hypotensive effect of carbidopa + DL-*m*-tyrosine. In conclusion, our findings and the findings of others cited conclusion, our findings and the findings of others cited[2-4,9-13] imply that *m*-tyrosine produces the hypotensive effect by a central mechanism after peripheral aromatic amino acid decarboxylase inhibition. The response is likely elicited by the metabolites of *m*-tyrosine.

Résumé. Chez des chiens anesthésiés, la *m*-tyrosine donne une réponse hypertensive à l'injection i.v. Cette réponse devient hypotensive lorsque les chiens sont traités au préalable par les inhibiteurs de la décarboxylase de l'acide aminé aromatique carbidopa ou Ro 4-4602. Chez les chiens traités au préalable par le carbidopa, la L-*m*-tyrosine est considérée comme un agent plus puissant que le racémate. Comme agent hypotenseur la L-Dopa est moins puissante que la DL- ou la L-*m*-tyrosine.

D. H. MINSKER and A. L. STOKES[15]

Merck Institute for Therapeutic Research, West Point (Pennsylvania 19486, USA), 20 November 1973.

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Ethanol Narcosis in Mice: Serotonergic Involvement

The possibility that biogenic amines or their respective metabolites may be involved in the soporific action of ethanol derives support from a number of investigators[1-4]. The findings of this report suggest that biogenic amine metabolites, rather than the amines per se, may be involved in the mechanism of action of ethanol. The data further imply that the observed synergistic effect of L-dopa or dopamine on ethanol-induced sleep time in mice may be due in part to a serotonergic rather than a direct, dopaminergic mechanism[5].

Ethanol narcosis was induced in 100 albino, male, Swiss-Webster mice (18-25 g) by administering 87 m*M*/kg of ethanol i.p. The measured response, sleeping time, was defined as the length of time required for each mouse to regain the righting reflex[6]. If a mouse did not recover this reflex within $2^1/_2$ h after the ethanol administration, a score of 150 min was assigned as its sleeping time.

Ethanol was combined with sodium chloride (0.9%) as a 25% v/v solution. Tryptophol was suspended in peanut oil, and all other compounds were dissolved in a saline solution. All doses are expressed in terms of the base, and injections were given i.p.

The duration of the ethanol-induced sleep response was compared for groups of mice pretreated with the saline solution or peanut oil, L-dopa, dopamine, 3,4-dihydroxyphenylethanol (DOPET), serotonin or the neutral metabolite, tryptophol, at various time intervals prior to ethanol injection. The effects on ethanol-induced sleep time after the administration of these 5 compounds, with methysergide, an antiserotonin compound[7], administered 30 min previously, were observed.

The effects of methysergide (0.28 m*M*/kg) on the synergistic action of serotonin (0.28 m*M*/kg)-dopamine (0.06 m*M*/kg)-and L-dopa (5.2 m*M*/kg)-ethanol (87 m*M*/kg)-combinations in mice are illustrated in the Figure. A significant synergistic effect ($P < 0.01$) as measured by the Student's *t*-test was observed in mice treated with methysergide and ethanol. Methysergide was found to significantly inhibit the augmentation of ethanol-induced sleep by dopamine ($P < 0.001$) and serotonin ($P < 0.001$).

The interpretation of the effect of methysergide and L-dopa on the sleep time response is a little more difficult to assess than the effects of methysergide in combination with dopamine or serotonin. L-dopa does augment the sleep time response as does L-dopa plus methysergide (41.8 ± 12.0 as compared to 32.5 ± 13.0). Upon reexamination of the Figure, it can be seen that methysergide alone augments sleep time to about the same magnitude as methysergide plus L-dopa. Thus it can be argued that

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Effects of methysergide alone and in combination with dopamine, L-DOPA, serotonin, 3,4-dihydroxyphenylethanol (DOPET) and tryptophol on blood and brain ETOH concentrations (mg/100 ml ± S.E.)

Pretreatment	Saline		Dopamine (0.06 m*M*/kg)		L-DOPA (5.2 m*M*/kg)		Serotonin (0.28 m*M*/kg)		DOPET (5.2 m*M*/kg)		Tryptophol (1.5 m*M*/kg)	
	Blood	Brain	Blood	Brain	Blood	Brain	Blood	Brain	Blood	Brain	Blood	Brain
Methysergide 0.28 m*M*/kg	321.0	139.1	286.2	167.7	285.3	146.2	224.6	184.6	207.6	176.8	310.0	176.2
	±	±	±	±	±	±	±	±	±	±		±
Injected I.P. 30 min. prior	10.9	2.94	22.9	19.9	36.1	19.1	13.2	12.6	23.5	14.7	33.8	23.1

Mg/100 ml ± S.E. values for saline + ETOH were: blood = 248.0 ± 28.2 and brain = 208.5 ± 9.0. These values did not differ significantly from the saline control. All agents listed in the table were administered in combination with 87 m*M*/kg ETOH. The animals were sacrificed and the blood and brain were collected at the following average sleep times: saline, 10 min; dopamine, 116 min; L-DOPA, 45 min; serotonin, 129 min; DOPET, 124 min; tryptophol, 52 min.

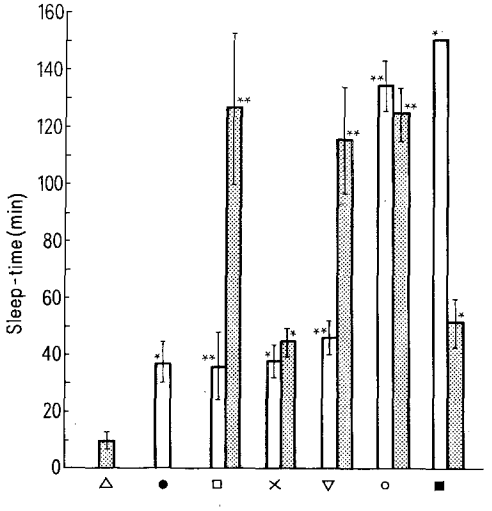
methysergide inhibits or at least masks any observed synergistic effect of L-dopa plus ethanol on induced narcosis by ethanol.

These results strongly suggest that the potentiative action of both dopamine and L-dopa may be the result of a serotonergic rather than dopaminergic mechanism. The enhancement of ethanol-induced sleep observed with the administration of dopamine may be conceivably due to the release of serotonin from neuronal stores by dopamine displacement[8].

Methysergide inhibited the augmentation action of L-dopa, dopamine and serotonin on ethanol narcosis. Singularly it augmented the soporific action of ethanol. It is speculative but the L-dopa, dopamine, and serotonin enhancement of sleep time induced by ethanol may be related to the formation and possible release of an aldehyde or alcohol-like metabolite such as tryptophol and DOPET, rather than the release of amines.

In support of this hypothesis we studied the effect of methysergide on the augmenting action of both tryptophol and DOPET on the soporific action of ethanol. We have found that methysergide failed to significantly inhibit the tryptopholethanol or the DOPET-ethanol sleep time response (Figure). In fact, the tryptophol effect was enhanced by methysergide, similar to the effect found for this antiserotonin compound on the ethanol response alone and unlike the inhibitory effect observed for methysergide on the L-DOPA, dopamine and serotonin augmenting action. These observations are supported by other studies[1,2] in which it has been shown that both tryptophol and DOPET potentiate the soporific action of ethanol.

To analyze the concentration of ethanol in the blood and brain, 6 additional groups of mice were injected with saline or peanut oil and ethanol (87 mM/kg), dopamine (0.06 mM/kg) and ethanol, L-DOPA (5.2 mM/kg) and ethanol, serotonin (0.82 mM/kg) and ethanol, tryptophol (1.5 mM/kg) and ethanol, DOPET (5.2 mM/kg) and ethanol, and methysergide (0.28 mM/kg) and ethanol. In another group, methysergide was combined with these treatments. Blood and brain samples were taken from these groups at the determined average sleep time after ethanol administration. After the mice were sacrificed, the blood and brain from each animal were analyzed for ethanol content by a modification of a gas chromatographic method[9]. At least 6 mice were used for each determination.



Effect of methysergide on monoamines' and neutral metabolites' augmentation of ethanol-induced sleep responses in mice. Standard errors of means are indicated by vertical brackets. Each bar represents average of 10 mice used in each experiment. 1 asterisk (*) indicates values significantly different from saline +ETOH treatment (at least $P < 0.01$ by student's t-test). 2 asterisks (**) indicate values significantly different from saline + ETOH treatment (at least $P < 0.001$ by student's t-test). △, ETOH + saline; ●, methysergide + ETOH; □, methysergide + dopamine + ETOH; ×, methysergide + L-DOPA + ETOH, ▽, methysergide + serotonin + ETOH; ○, methysergide + DOPET; ■, methysergide + tryptophol + ETOH. Methysergide was administered 30 min prior to additional drug treatments. The sleep response in the peanut oil-treated mice did not differ significantly from saline-treated animals; therefore, it is not shown in the figure. Shaded bars represent effect of either dopamine, L-DOPA, serotonin, DOPET or tryptophol with ETOH alone.

The concentrations of ethanol in the blood and brain after administration of the saline and ethanol combination did not differ significantly from the concentrations obtained after administration of the biogenic amines with their respective neutral metabolites and ethanol, or after injection of these agents in combination with methysergide. Since equivalent concentrations of ethanol in the blood and brain were obtained from mice treated with saline and ethanol, dopamine and ethanol, serotonin and ethanol, L-DOPA and ethanol, tryptophol and ethanol, and DOPET and ethanol, the synergistic effect induced by these agents is probably not related to an alteration of ethanol metabolism (Table).

The findings that methysergide reduces the synergistic effect of dopamine, L-DOPA and serotonin on ethanol-induced sleep lends further support to the possibility that the serotonergic system may play a role in ethanol narcosis. An alternative explanation may reside in the alteration of ethanol metabolism by the serotonin inhibitor methysergide. The distinct possibility exists that methysergide may inhibit alcohol dehydrogenase thereby preventing the increase of endogenous aldehyde levels which would in turn reduce the synergistic effect seen with dopamine, L-DOPA, and serotonin. Further studies to delineate this relationship are currently underway. However, since methysergide failed to significantly inhibit the potentiating action of the alcohol-like metabolites on ethanol sleep time, ethanol effects may be attributed to alcohol-like metabolites rather than to the release of biogenic amine precursors.

Zusammenfassung. Hinweis, dass biogene Amine eher als Amine selbst, im Narkoseprozess von Ethanol beteiligt sind und dass die beobachteten synergistischen Effekte von L-DOPA oder Dopamin bei Mäusen mit Ethanol induzierter Schlafzeit wahrscheinlich eher auf einem serotonergetischen als auf einem direkten, dopaminergetischen Mechanismus beruhen.

K. BLUM, J.E. WALLACE, W. CALHOUN
R.G. TABOR and J.D. EUBANKS[10]

Department of Pharmacology and Pathology,
The University of Texas Health Science Center,
7703 Flayd Curl Drive,
San Antonio (Texas, USA),
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