} and the apparent k_m. The results were expressed as means ± SEM of enzymatic specific activity, nmol/min/mg protein. The Student's *t*-test for independent means was used for the statistical evaluation of the data.

RESULTS

Figure 1 shows the in vivo effect of repeated administration or equal small dose regimens of RES or CPZ and a larger AMN dose on T-CT-ALDH as a function of mouse strain. The RES and CPZ treatment inhibited ICR mouse T-CT-ALDH by approximately 35% from saline control. However, the CPZ effect was not statistically significant ($p<0.09$). Pretreatment with AMN antagonized both CPZ- and RES-produced inhibition of ICR mouse T-CT-ALDH. No drug-related changes in specific activity of this enzyme was determined in the other mouse strains studied. Figure 1 also shows strain differences in endogenous T-CT-ALDH among the saline controls. This is indicated by a 75% ($p<0.002$) and 65% ($p<0.02$) greater ICR mouse L-CT-ALDH than these assayed in C_{57}BL/6 and BALB/C saline control mouse strains, respectively.

Table 1 shows the effect of LNK and THP on mouse and rat T-CT-ALDH, respectively. The LNK treatment resulted in 68% ($p<0.003$) induction of mouse T-CT-ALDH from saline controls. Short-term administration of THP did not alter rat T-CT-ALDH from corresponding control.

Table 2 shows the in vitro effect of PAP and SCP on specific activity of rat T-ADH, T-CT-ALDH and EP-CT-ALDH. The presence of 10^{-4} M PAP in the reaction mixture insignificantly ($p<0.1$) enhanced specific activity of T-ADH by approximately 60% compared to its absence. No other changes in testicle enzymes studied were determined in the presence of 10^{-3} M SCP concentration.

Table 3 shows the in vitro effect of CPZ, 5×10^{-5} M on T-ADH and T-ALDH of various subcellular fractions and EP-

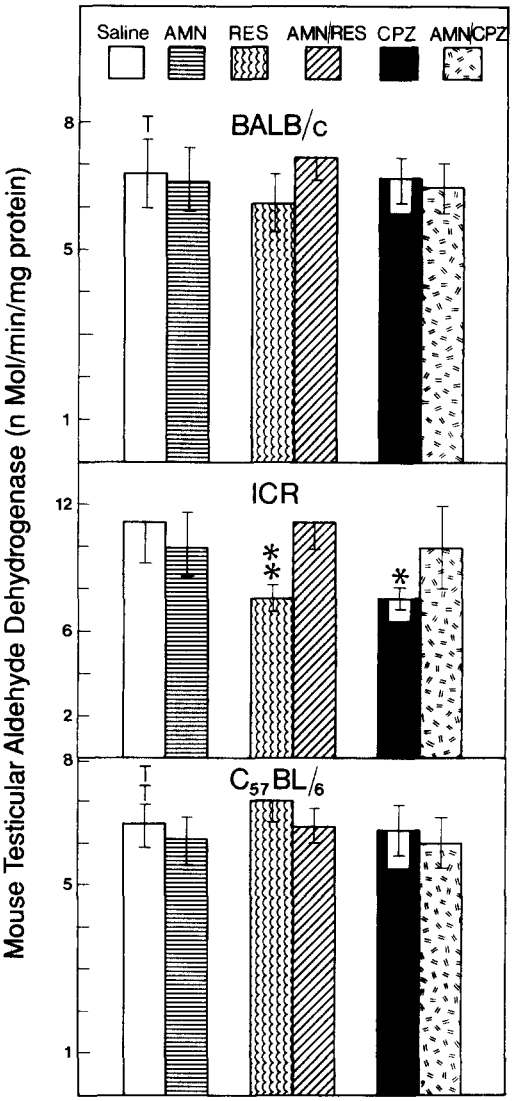

FIG. 1. The effect of separate and combined intraperitoneal injection of dose regimens of amantadine (AMN), reserpine (RES), and chlorpromazine (CPZ) on specific activity of testicular aldehyde dehydrogenase as a function of mouse strain. Saline was administered 15 min prior to AMN, 100 mg/kg, RES, 0.2 mg/kg, CPZ, 0.2 mg/kg. AMN was given 15 min before RES, 0.2 mg/kg (designated AMN/RES), or CPZ, 0.2 mg/kg (AMN/CPZ). These dose regimens were repeated for six trials over 30 hr and the mice were sacrificed 30 min post the terminal treatment. Each bar graph represents mean ± SEM of enzymatic specific activity (nmol/min/mg protein) of 15–18 determinations. Differences among mouse strain (saline controls): $^{TT}p<0.002$, $^{T}p<0.02$. Differences from corresponding controls: $^{**}p<0.003$, $^{*}p<0.09$ (not significant).

TABLE 1

THE IN VIVO EFFECTS OF LEU-ENKEPHALIN AND TETRAHYDROPAPAVERINE ON RODENT TESTICULAR ALDEHYDE DEHYDROGENASE

Treatment	Species	Dose (mg/kg, IP)	Duration (Days)	(n)	T-CT-ALDH (nMol/min/mg protein)
		Treatment			
Saline	Mouse	—	2	(8)	2.8 ± 0.3
LNK		100	2	(9)	4.7 ± 0.3*
Saline	Rat	—	6	(6)	4.4 ± 0.5
THP		50	6	(6)	4.7 ± 0.7

Leu-enkephalin (LNK) was injected, 100 mg/kg, IP, once daily for two consecutive days and the mice were sacrificed 24 hr post the second treatment. Tetrahydropapaverine (THP) was given, 50 mg/kg, IP/day, for 6 days and the male rats were sacrificed 30 min post the terminal injection. Values are means ± SEM of specific activity, nmol/min/mg protein, of testicular cytoplasmic aldehyde dehydrogenase (T-CT-ALDH) for the number of animals given between parentheses.

*$p<0.003$.

CT-ALDH. Testicular ADH was inhibited in the presence of CPZ by approximately 52% from the control sample ($p<0.004$). No further alteration in specific activities of the enzymes studied were found as a consequence of the CPZ addition to the reaction mixture.

Figure 2 shows the double reciprocal lineweaver and Burk plots for the relationship between the velocity of the reaction in the absence (control) and in presence of CPZ, 5×10^{-5} M, as a function of ET substrate concentration. The V_{max} was decreased by approximately 16% by CPZ. The apparent K_m value was increased by CPZ to 0.16 compared to the 0.03 units from the control. Figure 2 also shows that CPZ noncompetitively inhibited T-ADH in vitro.

DISCUSSION

The in vivo results demonstrate the sensitivity of the ICR mouse T-ALDH to the inhibitory actions of CPZ and RES compared to the other mouse strains studied. This suggests a strain-dependent sensitivity to these agents. Reduced elimination of testicular ET-derived acetaldehyde by these agents is likely to evoke gonadal toxicity which could be enhanced by ET consumption. Moreover, inhibition of T-ALDH by CPZ or RES will increase the formation of BA aldehydes which could condense with the unreacted parent amine to form tetrahydroisoquinolines, alkaloid-like substances (13,14). These alkaloids have been suggested in the pathogenesis of neuroleptic-induced extrapyramidal side effects (6, 7, 20) and in the addictive liability of ET (4, 5, 35). These compounds could adversely affect testicular function. The AMN antagonism of CPZ and RES-produced inhibition of T-CT-ALDH may be helpful when considering management of neuroleptic endocrine effects since AMN has been used in the treatment of their extrapyramidal side effects (1,8).

The lack of in vivo effect of THP on testicular enzymes studied contrasts to its in vitro inhibition of rat liver ADH and mitochondrial ALDH (23). This could be due to the difference between the in vivo and in vitro effect, requirement of larger in vivo THP doses to attain the same effect as observed in vitro by a 10^{-4} M THP concentration and/or due to existence of a blood-testis barrier for THP.

The determined induction of T-ADH by LNK in vivo could provide for an endocrine-mediated metabolic detoxification mechanism for rapid intracellular removal of ET from the testicles. Enkephalines have been shown to interact with the endocrine system, and certain BA as dopamine (9, 15, 30, 31), which are involved in some of ET-evoked responses. This suggests an interrelationship between peripheral LNK effect and ET as has been recently shown (21–23).

The in vitro results have demonstrated the sensitivity of T-ADH to the action of CPZ and the alkaloid PAP, an O-methylated analogue of THP. The inhibitory action of CPZ on T-ADH may produce metabolic adverse effects on both ET- and BA-derived aldehyde intermediates by the gonads, if occurred in vivo. For example, inhibition of ET metabolism by the testis of alcoholics undergoing antipsychotic therapy with CPZ would

TABLE 2

IN VITRO EFFECT OF PAPAVERINE AND SCOPOLAMINE ON RAT TESTICULAR AND EPIDIDYMAL ALCOHOL AND ALDEHYDE DEHYDROGENASE

In Vitro Drug Conc. (M)	T-ADH	T-CT-ALDH	EP-CT-ALDH
	(nmol/min/mg protein)		
—	1.0 ± 0.2 (5)	8.3 ± 0.7 (6)	7.6 ± 0.6 (11)
Papaverine (10^{-4} M)	1.6 ± 0.3* (4)	8.5 ± 1.1 (4)	7.5 ± 0.5 (18)
Scopolamine (10^{-3} M)	1.2 ± 0.1 (5)	8.5 ± 0.8 (5)	6.7 ± 0.4 (13)

Values are means ± SEM of enzymatic specific activity, nmol/min/mg protein, determined in vitro for the member of independent assays given between parentheses. Testicular alcohol dehydrogenase (T-ADH), aldehyde dehydrogenase (T-ALDH) and epididymal aldehyde dehydrogenase (EP-ALDH) were assayed in the cytoplasmic (CT) tissue preparations.

*$p<0.1$ (not significant).

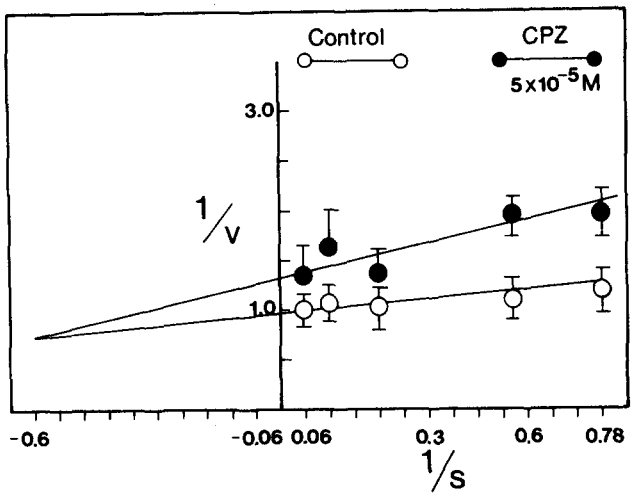

FIG. 2. Double reciprocal lineweaver and Burk plots for the in vitro measurements of the velocity of rat testicular alcohol dehydrogenase reaction as a function of ethanol concentration in the presence and absence of chlorpromazine (CPZ), 5×10^{-5} M. Each point represents mean ± SEM of 5–6 determinations.

TABLE 3

IN VITRO EFFECT OF CHLORPROMAZINE ON RAT TESTICULAR AND EPIDIDYMAL ALCOHOL DEHYDROGENASE AND ALDEHYDE DEHYDROGENASE

In Vitro CPZ (5×10^{-5} M)	T-ADH	T-ALDH NC	T-ALDH MT	T-ALDH CT	ED-CT-ALDH
—	0.84 ± 0.10 (8)	7.1 ± 0.8 (13)	3.3 ± 0.5 (11)	4.4 ± 0.3 (13)	6.74 ± 0.7 (16)
CPZ	0.40 ± 0.22* (8)	6.5 ± 0.8 (13)	2.9 ± 0.4 (11)	4.3 ± 0.3 (13)	6.37 ± 0.6 (16)

Values represent means ± SEM of enzymatic specific activities, nmol/min/mg protein, determined in vitro for the number of independent assays given between parentheses. Testicular aldehyde dehydrogenase (T-ALDH) was assayed in the nuclear (NC), mitochondrial (MT) and cytoplasmic (CT) subcellular fractions of the rat testis. Both testicular (T) alcohol dehydrogenase (T-ADH) and epididymal aldehyde dehydrogenase (EP-ALDH) were determined in CT tissue preparation. Assays were performed in duplicates in the absence and presence of 5×10^{-5} M chlorpromazine (CPZ).

*$p<0.004$.

reduce testicular elimination of ET and thereby augment its gonadal toxicity. This could result in early induction of abnormal endocrine function in alcoholics, since both chronic use of ET (33) and CPZ (2, 3, 10–12, 29) independently produce gynecomastia, decreased libido and sterility in the susceptible patients.

The PAP produced in vitro increase of specific activity of T-ADH is consistent with that reported for the hepatic enzyme (23). The enhancement of liver ADH (23) and probably testicular ADH by PAP is of particular interest in view of the use of PAP in the diagnosis and management of erectile impotence (32,34) compared to ET liability to evoke impotence in alcoholic men (24). It remains to be determined, however, if PAP can alter ET-produced effects on the gonads.

In conclusion, it appears that the testicular enzymes studied are sensitive to endocrine-acting agents and they may be involved in mediating some of the endocrine dysfunction induced by certain antipsychotic drugs and by ET. The use of such psychoactive agents during chronic ET consumption may facilitate and/or augment the development of such endocrine side effects and their use should be contraindicated in chronic alcoholism.

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