*Pharmacology Biochemistry & Behavior*, Vol. 14, pp. 293–298. Printed in the U.S.A.

# Antagonism of Ethanol-Induced Decrease in LH by Para-Chlorophenylalanine: Lack of Correlation with Altered Serotonergic Mechanisms

ROBERT E. CHAPIN, GEORGE R. BREESE AND ROBERT A. MUELLER[1]

*Departments of Anesthesiology, Pharmacology and Psychiatry and the Biological Sciences Research Center UNC School of Medicine, Chapel Hill, NC 27514*

Received 10 September 1980

CHAPIN, R. E., G. R. BREESE AND R. A. MUELLER. *Antagonism of ethanol-induced decrease in LH by para-chlorophenylalanine: Lack of correlation with altered serotonergic mechanisms.* PHARMAC. BIOCHEM. BEHAV. 14(3) 293–298, 1981.—Acute administration of ethanol lowers plasma levels of luteinizing hormone (LH) in several species. Since ethanol may interact with central serotonergic (5HT) neurons, and since 5HT systems have been found to play a role in modulating LH release, we examined the possible role of central serotonergic neurons in the ethanol-induced depression of LH. Acute PCPA (400 mg/kg, 20 hr before 2.0 g/kg ethanol) was effective in preventing the ethanol-induced depression of LH, suggesting that ethanol activates 5HT systems to lower LH. In support of this, the central 5HT agonist 5-methoxy-N, N-dimethyltryptamine (5MDMT) depressed LH in a dose-dependent manner. However, while the effects of a sub-maximal dose of 5MDMT were blocked by prior administration of methysergide, this 5HT receptor antagonist was unable to prevent the post-ethanol fall in LH. Additionally, because other doses of PCPA (250 mg/kg 20 hr prior to ethanol, and 100 mg/kg P.O.×3 days before ethanol) produced similar reductions in hypothalamic 5HT but did not block the ethanol effect, and because electrolytic lesions of the median raphe nucleus were also ineffective in preventing the post-ethanol depression of LH, we conclude that activation of serotonergic systems does not play a major role in the ethanol induced depression of LH.

Ethanol LH Serotonin PCPA Castrated rats

THE mammalian reproductive system provides a good example of the pervasiveness of ethanol's effects *in vivo*. Ethanol acts directly on the testis to decrease steroidogenesis [9,12], and thus lower circulating testosterone levels [26]. In addition, plasma levels of luteinizing hormone (LH), the stimulus for testosterone production, are also decreased [8]. Since LH release elicited by administering LH-releasing-hormone (LHRH) is unchanged by ethanol [6, 9, 35], it would appear that the pituitary is not the primary target for lowering LH, but rather that ethanol acts at a supra-sellar site to depress LH levels.

There is evidence to indicate that ethanol interacts with serotonergic neurons in the brain, although the nature of the interaction is in dispute (see [23] for review). Because serotonergic systems may modulate plasma LH levels [9,33], it seemed plausible that ethanol might decrease LHRH release by altering serotonergic neuronal function. This report demonstrates that although the depressant activity of ethanol on LH is antagonized by the tryptophan hydroxylase inhibitor p-chlorophenylalanine, the LH decrease after ethanol is probably not due to alteration in serotonin availability.

## METHOD

### *Animals*

The methods have been previously described [6]. Briefly, diurnally cycled (0700–1900 hr light) Sprague-Dawley rats (200–400 g), castrated 5 days previously, were anesthetized with ether, implanted with a cannula (PE 50, Clay Adams) in the ventral tail artery, and attached to a balanced feed-through swivel (Tech-Serv). This allowed samples of arterial blood (0.44 ml) to be drawn (from 0900–1600 hr) without disturbing the animal. Sampling was started no sooner than 2 hr after cannulation. Four sequential samples were drawn at 20 min intervals before drug administration, followed by 6 more samples at equal intervals; each sample was immediately replaced with an equal volume of physiological saline.

[1]Send reprint requests to Dr. Robert A. Mueller, Professor of Anesthesiology and Pharmacology, 223 Clinical Sciences Building, UNC School of Medicine, Chapel Hill, NC 27514.

*Drugs*

Since 2.0 g/kg ethanol IP (20% w/v) was found to be the lowest dose to consistently depress LH [6], this dose was used to evaluate the effects of various treatment regimens. Para-chlorophenylalanine methylester (PCPA, Sigma Chem. Co., St. Louis) was given IP in saline (3 ml/kg) 20 hr before ethanol, or PO at 100 mg/kg for 3 days prior to ethanol. 5 Methyoxy-N,N-dimethyltryptamine (5MDMT, Sigma), the putative 5HT receptor agonist [13], was dissolved in acid saline and neutralized before IP injection in a volume of 1.0 ml/kg. Methysergide maleate (Sandoz, Hanover, NJ) was dissolved in saline (2 ml/kg) and administered SC 10 min prior to ethanol or 5MDMT (2.5 mg/kg).

In order to reduce the population of serotonin-containing neurons in brain, some animals were given 5,7-dihydroxytryptamine creatinine sulfate (5,7-DHT, 50 $\mu$g free base) intracisternally 30 min after IP treatment with pargyline (40 mg/kg). These 3-day-old animals were returned to their mothers and allowed an undisturbed development. They were tested as adults (300–400 g) for 5HT receptor supersensitivity by the method of Breese *et al.*, [5]; those who demonstrated a supersensitive response to 5-hydroxytryptophan (5-HTP) were subsequently castrated 5 days before sampling, and treated with PCPA (50 or 75 mg/kg, PO) 3 days before examination of their LH response to ethanol.

*LHRH Challenge*

To test an effect of PCPA on the pituitary, castrated males (n=6) were given 400 mg/kg PCPA 20 hr prior to 200 $\mu$g LHRH. The animals were sacrificed by decapitation 20 min after LHRH injection, and the plasma assayed for LH.

*Lesions*

Electrolytic lesions of the median raphe nucleus were performed under ether anesthetic before castration and at least one week before cannulation. With the incisor bar 2.5 mm below the inter-aural line, the angled electrode (16° from vertical) was lowered to reach the following coordinates: A: 0.2 mm, L: 9.9 mm, and V: −2.8 mm [22]. Current (1 mA) was passed for 10 sec; in sham lesioned animals, the electrode was lowered but no current was passed. Lesion placement was verified and recorded by placing frozen thin sections in an enlarger and exposing photographic paper to the image thus formed.

*Hormone and Amine Determination*

Plasma was assayed for LH in duplicate or triplicate using the kits kindly provided by the N.I.A.M.D.D. Rat Pituitary Hormone Distribution Program and Dr. A. F. Parlow. Ovine LH for iodination and anti-ovine serum were the generous gifts of Dr. L. E. Reichert and Dr. G. D. Niswender, respectively. Results were expressed as ng/ml LH-RP-1. The LH assay had an inter-assay coefficient of variation (CV) of 16%, and an average intra-assay CV of 9.4%.

For amine determination, animals were decapitated and the brains dissected quickly on ice-chilled glass following the subdivision of Glowinski and Iversen [17]; tissue was frozen at −40°C until assayed. Samples were homogenized in 0.4 N perchloric acid, and catecholamines analyzed in aliquots of supernatant. Dopamine (DA) and norepinephrine (NE) were isolated by adsorption onto alumina, eluted with acetic acid, and assayed fluorometrically [4]. Serotonin was isolated from supernatant by the method of Bogdanski *et al.* [3] and measured fluorometrically [34].

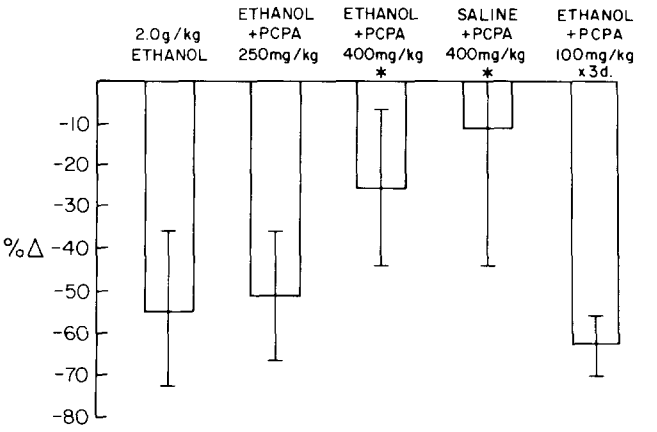


FIG. 1. Antagonism by PCPA of the effect of intraperitoneal ethanol (2.0 g/kg) on plasma LH. PCPA at 250 mg/kg and 400 mg/kg was injected IP 20 hr before ethanol, or at 100 mg/kg by gastric intubation for three days prior to ethanol. Abscissa is the percent change from control for the AUC (see Methods); each column length represents the mean ± SD (brackets) of 6–8 rats. *Different from ethanol alone, $p<0.05$.

*Data Treatment*

Hormone values were plotted sequentially for each animal. The area under the curve (AUC) before drug administration was compared to the AUC for the four samples immediately following drug administration; this ratio is expressed as the percent change from control (%Δ) These ratios were grouped by treatment and the group means compared by Student's *t*-test [11].

## RESULTS

*Effect of PCPA on Ethanol-Induced Reduction of LH*

Pre-drug LH levels in individual animals cycled between 200 ng/ml and 600 ng/ml, with a period of 20–60 min. Figure 1 shows that 2.0 g/kg of ethanol produces a submaximal depression of LH, as reported earlier [6], reducing LH to approximately 50–60 ng/ml, whereas plasma LH in saline controls was only slightly depressed. Although neither 100 mg/kg PCPA for 3 days nor 200 mg/kg PCPA acutely blocked the ethanol effect on LH, 400 mg/kg PCPA was effective in preventing the ethanol-induced fall. There were no apparent behavioral differences between any of the groups that received ethanol; all animals were sedated and quiescent.

Regional levels of amines from rats given PCPA only are shown in Table 1. While the highest dose of PCPA reduced hypothalamic 5HT more than the 250 mg/kg dose, there was no difference in 5HT between the single 400 mg/kg dose and the 100 mg/kg × 3 days dose. While catecholamine levels were also altered by the different PCPA regimens, these changes could not be associated with consistent changes in LH after ethanol.

Table 2 shows that animals treated with 400 mg/kg PCPA 20 hrs prior to LHRH released significantly less LH after LHRH than animals who received PCPA vehicle.

TABLE 1

EFFECT OF P-CHLOROPHENYLALANINE ON LEVELS OF SEROTONIN, NOREPINEPHRINE, AND DOPAMINE IN HYPOTHALAMUS AND BRAIN STEM

| | | PCPA 400 mg/kg | PCPA 250 mg/kg | PCPA 100 mg/kg × 3D | Saline |
|---|---|---|---|---|---|
| Serotonin ng/kg Tissue | Hypothalamus | 145 ± 15*§ | 280 ± 40*† | 137 ± 15*§ | 580 ± 87 |
| Mean ± SEM | Brain Stem | 232 ± 37*† | 243 ± 52†* | 121 ± 11* | 454 ± 19 |
| | Hypothalamus | 514.2 ± 50† | 408 ± 42*‡ | 707 ± 51 | 606 ± 28 |
| Norepinephrine ng/g | Brain Stem | 196 ± 11‡ | 219 ± 8‡ | 298 ± 20* | 214 ± 15 |
| | Hypothalamus | 1409 ± 170† | 1278 ± 85 | 763 ± 210 | 1071 ± 125 |
| Dopamine ng/g | Brain Stem | 182 ± 15 | 288 ± 67 | 157 ± 37* | 273 ± 50 |

All values represent the mean ± SD of 5–7 animals, measured 20 hrs after IP injection of p-chlorophenylalanine or saline.
*=different from saline, $p<0.05$.
†=different from PCPA, 100 mg/kg × 3D, $p<0.05$.
‡=different from PCPA, 100 mg/kg × 3D, $p<0.005$.
§=different from PCPA, 250 mg/kg, $p<0.005$.

TABLE 2

PLASMA LH LEVELS AFTER LHRH IN ANIMALS PRETREATED WITH PCPA OR VEHICLE

| PCPA | Vehicle |
|---|---|
| 750 ± 123* | 1069 ± 69 |

PCPA (400 mg/kg) or vehicle was given 20 hr before LHRH (200 μg, SC). Values are mean ± SD FOR 5–7 animals. *=significantly different, $p<0.01$.

*Effect of Median Raphe Lesions on Ethanol-Induced Reduction of LH*

Figure 2 depicts the extent of the electrolytic lesions of the median raphe in rats, subsequently given ethanol. Table 3 indicates that the lesion significantly decreased hypothalamic 5HT and NE levels 10–14 days after the lesion, although the percent reduction for 5HT is greater. Lesions were not as effective as PCPA in lowering 5HT, nor were the lesions able to prevent the ethanol-induced decrease in plasma LH (Fig. 3).

*Effect of 5MDMT and Methysergide on Plasma LH*

Figure 4 demonstrates that 5MDMT decreases plasma LH in a dose-dependent manner. While the effect of a submaximal dose of 5MDMT was blocked by the putative 5HT receptor antagonist methysergide (Fig. 5), methysergide was unable to prevent the post-ethanol reduction of LH (Fig. 5).

*Effect of 5,7-DHT Treatment on the Post-Ethanol Reduction of LH*

In order to see if the depletion of serotonin availability by other means might also inhibit the LH response to ethanol, this response was tested in adult animals treated neonatally with 5,7-DHT, and subsequently given PCPA as adults. Hy-

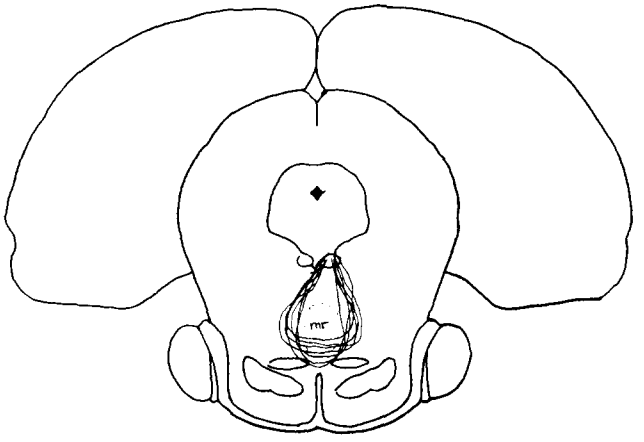


FIG. 2. Schematic diagram of the rat brain stem, 650 microns anterior to the inter-aural line, after König and Klippel [22]. The figure depicts the placement of lesions in the 7 animals bearing electrolytic median raphé lesions and receiving 2.0 g/kg ethanol IP. m.r.= median raphé nuc.

TABLE 3

LEVELS OF HYPOTHALAMIC AMINES IN RATS BEARING ELECTROLYTIC OR SHAM LESIONS OF THE MEDIAN RAPHE NUCLEUS

| Treatment | Serotonin | Norepinephrine | Dopamine |
|---|---|---|---|
| Lesion + EtOH | 188 ± 32* | 908 ± 132* | 801 ± 315 |
| Lesion + NaCl | 201 ± 26* | | |
| Sham +EtOH | 388 ± 192 | 1199 ± 198 | 704 ± 282 |
| Sham +NaCl | 486 ± 70 | 1290 ± 184 | 993 ± 192 |

Values given as the mean ± SD for 6–8 animals, expressed as ng/g tissue, wet weight. *=different from sham-operated rats, $p<0.05$.

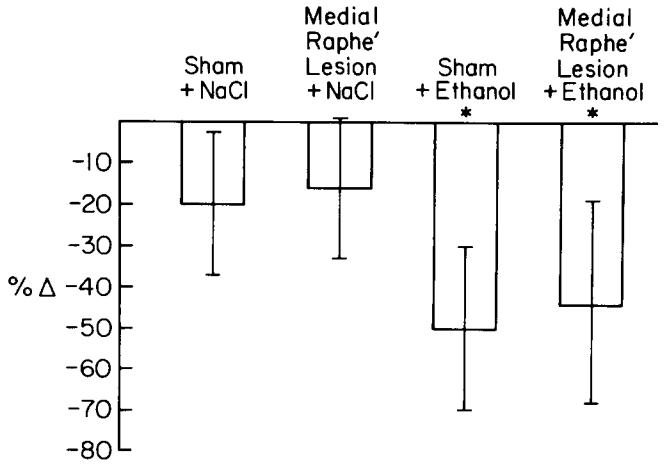


FIG. 3. The effect of intraperitoneal ethanol (2.0 g/kg) or saline on plasma LH in animals bearing electrolytic or sham lesions of the median raphé nucleus. Abscissa is the percent change from control for AUC (see Method); each column length represents the mean ± SD for 6–8 rats. *Difference from saline injected controls ($p<0.05$).

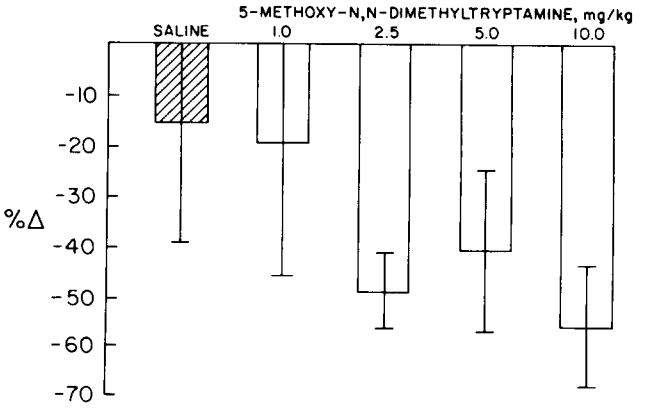


FIG. 4. The effect of intraperitoneal 5-methoxy-N,N-dimethyltryptamine or saline on plasma LH. Saline=pool of all vehicle controls. Abscissa is the percent change from control for AUC (see Method); each column length represents the mean ± SD for 6–8 rats.

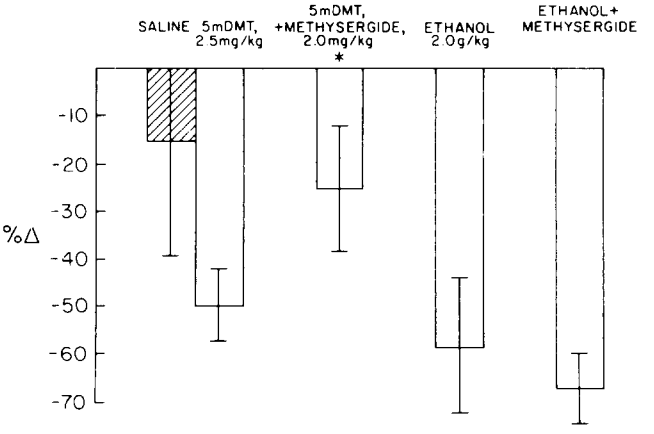


FIG. 5. The effect of intraperitoneal 5-methoxy-N,N-dimethyltryptamine (5MDMT), methysergide, and ethanol on plasma LH. Saline = pool of all vehicle controls for 5MDMT and 5MDMT + methysergide groups. Abscissa is the percent change from control for AUC (see Method); each column length represents the mean ± SD for 6–8 animals. *Different from 5MDMT alone, $p<0.05$.

pothalamic 5HT was depleted to 20–40% of normal in these animals, and they all demonstrated supersensitivity to a large dose of the 5HT precursor 5-hydroxytryptophan several days before the ethanol was administered, [5]. Furthermore, despite these perturbations, ethanol was still effective in lowering plasma LH, producing a percent change of −51.4 ± 20. Pearson's product moment correlation coefficient (r) was calculated to test the strength of the relationship between the change in LH after ethanol and hypothalamic 5HT levels. For these rats treated with 5,7-DHT, PCPA, and ethanol, r=0.267, indicating a very weak relationship between hypothalamic 5HT levels and plasma LH levels after ethanol.

## DISCUSSION

These results confirm previous reports [8,26] that ethanol depresses plasma LH. Since serotonergic stimulation was previously observed to be inhibitory to both LH release *in vivo* and *in vitro* [7,24] it is possible that ethanol could act via a serotonergic mechanism to lower LH. In the present study we found that the putative serotonin receptor agonist 5MDMT reduced plasma LH, thus supporting an inhibitory role for serotonin on LH. Gallo and Drouva [15] and Pilotte and Porter [30], found LH lowered in both intact rats receiving third ventricle 5HT infusions [33] and in ovariectomized rats undergoing electrical stimulation [1]. Nevertheless, some investigators have found serotonergic systems to be permissive of LH release [19, 27, 38, 39], and some data from intact animals suggest both inhibitory [7,24] and stimulatory [27, 38, 41] roles for serotonin in LH release.

Additional studies were intended to test the theory that ethanol was influencing serotonergic pathways to reduce LH. However, these studies did not support a serotonergic mechanism for the ethanol-induced fall in LH.

The initial conflict arose with the observation that, while 400 mg/kg PCPA antagonized the ethanol-induced fall in LH, repeated injections of lower doses of this tryptophan hydroxylase inhibitor did not block the ethanol effect, despite equal reductions in hypothalamic serotonin levels by both treatment regimens. Although determination of transmitter levels does not measure neuronal activity or amine release rate (see [40]), adequate transmitter availability no doubt plays an important role in normal neuronal functioning. Thus it is possible that the 400 mg/kg dose was more effective in decreasing 5HT neuronal release, an effect not measured by the present method.

The blockade of the effect of ethanol on LH produced by 400 mg/kg PCPA might be the result of increased pituitary sensitivity to LHRH. However, the fact that castrates treated with PCPA released significantly less LH in response to LHRH than did rats treated with vehicle suggests that, on the contrary, PCPA decreases pituitary responsiveness to LHRH. The action of PCPA could also be a result of increased metabolism of ethanol due to enhanced alcohol dehydrogenase activity, although this seems unlikely. Because the peripheral clearance rate of LH is insignificantly decreased by ethanol (data not shown), PCPA is probably not

reversing a peripheral action of ethanol, but having an effect on the CNS to modify LH secretion.

The percent of hypothalamic 5HT depletion seen in the lesioned animals is in accord with previous reports [14,21]. Although both the dorsal and median raphe send fibers to the hypothalamus [2,28], selective lesions of the median raphe have been found to specifically diminish hypothalamic 5HT uptake [14]; thus, median raphe lesions may provide the most localized perturbation of hypothalamic serotonergic systems. However, dorsal raphe neurons were spared by the lesion, and significant hypothalamic 5HT remained after the lesion, despite a slight lowering of hypothalamic NE after the lesion, indicating again the difficulty of attaining both thorough and specific lesions of specific transmitter neurons. Hypothalamic NE levels did not correlate with LH changes after ethanol in either the lesioned animals or in animals receiving PCPA, and we conclude that the post-lesion changes in NE are probably unimportant.

It appears that 5MDMT stimulates central serotonin receptors, since the drug decreases central 5HT turnover [13], and dose-dependently decreases firing rates in dorsal raphe units [37], actions which are common to other 5HT receptor agonists. Methysergide has been found effective in some [20], but not all the CNS responses provoked by serotonin [10, 16, 18]. Nevertheless, methysergide was effective in blocking the depression of plasma LH produced by 5MDMT, although it was unable to prevent the ethanol-induced fall in LH. This would suggest that ethanol was not lowering LH secondary to serotonergic stimulation.

All the animals treated neonatally with 5,7-DHT displayed characteristic signs of 5HT receptor supersensitivity after an intraperitoneal injection of 7.5, 15 or 30 mg/kg 5-HTP. This behavioral syndrome consisted of tremor, muscular rigidity, splayed feet, stiff tail, forepaw treading, head weaving, and salivation [5]. Although 5-HTP does displace endogenous dopamine from intracellular stores [29], this syndrome is generally considered to be 5-HT-mediated, for drugs which increase 5HT availability or receptor activation produce the syndrome, and drugs which decrease availability or block 5HT receptors prevent the syndrome (see [36]). Small doses of PCPA were administered to these animals to further deplete central 5HT stores; however, the ethanol-induced depression of LH was unchanged. Also, the lack of correlation between each animal's LH response to ethanol and that animal's hypothalamic 5HT levels argues against serotonergic mediation of the ethanol effect on LH.

It is possible that the PCPA block is a result of non-specific effects. PCPA raises serum phenylalanine levels [25], and may elevate central levels of $\beta$-phenethylamine [31] and phenylethanolamine [32]. Exogenous $\beta$-phenethylamine can stimulate NE release; these and/or other unexamined effects of PCPA may contribute to the blockade of the LH-depressing effect of ethanol. It is also possible that some other 5-hydroxyindoleamine, whose synthesis is blocked by PCPA, but whose receptor stimulation is not antagonized by methysergide, mediates the ethanol-induced fall of LH. Preliminary results suggest that pineal melatonin is not involved, nor is the effect on LH release altered by administration of ethanol during light or dark circadian periods.

In summary, although the ethanol effect on LH was blocked by PCPA, an inhibitor of tryptophan hydroxylase, a 5HT antagonist and chemical or electrolytic lesion-induced alterations in 5HT availability did not modify the post-ethanol fall in LH. Thus, we conclude that serotonergic systems do not play a major role in the ethanol-induced depression of LH.

ACKNOWLEDGEMENTS

The authors are indebted to Jeanne Crowle, Eva Hoke, Edna Edwards, and Marcine Garrison for expert technical assistance, and to Laure Wagner for tireless manuscript preparation. R. E. C. is the recipient of a fellowship from the N.C. Alcohol Research Authority and G. R. B. is supported by a Research Career Development Award (MH-00013).

This work was supported by USPHS Grants HD 10570 and AA 02334.

## REFERENCES

1. Arendash, G. W. and R. V. Gallo. Serotonin involvement in the inhibition of episodic luteinizing hormone release during electrical stimulation of the midbrain dorsal raphé nucleus in overiectomized rats. *Endocrinology* **102:** 1199–1206, 1978.
2. Azmitia, E. C. and M. Segal. An autoradiographic analysis of the differential ascending projections of the dorsal and median raphé nuclei in the rat. *J. comp. Neurol.* **170:** 641–668, 1978.
3. Bogdanski, D. F., A. Pletscher, B. B. Brodie and S. Undenfriend. Identification and assay of serotonin in brain. *J. Pharmac. exp. Ther.* **117:** 82–88, 1956.
4. Breese, G. R. and T. D. Traylor. Effect of 6-hydroxydropamine on brain norepinephrine and dopamine: Evidence for selective degeneration of catecholamine neurons. *J. Pharmac. exp. Ther.* **174:** 413–420, 1970.
5. Breese, G. R., R. A. Vogel, C. M. Kuhn, R. B. Mailman, R. A. Mueller and S. M. Shanberg. Behavioral and prolactin responses to 5-hydroxytryptophan in rats treated during development with 5,7-dihydroxytryptamine. *Brain Res.* **155:** 263–275, 1978.
6. Chapin, R. E., G. R. Breese and R. A. Mueller. Possible mechanism of reduction of plasma luteinizing hormone by ethanol. *J. Pharmac. exp. Ther.* **212:** 6–10, 1980.
7. Charli, J. L., W. H. Rotsztejn, E. Pattou and C. Kordon. Effect of neurotransmitter on *in vitro* release of luteinizing-hormone-releasing hormone from the mediobasal hypothalamus of male rats. *Neurosci. Lett.* **10:** 159–163, 1978.
8. Cicero, T. J. and T. M. Badger. Effects of alcohol on the hypothalamic pituitary-gonadal axis in the male rat. *J. Pharmac. exp. Ther.* **201:** 427–433, 1977.
9. Cicero, T. J., E. R. Meyer and R. D. Bell. Effects of ethanol on the hypothalamic-pituitary-luteinizing hormone axis and testicular steroidogenesis. *J. Pharmac. exp. Ther.* **205:** 210–215, 1979.
10. D'Amico, D. J., B. C. Patel and H. L. Klawans. The effect of methysergide on 5-hydroxytryptamine turnover in whole brain. *J. Pharm. Pharmac.* **28:** 454–455, 1976.
11. Dunn, O. J. and V. A. Clark. *Applied Statistics: Analysis of Variance and Regression*, first edition. New York: J. Wiley and Sons, 1974.
12. Ellingboe, J. and C. C. Varanelli. Ethanol inhibits testosterone biosynthesis by direct action on Leydig cells. *Res. Communs chem. pathol. Pharmac.* **204:** 87–102, 1979.
13. Fuxe, K., B. Holmstedt and G. Jonsson. Effects of 5-methoxy-N, N-dimethyltryptamine on central monoamine neurons. *Eur. J. Pharmac.* **19:** 25–34, 1972.
14. Fuxe, K. and G. Jonsson. Further mapping of central 5-hydroxytryptamine neurons: Studies with the neurotoxic dihydroxytryptamines. *Adv. Biochem. Psychopharmac.* **10:** 1–33, 1974.
15. Gallo, R. V. and S. P. Drouva. Effect of intraventricular infusion of catecholamines on luteinizing hormone release in ovariectomized and ovariectomized, steroid-primed rats. *Neuroendocrinology* **29:** 149–162, 1979.

16. Geyermeck, L. 5-hydroxytryptamine antagonists. *Pharmac. Rev.* **13:** 399–439, 1961.
17. Glowinski, J. and L. L. Iversen. Regional studies of catecholamines in the rat brain. *J. Neurochem.* **13:** 655–669, 1966.
18. Haigler, H. I. and G. K. Aghajanian. Peripheral serotonin antagonists: Failure to antagonize serotonin in brain areas receiving a prominent serotonin input. *J. Neural. Trans.* **35:** 257–273, 1974.
19. Hery, M., E. Laplante and C. Kordon. Participation of serotonin in the phasic release of LH. I. Evidence from pharmacological experiments. *Endocrinology* **99:** 469–503, 1976.
20. Hollister, A. S., G. R. Breese, C. M. Kuhn, B. R. Cooper and S. M. Shanberg. An inhibitory role for brain serotonin-containing systems in the locomotor effects of d-amphetamine. *J. Pharmac. exp. Ther.* **198:** 12–22, 1976.
21. Jacobs, B. L., W. D. Wise and K. M. Taylor. Differential behavioral and neurochemical effects following lesions of the dorsal or median raphé nuclei in rats. *Brain Res.* **79:** 353–361, 1974.
22. König, J. F. R. and R. A. Klippel. *The Rat Brain, A Stereotaxic Atlas of the Forebrain and Lower Parts of the Brain Stem.* Baltimore: Williams and Wilkins, 1963.
23. Lahti, R. A. Alcohol, aldehydes, and biogenic amines. *Adv. expl. biol. Med.* **56:** 239–254, 1975.
24. Leonardelli, J., M. P. Dubois and P. Poulain. Effect of exogenous serotonin on LH-RH secreting neurons in the guinea pig hypothalamus as revealed by immunofluorescence. *Neuroendocrinology* **15:** 69–72, 1974.
25. Lipton, M. A., R. Gordon, G. Guroff and S. Undenfriend. p-Chlorophenylalanine-induced chemical manifestations of phenylketonuria in rats. *Science* **156:** 248–250, 1967.
26. Mendelson, J. H., J. Ellingboe, N. K. Meloo and J. Kuehnle. Effects of alcohol on plasma testosterone and luteinizing hormone levels. *Alcoholism: Clin. exp. Res.* **2:** 255–258, 1978.
27. Meyer, D. C. Hypothalamic and raphé serotonin systems in ovulation control. *Endocrinology* **103:** 1067–1074, 1978.
28. Moore, R. Y., A. E. Halaris and B. E. Jones. Serotonin neurons of the mid-brain raphé: Ascending projections. *J. comp. Neurol.* **180:** 417–438, 1978.
29. Ng, L. K. Y., T. N. Chase, R. W. Colburn and I. J. Kopin. Release of ($^3$H)-dopamine by L-5-hydroxytryptophan. *Brain Res.* **45:** 499–505, 1972.
30. Pilotte, N. S. and J. C. Porter. Serum LH and prolactin concentrations in intact and castrated rats treated with 5-hydroxytryptamine. *Soc. Neurosci. M Abstr.* **4:** 352, 1978.
31. Saavedra, J. M. Enzymatic isotopic assay for and presence of β-phenethylamine in brain. *J. Neurochem.* **22:** 216–221, 1974.
32. Saavedra, J. M., J. T. Coyle and J. Axelrod. Developmental characteristics of phenlethanolamine and octopamine in the rat brain. *J. Neurochem.* **23:** 511–513, 1974.
33. Schneider, H. P. G. and S. M. McCann. Mono- and indolamines and control of LH secretion. *Endocrinology* **86:** 1127-1133, 1970.
34. Snyder, S. H., J. Axelrod and M. Zweig. A sensitive and specific fluorescence assay for tissue serotonin. *Biochem. Pharmac.* **14:** 832–835, 1965.
35. Symons, A. M. and V. Marks. The effects of alcohol on weight gain and the hypothalamic-pituitary-gonadotropin axis in the maturing male rat. *Biochem. Pharmac.* **24:** 955–958, 1975.
36. Trulson, M. E., E. E. Eubanks and B. L. Jacobs. Behavioral evidence for supersensitivity following destruction of central serotonergic nerve terminals by 5,7-dihydroxytryptamine. *J. Pharmac. exp. Ther.* **198:** 23–32, 1976.
37. Trulson, M. E. and B. L. Jacobs. Effects of 5-methoxy-N, N-dimethyltryptamine on behavior and raphé unit activity in freely moving cats. *Eur. J. Pharmac.* **54:** 43–50, 1978.
38. van de Kar, L., S. A. Lorens, A. Vodraska, G. Allers and L. S. Van Orden, III. A stimulatory role of the serotonergic dorsal raphé-hypothalamic projection in LH secretion. *Soc. Neurosci. Abstr.* **4:** 357, 1978.
39. Vane, J. R., H. O. J. Collier, S. J. Corne, E. Marley and P. B. Bradley. Tryptamine receptors in the central nervous system. *Nature* **191:** 1068–1069, 1961.
40. Weiner, N. Regulation of norepinephrine biosynthesis. *A. Rev. Pharmac.* **10:** 273–290, 1970.
41. Wuttke, W., A. Bjorklund, H. G. Baumgarten, L. Lachenmeyer, M. Fenske and H. P. Klemmn. De- and regeneration of brain serotonin neurons following 5,7-dihydroxytryptamine treatment: Effects on serum LH, FSH, and prolactin levels in male rats. *Brain Res.* **134:** 317–331, 1977.