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Soporific Action of Ethanol in Mice: Possible Role of Biogenic Amines[1]

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BLUM, K., W. CALHOUN, J. E. WALLACE, J. H. MERRITT AND I. GELLER. *Soporific action of ethanol in mice: Possible role of biogenic amines.* PHARMAC. BIOCHEM. BEHAV. 1(3) 271–276, 1973.–Ethanol-induced sleep time was measured in mice after administration of L–3,4-dihydroxyphenylalanine (L-DOPA), L-tryptophan (L-TP), DL-5-hydroxytryptophan (5-HTP), serotonin (5HT), DL-5-hydroxyindole-3-acetic acid (5HIAA), and DL-parachlorophenylalanine (pCPA), a serotonin depletor. The pCPA administration, with concomitant reduction of brain serotonin, had no effect on ethanol-induced sleep; TP, 5-HTP and 5HIAA failed also to significantly enhance ethanol sleep in mice. However, serotonin significantly enhanced sleeping time of mice administered an ineffective dose of ethanol. Pretreatment with L-DOPA produced a marked prolongation of ethanol narcosis with a concomitant large increase in whole brain dopamine (DA). Administration of L-DOPA and pCPA, together, produced a smaller augmentation of ethanol effects.

Ethanol narcosis Biogenic amines

STUDIES dealing with the effects of ethanol on brain biogenic amines have yielded findings of a controversial nature. Gursey and Olson [19] demonstrated that intravenous infusion of ethanol in rabbits produced depression of 5HT and norepinephrine (NE) levels. These results were not confirmed by Efron and Gessa [10] who measured the effect of ethanol on brainstem of rats and found no effect of ethanol on 5HT and 5HIAA in rat brain slices. Other investigators [14,20] have reported that ethanol alters both brain 5-hydroxyindoleacetaldehyde and 5HIAA.

On the other hand, Bonnycastle *et al.* [7] reported that ethanol produced an increase in rat whole brain 5HT. Most recently, Palaic *et al.* [30] and Reichle *et al.* [31] both reported that acute injections of ethanol signifantly increased the 5HT level of rat brain.

The possibility that biogenic amines may be involved in the actions of ethanol derives support from a number of investigators [11,17]. Rosenfeld [3,32] reported an increase in ethanol-induced sleep in mice pretreated with either 5HT or DA. A similar potentiation of ethanol's action also was found in mice pretreated with L-DOPA [6,33]. The qualitatively similar effects, after L-DOPA, may be explained in view of a recent observation by Ng *et al.* [29] that L-DOPA at high concentrations may enter serotonergic neurons, displace 5HT and act as a false transmitter substance. Perhaps L-DOPA's effect is attributable to a release of 5HT which is known to enhance the behavioral action of ethanol.

In an attempt to examine this possibility, we studied the ethanol augmentation effect of L-DOPA in mice in which

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brain content of 5HT was reduced to approximately half of its normal value by pretreatment with parachlorophenylalanine (pCPA). We have also studied the effects of various indolealkylamines on ethanol-induced narcosis in mice.

METHOD

Albino male Swiss Webster mice, ranging in weight from 15–25 g, were used in all experiments. The sleeping time test [23], the length of time required for an animal to regain the righting reflex, was used to measure the effect of altered brain monoamine levels on ethanol-induced narcosis. If a mouse did not awaken within 2.5 hr post-ethanol administration, a score of 150 min was assigned as its sleep time. Mice were given intraperitoneal injections of ethanol in doses ranging from 4.0–7.0 g/kg.

The duration of ethanol-induced sleep time was compared for groups of mice pretreated with saline, L-DOPA, L-TP, DL-5-HTP, 5HT, 5HIAA, pCPA and L-DOPA plus pCPA. Ten mice were used for each ethanol dose. Log dose-response curves were plotted for each drug pretreatment group and data were compared by statistical analysis with a combined saline pretreatment control group. The saline control animals were grouped together because their means did not differ significantly [15].

Ethanol solutions were prepared in saline as a 25% solution (v/v) except for the 7.0 g/kg dose which was administered as a 50% solution (v/v) so that no mouse ever received a volume greater than 0.75 cc. 5HIAA and pCPA were suspended in 0.5% carboxymethylcellulose and administered at a concentration of 4 mg/cc and 25 mg/cc. L-TP, 5-HTP and L-DOPA (adminstered as the methyl ester salt) were dissolved in physiological salt solution and administered at concentrations of 4 mg/cc and 25 mg/cc, respectively. All doses are expressed in terms of the base and all injections were given intraperitoneally.

Groups of mice were given: (a) a single administration of 400 mg/kg of L-DOPA followed 45 min later by an injection of ethanol; (b) a single administration of 100 mg/kg of L-TP followed one hr later by an injection of ethanol (4.0 g/kg); (c) a single administration of 200 mg/kg of 5-HTP followed one hr later by an injection of ethanol (4.0 g/kg); (d) 100 mg/kg of 5-HTP daily for three consecutive days followed by ethanol (4.0–7.0 g/kg) two hr after the last drug treatment; (e) combined administration of 12.3 mg/kg or 49.2 mg/kg of 5HT and 4.0 m/kg of ethanol; (f) a single injection of 5HIAA at 200 mg/kg followed immediately or one hr later by 4.0 g/kg of ethanol; (g) 320 or 620 mg/kg of pCPA daily for three consecutive days followed by ethanol (4.0–7.0 g/kg) 24 hr after the last drug treatment; (h) 620 mg/kg pCPA daily for three consecutive days plus 400 mg/kg L-DOPA on the fourth day followed by ethanol 45 min later; or (i) saline followed by ethanol at intervals of 45 min, two hr or 24 hr.

To study further the augmentation effects, brain amine analyses were conducted in mice subjected to the same treatment conditions without administration of ethanol. The mice were sacrificed by immersion in liquid nitrogen according to the time sequence used for the measurement of sleep time. The brains of three mice were pooled and homogenized in cold 0.4 N perchloric acid. Norepinephrine, DOPA and dopamine in the acid supernatant were abosrbed to and eluted from aluminum oxide by the method of Anton and Sayre [1]. Aliquots of the eluate were applied to Dowex columns to separate DOPA and dopamine for subsequent assay [27]. Another aliquot of the eluate was used to determine norepinephrine content [25]. Serotonin was assayed in single mouse brains [16].

RESULTS

Figure 1 illustrates the effects of L-DOPA and pCPA together on ethanol-induced sleep. The curve for control animals represents the effects of saline on ethanol-induced sleep time at dosage levels of 4.4, 5.5 and 7.0 g/kg of ethanol. Pretreatment with L-DOPA produced the maximum augmentation of ethanol-induced narcosis. Direct observation of the mice 45 min after L-DOPA administration showed them to be alert, irritable and aggressive. Combined pretreatment with L-DOPA and pCPA elicited a significant increase in sleep time at 4.4 and 5.5 g/kg of ethanol over saline control. The observed increases were 70% ($p<0.05$) and 110% ($p<0.001$), respectively. The enhancement of sleep time for pCPA and L-DOPA at 5.5 g/kg of ethanol is significantly less than the sleep time associated with L-DOPA alone at the same dose ($p<0.05>0.02$).

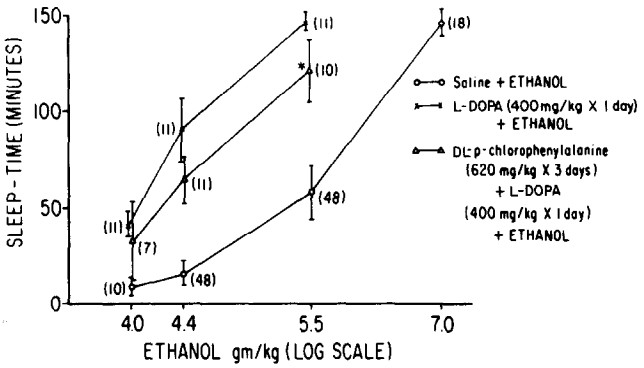

FIG. 1. Effects of L-DOPA and DL-parachlorophenylalanine on ethanol-induced sleep time in mice. Standard errors of means are indicated by vertical brackets. The number of animals used is shown in parenthesis at each average data point. The asterisk indicates that this value was significantly different from L-DOPA alone treatment ($p<0.05>0.02$).

Figure 2 shows that pCPA produced no significant effect on ethanol-induced sleep time while 5-HTP pretreatment resulted in a slight but insignificant effect at the 5.5 g/kg ethanol dose.

Results of a study of indolealkylamine-ETOH interactions in mice are shown in Fig. 3. These data illustrate that TP, 5-HTP and 5HIAA failed to significantly enhance ethanol sleep in mice. However, successful replication, as shown in Fig. 4, of a previously reported study [32] confirmed the findings that 49.2 mg/kg of 5HT significantly (at least $p<0.01$) enhanced sleep time of mice administered an ineffective dose of ethanol.

Brain Monoamine Analysis

In Table 1 are shown whole brain levels of 5HT, DA and NE obtained from mice after administration of 5-HTP and pCPA.

The administration of 5-HTP caused a significant in-

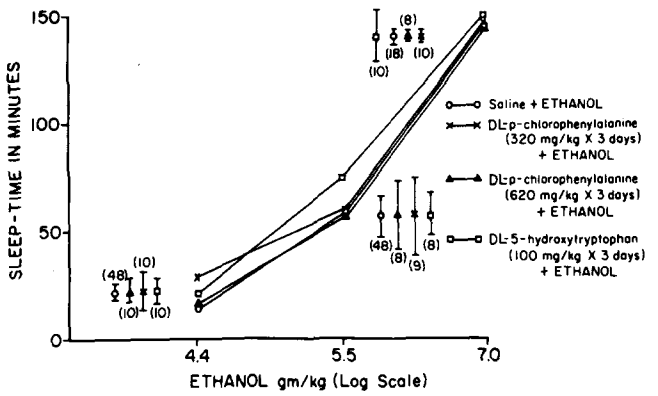

FIG. 2. Effects of DL-parachlorophenylalanine and 5-hydroxytryptamine on ethanol-induced narcosis in mice. Standard errors of means are indicated by vertical brackets. The number of animals used in each experiment is shown in parenthesis under each vertical bracket.

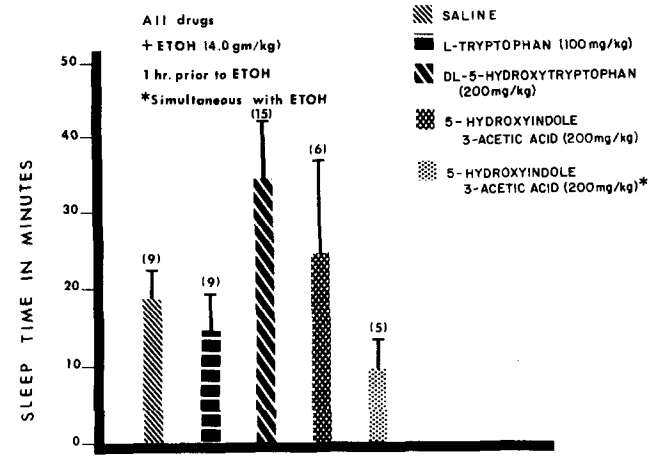

FIG. 3. Effects of various alkylamines on ethanol-induced narcosis in mice. Standard errors of means are indicated by vertical brackets. Each point represents average data and the number of animals used in each experiment is indicated in parenthesis. The asterisk indicates that the 5-hydroxyindole-3-acetic acid was given together with ethanol (4.0 g/kg). The other animals received 5-hydroxyindole-3-acetic acid one hour prior to ethanol. All other animals received either L-tryptophan (100 mg/kg), DL-5-hydroxytryptophan (200 mg/kg), or saline 1 hr prior to ethanol (4.0 g/kg).

crease of 244% in assayed 5HT and no significant change in either brain DA or NE levels. Mice receiving pCPA at 620 mg/kg had a significant reduction (48%) of brain 5HT but no significant change in brain DA and NE content. The finding that 620 mg/kg of pCPA reduced brain 5HT levels to only 50% of control lends further support to previous studies [24] which suggest that mice are more resistant than rats to pCPA effects. Although pCPA in our experiment increased brain DA by about 36%, it was not significant ($t = 1.82$ $p > 0.05$ at 13 degrees of freedom).

Similar data for 5HT, DA and NE whole brain levels following the administration of L-DOPA and L-DOPA plus pCPA are shown in Table 2.

Mice pretreated with L-DOPA alone showed a significant

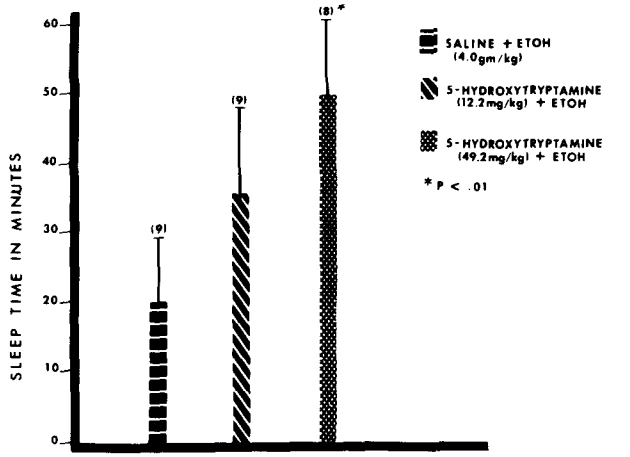

FIG. 4. Effects of serotonin on ethanol-induced narcosis in mice. Standard errors of means are indicated by vertical brackets. Each point represents average data and the number of animals used in each experiment is indicated in parenthesis. Asterisks indicate values significantly different from saline treatment ($p<0.01$). Ethanol was given in a dose of 4.0 g/kg.

reduction (52%) in brain 5HT, a marked increase (1114%) in brain DA and a less, but significant increase (76%) in brain NE. The animals receiving L-DOPA and pCPA together showed a significant decrease (57%) in brain 5HT and a significant increase in both brain DA (131%) and brain NE (117%).

DISCUSSION

The concept of a relationship between brain 5HT and ethanol-induced behavioral depression has generated many significant, but controversial contributions to the literature [11]. It is well established that in man and experimental animals, ethanol blocks the degradation of 5HT with a resultant decrease in the urinary excretion of 5HIAA [10,25]. Davis *et al.* [9] found that in man the 5HIAA level was reduced in the urine while the level of 5-hydroxytryptophol was increased following acute ethanol ingestion, indicating a shift of 5HT metabolism from the oxidative to the reductive pathway. Recently, Palaic *et al.* [30] reported that ethanol inhibits brain 5HT turnover rate and depletes endogenous 5HT from its storage site.

The lowering or raising of whole brain serotonin with pCPA or 5-HTP, respectively, did not modify the actions of ethanol. This finding was unexpected in view of previous reports that 5HT increased ethanol-induced sleep time of mice [3, 32, 34]. Perhaps the level of serotonin, which remained after pCPA treatment, was sufficient to maintain the normal pharmacologic activity of ethanol and, for this reason, ethanol's effect on sleep time was not diminished.

Failure to enhance ethanol's action with 5-HTP may indicate that 5HT levels were increased at sites that are not critical for ethanol's action on sleep time. Support for this speculation is derived from Aghajanian and Asher [2] who showed that, although 5-HTP loading increased levels of whole brain serotonin, it failed to increase fluorescence of neurons in brain stem raphe nuclei, an area which has been implicated in slow-wave sleep [21]. An increase of raphe

TABLE 1

EFFECTS OF DL-5-HYDROXYTRYPTOPHAN (5-HTP) AND DL-PARACHLOROPHENYLALANINE (pCPA) ON WHOLE BRAIN MONOAMINE CONTENT IN MICE*

Treatment	5HT (μg/g ± S.E.)	Percent Change from Control	NE (μg/g ± S.E.)	Percent Change from Control	DA (μg/g ± S.E.)	Percent Change from Control
Saline	0.63 ± 0.02	--	0.29 ± 0.01	--	1.54 ± 0.24	--
DL-5-HTP† (100 mg/kg) X 2 days	1.54 ± 0.05	+244§	0.33 ± 0.01	+14	1.37 ± 0.10	−11
DL-pCPA‡ (620 mg/kg) X 3 days	0.33 ± 0.03	−48§	0.23 ± 0.03	−24	0.98 ± 0.15	−36

*Each value is an average of at least 4 determinations read in triplicate.
†Sacrificed at 2 hr after last injection.
‡Sacrificed at 24 hr after last injection.
§Values significantly different from saline treatment ($p<0.05$ by student t test).

TABLE 2

EFFECTS OF L-3,4-DIHYDROXYPHENYLALANINE (L-DOPA) AND DL-PARACHLOROPHENYLELENINE (pCPA) ON WHOLE BRAIN MONOAMINE CONTENT IN MICE*

Treatment	5-HT (μg/g ± S.E.)	Percent Change from Control	NE (μg/g ± S.E.)	Percent Change from Control	DA (μg/g ± S.E.)	Percent Change from Control
Saline	0.63 ± 0.02	--	0.29 ± 0.01	--	1.54 ± 0.24	--
L-DOPA† (400 mg/kg) X 1 day	0.30 ± 0.04	−52§	0.51 ± 0.001	+76§	18.7 ± 2.0	1114§
DL-pCPA‡ (620 mg/kg) X 3 days	0.33 ± 0.03	−48§	0.23 ± 0.03	−24	0.98 ± 0.15	−36
L-DOPA (400 mg/kg) X 1 day DL-pCPA (620 mg/kg) X 3 days	0.27 ± 0.04	−57§	0.63 ± 0.04	+117§	3.6 ± 0.25	+131§

*Each value is an average of at least 4 determinations read in triplicate.
†Sacrificed at 45 min after last injection.
‡Sacrificed at 24 hr after last injection.
§Values significantly different from saline treatment ($p<0.05$ by student t test).

nuclei serotonin may be critical for an enhancement of ethanol activity.

L-tryptophan (TP), administered at a dose previously reported to enhance histochemical fluorescence of raphe neurons [14], also had no effect on ethanol-induced sleep time. Since L-tryptophan-induced fluorescence of raphe neurons is not prevented by pCPA [14], it is possible that tryptophan derivatives other than 5HT are found in raphe neurons after L-tryptophan administration. This would account for the lack of effect with L-tryptophan, if 5HT is critical for enhancement of ethanol-induced sleep time.

Successful replication of a previously reported study [32] confirmed the findings that 50 mg/kg of 5HT significantly enhanced sleeping time of mice administered an ineffective dose of ethanol. It should be of interest to determine whether administration of 5HT raises selectively 5HT and TP and the obtained effect with 5HT. In support of the above speculation, Bulat and Supek [8] reported that serotonin at 10 mg/kg given intraperitoneally to mice increased brain 5HT. Therefore, 5HT could cross the blood-brain barrier. Other investigators [4], however, demonstrated that serotonin has difficulty penetrating the

blood-brain barrier.

A possible role for DA in the pharmacologic actions of ethanol is suggested by the finding that L-DOPA enhanced ethanol-induced sleep time. Potentiation of ethanol's action by L-DOPA may relfect an indirect serotonergic involvement. Dopamine has been reported to displace 5HT from neuronal stores. Serotonin released in this manner might add to ethanol's effect on sleep time since, in the presence of ethanol, serotonin metabolism is diverted from a predominantly oxidative to a reductive pathway with concomitant formation of tryptophols [9]. These agents also have been reported to produce sleep in mice [12,13]. A lesser ethanol enhancement after pCPA and L-DOPA relative to the enhancement after L-DOPA alone may be due in part to the fact that insufficient 5HT was available for displacement by dopamine in mice that were administered pCPA, the serotonin depletor. Further support for this contention is derived from the preliminary experiment [5] showing that DA-induced enhancement of ethanol narcosis in mice was blocked by the anti-serotonin compound, methysergide, but not by haloperidol, a DA receptor blocker.

Based on our preliminary findings [19], McCabe *et al.* [26] speculated that an increase in brain dopamine may enhance the soporific action of ethanol. Since, after pCPA plus L-DOPA, the increase in brain dopamine is significantly ($p<0.001$) less than the increase following L-DOPA alone, one might explain this diminished ethanol enhancement effect by postulating a dopaminergic rather than a serotonergic mechanism.

It is possible that ethanol may divert the metabolism of dopamine from a predominately oxidative to a reductive pathway with formation of neutral or alcohol-like metabolites such as 3,4-dihydroxyphenylethanol (DOPET) [11,12]. The augmentation effect on ethanol may be due in part to the overproduction of these alcohol metabolites. In support of this notion in a preliminary experiment [5], it has been demonstrated that DOPET, when administered simultaneously with ethanol, significantly ($p<0.001$) enhanced ethanol-induced narcosis in mice. In this regard, it must be noted that very high doses of DOPET or tryptophol are required to produce sleep in mice. Direct cerebral application of these metabolites would increase our sphere of knowledge regarding the role of the alcohol-like metabolites in ethanol-induced sleep.

The role of the increased NE (76%) after L-DOPA administration in contributing to the effects of ethanol appears to be quantitatively less important than the increased dopamine (1114%). However, further experimentation utilizing specific DA blockers, dopa decarboxylase inhibitors and beta-hydroxylase inhibitors would be necessary in order to delineate the relative significance of these catecholamines in ethanol-induced narcosis. A suggestion by Miller and Maickel [28] provides the basis for an alternate interpretation to account for these data. They postulated that the balance of free 5HT and free NE is related to behavioral depression. This balance between serotonergic and adrenergic systems may also be a critical determinant in the actions of ethanol in the CNS.

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