Pharmacology Biochemistry & Behavior, Vol. 10, pp 947–949 Printed in the U S A.

Cyclobenzaprine and Ethanol Interaction

F. S. MESSIHA AND C. D. BARNES

Departments of Pathology, Physiology and Psychiatry
Texas Tech University School of Medicine, Lubbock TX 79430

(Received 26 February 1979)

MESSIHA, F S. AND C D. BARNES. *Cyclobenzaprine and ethanol interaction.* PHARMAC. BIOCHEM. BEHAV. **10**(6) 947–949, 1979.—The effects of cyclobenzaprine, a tricyclic compound, on the central depressant action of ethanol and on hepatic ethanol metabolizing enzymes were studied in rodents. Administration of cyclobenzaprine, 5 mg/kg, IP, 30 min prior to a narcotic dose of ethanol solution, 5 g/kg, IP, enhanced ethanol-produced narcosis in mice. This effect was greater in male than in female mice. Cyclobenzaprine inhibited endogenous rat liver alcohol dehydrogenase in vitro in the concentration range between 10^{-5}M and 10^{-6}M. Cyclobenzaprine exerted little effect on hepatic aldehyde dehydrogenase in vitro The results suggest that cyclobenzaprine possesses depressant properties and inhibition of liver alcohol dehydrogenase may underlie the observed behavioral response studied. It is concluded that alteration of endogenous liver alcohol dehydrogenase by certain tricyclic antidepressant drugs may be involved in the mechanism(s) of their toxic interaction with ethanol.

Alcohol dehydrogenase Cyclobenzaprine Ethanol-narcosis

CYCLOBENZAPRINE (CBZ), a dibenzocycloheptadiene derivative (see Fig. 1), is structurally related to the tricyclic antidepressant amitriptyline, except for the absence of two hydrogen atoms substitutions in the central ring structure. Pharmacologic properties of CBZ include mainly central skeletal muscle relaxant activity, some sedative action, and peripheral cholinergic and histaminergic blockade [1, 5, 6, 7].

The well-known interaction between certain tricyclic antidepressant drugs and ethanol (ETOH) coupled with the structural similarities between CBZ and these agents prompted the study of the effect of CBZ on the central depressant action of ETOH, as measured by ETOH-mediated narcosis test in mice, and the investigation of the possible underlying mechanism.

METHOD

Adult male and female Sprague-Dawley mice (Texas Inbred co., Houston TX) weighing 25–33 g, were used. Male Sprague-Dawley rats weighing 300–350 g were obtained from Holtzman Farms, Madison WI. Animals were fed Purina pellet food, and maintained at 23–25°C, in a room with 12 hr light and dark cycles. Drugs were injected intraperitoneally (IP).

Behavioral Studies

CBZ was dissolved in saline and given to mice, 5 mg/kg, IP, 30 min prior to injection of 25% (w/w) ETOH solution, 5.0 g/kg, IP, which was prepared by diluting 95% ETOH with saline. The controls received the vehicle, physiological saline, 30 min before injection of ETOH. Duration of ETOH-narcosis was considered as the time from the loss to the regaining of the righting reflex and is expressed in minutes. Experimental variables which can contribute to alteration in ETOH-narcosis were also considered [8].

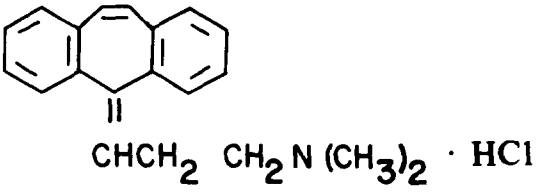

FIG 1. Cyclobenzaprine hydrochloride: N,N-dimethyl-5H-dibenzo [a,d] cycloheptene-Δ^5-propylamine hydrochloride.

Biochemical Studies

The in vitro effect of CBZ on rat liver alcohol dehydrogenase (L-ADH) and aldehyde dehydrogenase (L-ALDH) were studied in cytoplasmic and mitochondrial preparations as the source of the enzymes. For the in vitro determination of these enzymes, rats were sacrificed by decapitation, their livers removed and individually homogenized in 6.5 volumes of ice cold 0.1 M KCl solution in a Waring blender to obtain 15% (w/v) homogenates. These were then subjected to differential centrifugation to obtain the mitochondrial and the cytoplasmic fractions as described in details elsewhere [11]. Determinations of L-ADH were made by a spectrophotometric assay [2] using the cytoplasmic fraction as the source of the enzyme, while rat L-ALDH was assayed [3] both in the cytoplasmic and in the mitochondrial preparations. Protein determinations were made according to the Biuret method. In the spectrophotometric assay, the reference samples (controls) contained all reaction's mixture but the substrate. CBZ was dissolved in water to obtain the molar (M) concentration required in 0.1 ml volume. The activity of the enzymes studied are expressed as specific activ-

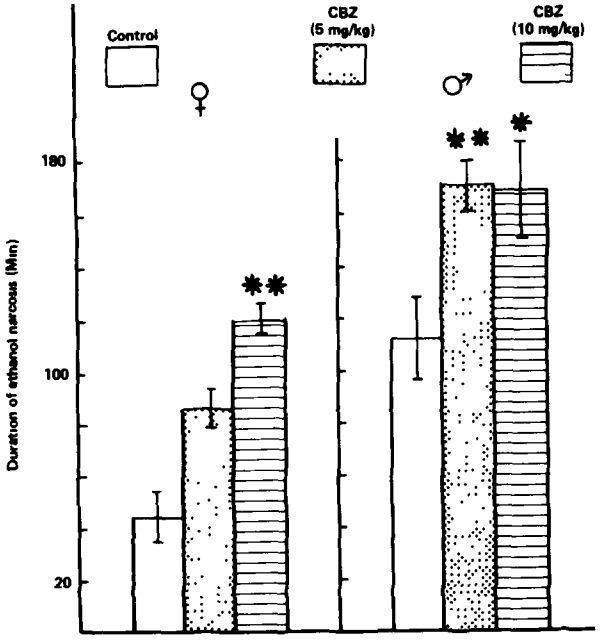

FIG. 2 The effect of cyclobenzaprine (CBZ) on ethanol produced narcosis in female (left panel) and male (right panel) mice The duration of narcosis, measured as the time elapsed from the loss to the regaining of righting reflex, is given in min. Each bar graph represents means ± SE derived from 13–20 mice $^{**}p<0.01$, $^{*}p<0.02$

ity, n/Mol/min/mg protein, measured at 30°C. Student's t-test for independent means was used for the statistical evaluation of the data.

RESULTS

Figure 2 shows the effect of CBZ on duration of ETOH-mediated narcosis in male (left panel) and female (right panel) mice. Administration of CBZ, 5 mg/kg, enhanced ETOH narcosis by approximately 92% and 53% ($p<0.01$) from saline-controls in female and male mice, respectively. This is compared to 2.7 fold ($p<0.01$) rise in ETOH narcosis in female mice and 1.6 fold ($p<0.02$) increase in male mice from corresponding controls as a function of pretreatment with CBZ, 10 mg/kg/IP.

Figure 3 shows the in vitro effect of CBZ on specific activity of L-ADH. Addition of CBZ, 10^{-5}M, to the reaction mixture inhibited specific activity of rat L-ADH from 14.44 ± 1.33 to 8.22 ± 1.36 nMol/min/mg protein ($p<0.01$). A similar inhibition of rat L-ADH occurred by 10^{-6}M concentration of CBZ ($p<0.01$). Rat liver mitochondrial and cytoplasmic ALDH were not altered by CBZ concentration as high as 10^{-3}M in vitro.

Figure 4 shows the reciprocal plots of control and inhibition of rat L-ADH by 10^{-6}M CBZ. The utilization of the Lineweaver-Burk reciprocal plots for this kinetic study reveals that CBZ exerted non-competitive type of inhibition on L-ADH with a V_{max} of 9.6 compared to 14.3 μM of controls and mean K_m value of 0.31 μM which was not different from that of control value of 0.31 μM.

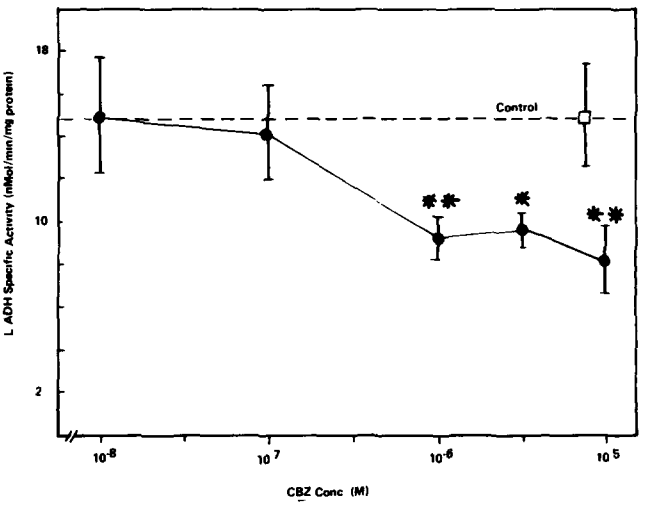

FIG 3 The in vitro effect of cyclobenzaprine (CBZ) on rat liver alcohol dehydrogenase (L-ADH) Liver ADH is expressed as specific activity, nMol/min/mg protein in the absence (control) and in the presence of CBZ, in various molar (M) concentrations. Each point represents means ± SE of 6 independent experiments $^{**}p<0.01$, $^{*}p<0.02$

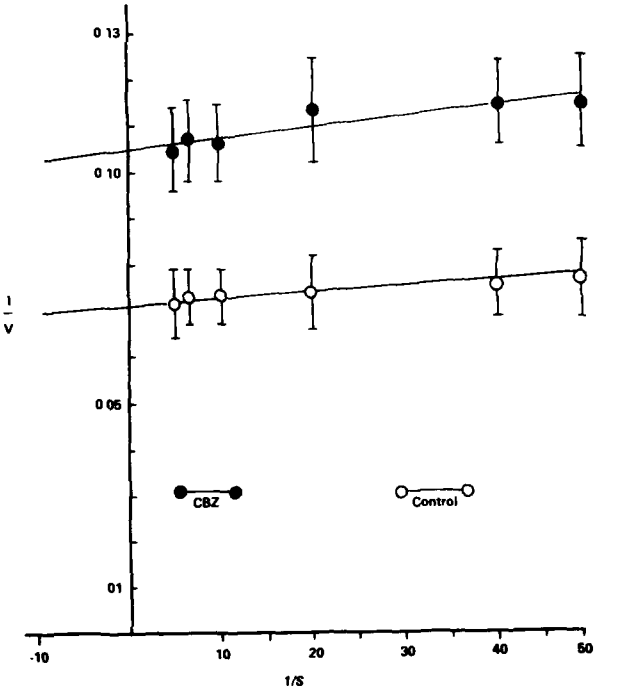

FIG. 4 Reciprocal plots of rat liver alcohol dehydrogenase inhibition by cyclobenzaprine (CBZ), 10^{-6} Molar (M) Each point represent mean ± SE of 6 independent experiments.

DISCUSSION

The present results indicate that CBZ prossesses depressant properties as evidenced by CBZ enhancements of ETOH-mediated narcosis in mice and the previously reported prolongation of pentobarbital-induced narcosis in mice with identical doses of CBZ [10]. In this behavioral performance test used, CBZ seems to exert central de-

pressant action similar to that of the tricyclic depressant drugs, e.g., imipramine [4] and other drugs possessing behavioral depressant properties.

The in vitro inhibition of L-ADH by CBZ is of particular interest. For example, if CBZ produces such inhibition in vivo then this may increase blood ETOH concentrations which can cause prolongation of ETOH narcosis. Furthermore, it has been shown that short-term administration of imipramine did not alter endogenous rat L-ADH from saline control in vivo [9]. Thus, it seems likely that minor changes in the N-substitution and/or hydrogen saturation of the central ring structure of the tricyclic anti-depressant may provide alternative experimental approaches for evaluating new clinically useful inhibitors for hepatic ETOH oxidation other than that of the toxic pyrazol derivatives. Alternatively, studying the effect of tricyclic antidepressant and other drugs which interact with ETOH on hepatic ETOH metabolizing enzymes may be a useful approach for evaluating additional mechanisms underlying ETOH-drugs interaction.

REFERENCES

1. Barnes, C. D. Effects of cyclobenzaprine on brainstem motor systems. *Neuropharmacology* **15:** 643–652, 1976.
2. Blair, A H. and B. L. Valee. Some catalytic properties of human liver alcohol dehydrogenase. *Biochemistry* **5:** 2026–2034, 1966.
3. Blair, A. H. and F H. Bodley. Human liver aldehyde dehydrogenase. partial purification and properties. *Can. J Biochem.* **47:** 265–272, 1969.
4. Byck, R. Drugs and the treatment of psychiatric disorders. In: *The Pharmacological Basis of Therapeutics,* edited by L. S. Goodman and A. Gilman. New York: Macmillan Pub. Co., Inc., 1975, pp. 152–200
5 Hughes, M. J., S. Lemons and C. D. Barnes. Cyclobenzaprine· some pharmacological cardiac actions *Life Sci* **23:** 2779–2786, 1978
6. Korten, J. J. and J. vanHemert. Pilot study with cyclobenzaprine in idiopathic Parkinsonism. *Psychiat Neurol Neurochir* **76:** 119–128, 1973
7. Share, N. N. and C. S. McFarlane. Cyclobenzaprine: A novel centrally acting skeletal muscle relaxant. *Neuropharmacology* **14:** 675–684, 1975.
8. Messiha, F. S., M. Morgan and I. Geller. Ethanol narcosis in mice: Effects of L-dopa, its metabolites and other experimental variables. *Pharmacology* **13:** 340–351, 1975.
9. Messiha, F. S., J. W Larson and C. D Lox. d-Amphetamine, imipramine and ethanol metabolizing enzymes *Proc west Pharmac* **21:** 277–280, 1978.
10. Messiha, F. S., C. D. Barnes, M. J. Hughes and K. E. Light. Cyclobenzaprine: A behavioral profile of a skeletal muscle relaxant. *Proc west Pharmac* , (in press).
11 Messiha, F. S. and M. J. Hughes. Liver alcohol and aldehyde dehydrogenase: Inhibition and potentiation by histamine agonists and antagonists *Clin. exp Pharmac Physiol* , (in press).