Biochemical Pharmacology, 1966, Vol. 15, pp. 711–721. Pergamon Press Ltd., Printed in Great Britain.

IMPORTANCE OF ACETALDEHYDE IN THE ACTION OF ETHANOL ON BRAIN NOREPINEPHRINE AND 5-HYDROXYTRYPTAMINE*

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(*Received* 15 *October* 1965; *accepted* 14 *December* 1965)

Abstract—Ethanol alone produced small decreases in rat and rabbit brain norepinephrine (NE) and 5-hydroxytryptamine (5-HT) concentrations that were no greater than the effects of intraperitoneal injections of similar volumes of isotonic saline. However, increased acetaldehyde blood levels produced by disulfiram pretreatment before the ethanol doses or by the administration of acetaldehyde itself caused statistically significant decreases in brain NE but no further effects on 5-HT. Chlorpromazine blocked decreases of both 5-HT and NE without producing any change in the blood levels of acetaldehyde or ethanol. The depletion of NE coincided temporally with peak concentrations of acetaldehyde in the brain rather than with blood levels.

MANY studies have shown that alcohol produces a stress-like increase in the urinary excretion of catecholamines and their metabolites in animals[1–4] and in man.[5–10] Ethanol also may produce an inhibition of 5-hydroxytryptamine (5-HT) metabolism.[11–13] Concomitant decreases in brain concentrations of norepinephrine (NE) and 5-HT have been claimed[14, 15] but not substantiated.[16–20]

Previous investigations in this department have examined the role of acetaldehyde in the actions of ethanol. Very low concentrations of acetaldehyde exert an inhibitory effect on oxidative phosphorylation by brain mitochondria, whereas ethanol, even in lethal concentrations, has no such effect.[21, 22] This action is related to deprivation of pyruvate as a mitochondrial energy source through coupling to acetoin formation and has been observed by others.[23–25]

The role of acetaldehyde was investigated in the disulfiram-like antialcohol reactions produced by the hypoglycemic sulfonylurea drugs, tolbutamide and chlorpropamide.[26] The conversion of acetaldehyde vasopressor responses to depressor responses drew attention to the observations of Eade,[27] who showed that this sympathomimetic action of acetaldehyde was mediated by catecholamine release. These findings, coupled with previous interest in the actions of acetaldehyde led to the following study of its effects on brain NE and 5-HT as a possible answer to the discrepancies reported in studies with alcohol on these brain amines.

MATERIALS AND METHODS

Animals. Male albino Wistar rats weighing approximately 150 g, and male albino New Zealand rabbits weighing approximately 2·0 kg, were used. The animals were on hand at least 4 days prior to use and were maintained on Purina chow diet.

* Supported by Grant MH-O-4012, from the United States Public Health Service.

Drugs. Ethanol (2 or 4 g/kg) and acetaldehyde (300 mg/kg), both diluted with isotonic saline to 20% and 2%, respectively, and isotonic saline control injections of 11·25 ml/kg and 15 ml/kg were administered i.p. The first saline volume is equivalent to the ethanol dose of 2 g/kg, and the second is equivalent to the acetaldehyde dose of 300 mg/kg. Disulfiram (200 mg/kg) was given orally in a 10% acacia suspension 49, 25, and 1 hr before the administration of ethanol or its equivalent saline control. Chlorpromazine was injected i.p. in a dose of 10 mg/kg half an hour before administration of ethanol or saline.

Catecholamine determination. Norepinephrine and 5-HT were determined by the Mead and Finger modification[28] of the spectrophotofluorimetric methods of Bogdanski *et al.*[29] and Shore and Olin.[30] The whole rat brain and the rabbit brain stem (brain minus cerebral cortex and cerebellum) were analyzed in untreated and saline-treated controls and in the experimental animals The possibility that acetaldehyde and ethanol affected the solvent extraction method for NE or 5-HT was examined as a possible interference. Concentrations of acetaldehyde and ethanol expected to occur in animal tissues, as well as a high amount of formaldehyde, were added to aqueous solutions and to pooled rat brain homogenate containing 0·5 μg 5-HT and NE per ml and per g respectively.

TABLE 1. EFFECT OF ACETALDEHYDE (AcH), ETHANOL (EtOH) AND FORMALDEHYDE (HCHO) ON THE RECOVERY OF 0·5 μg/ml CONCENTRATIONS OF NOREPINEPHRINE (NE) AND 5-HYDROXYTRYPTAMINE (5-HT) FROM AQUEOUS SOLUTIONS AND ON THE ENDOGENOUS LEVELS OF NE AND 5-HT FOUND IN A POOLED RAT BRAIN HOMOGENATE

Treatment	Added drug conc. (μg/g wet wt. brain tissue or per ml)	Aqueous solution 5-HT (μg/ml)	Aqueous solution NE (μg/ml)	Brain homogenates* 5-HT (μg/g)	Brain homogenates* NE (μg/g)
Control		0·50† ±0·01	0·50† ±0·02	0·45 0·45	0·42 0·42
AcH	40	0·51†	0·51†	0·44 0·43	0·41 0·42
AcH	400	0·50†	0·50†	0·46 0·46	0·42 0·42
EtOH	2000	0·51‡	0·48‡	0·46 0·44	0·42 0·42
EtOH	4000	0·51‡	0·51‡	0·46 0·46	0·42 0·40
HCHO	500	0·50§	0·50§	0·44 0·44	0·42 0·41
Mean				$\bar{x}$ = 0·450 ±0·012	0·417 ±0·006

* Pool of 14 rat brains.
† n = 6.
‡ n = 2.
§ n = 4.

Data shown in Table 1 indicate that altered recovery of added or endogenous NE and 5-HT does not occur with these concentrations of acetaldehyde, ethanol, or formaldehyde. The activation and fluorescence emission peaks were not shifted in wavelength for either NE or 5-HT as a result of this treatment.

Blood analysis for ethanol and acetaldehyde. Blood levels of acetaldehyde and ethanol were analyzed simultaneously by the gas chromatographic method of Duritz and Truitt.[31] Blood was obtained after decapitation, and no difference in levels was noted when it was compared to blood obtained from direct cardiac puncture.

Brain analysis for ethanol and acetaldehyde. Gas chromatographic analyses of acetaldehyde and ethanol in brain were made by a modification of the method of Duritz and Truitt for blood.[31] An animal was sacrificed by decapitation and the brain quickly removed from the skull and placed in iced water. The brain was rapidly blotted on filter paper, weighed, and placed in an ice-cold homogenizing tube previously kept in ice.

Two ml of cold distilled water was added for every gram of brain tissue present, and the tissue was immediately homogenized at 5°. One-ml samples of the resulting homogenates were added to 25-ml serum bottles which contained 0·25 ml of 5% $ZnSO_4$. The contents were mixed, and 0·25 ml of 0·3 N $Ba(OH)_2$ was added to complete the deproteinizing step. The serum bottle was fitted with a self-sealing rubber stopper and the analysis of acetaldehyde and ethanol was performed as previously described for blood.

RESULTS

Brain NE and 5-HT levels

Figure 1 is a time study of the effects of ethanol (2 g/kg, i.p.) administration to disulfiram (TETD)-pretreated rats to determine the rapidity and peak time of action

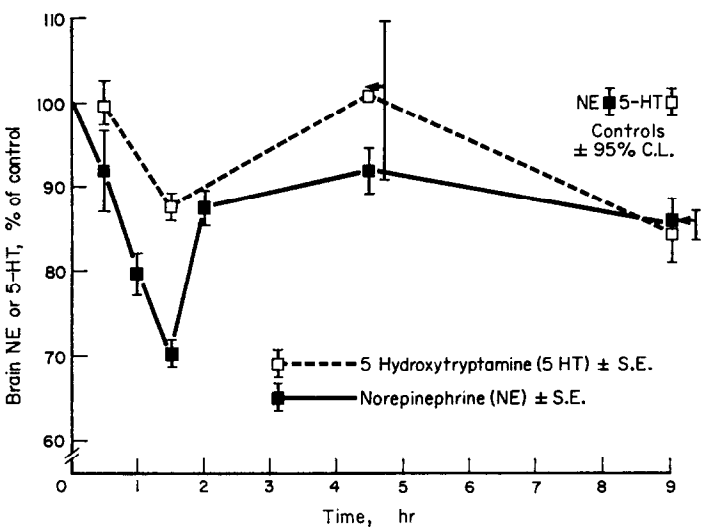

FIG. 1. Time study of whole-brain norepinephrine and 5-hydroxytryptamine after ethanol (2 g/kg i.p.) in disulfiram-pretreated rats. Range for 95% confidence limits of untreated control rat brains is shown at upper right.

on brain levels of NE and 5-HT. The changes are expressed as a per cent of untreated control brain levels with the range for 95 per cent confidence limits bracketed at the extreme right. By comparison with untreated brain levels, all the time points for NE and the changes at 90 min and 9 hr for 5-HT are statistically significant. However, a

more stringent comparison should be made to the maximal effect produced in TETD-saline treated animals which fell to 88·5 per cent ± 1·47 for NE, and 87·6 per cent ± 1·47 for 5-HT. With this evaluation, only the decrease in brain NE at 60 and 90 min and the 5-HT effect at 9 hr are statistically significant. Although the 9-hr decrease in 5-HT is valid at the 5% level, it is not significant when compared to ethanol alone or even to saline-treated controls.

Blood and brain acetaldehyde and ethanol levels

Figure 2 is a time study of the blood acetaldehyde and ethanol levels after ethanol administration to untreated and TETD-pretreated rats. After the administration of

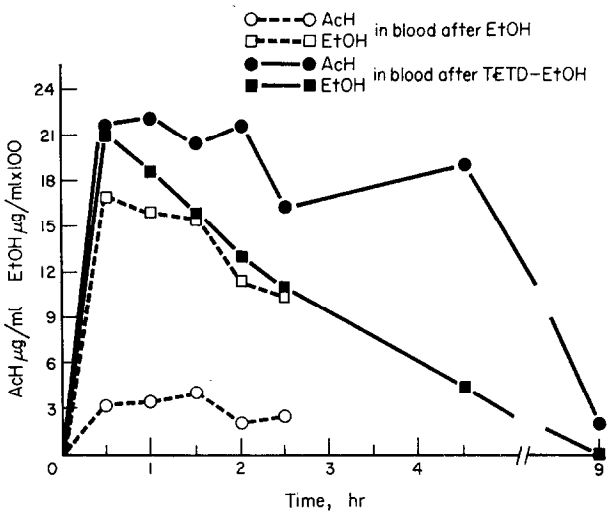

FIG. 2. Time study of ethanol (EtOH) and acetaldehyde (AcH) blood levels in rats after administration of EtOH alone with disulfiram (TETD) pretreatment.

ethanol alone, it can be seen that blood ethanol is at its highest concentration in 30 min, whereas acetaldehyde does not reach its highest blood level until 90 min. Figure 2 also shows that ethanol given to TETD-pretreated rats reaches its highest blood concentration in 30 min and then declines as it does when ethanol is given alone. On the other hand, the blood acetaldehyde levels after ethanol in TETD-pretreated animals are maintained at high levels throughout the first 4·5 hr and then decline sharply by 9 hr, whereas rats given only ethanol and peak acetaldehyde levels at 90 min.

Data shown in Table 2 concerning brain acetaldehyde and ethanol levels after ethanol administration to TETD-pretreated rats indicate that both acetaldehyde and ethanol attain high brain levels at 30 min, reach their peak at 90 min, and then decline by 270 min. The brain ethanol decrease is much sharper and faster than the decrease observed with brain levels of acetaldehyde.

Since NE shows its greatest decrease, blood levels of acetaldehyde are maximal, and acetaldehyde and ethanol attain their highest brain levels 90 min after ethanol is given to TETD-pretreated rats, these findings prompted the selection of 90 min as a fixed interval for comparison of factors affecting the NE and 5-HT levels of brain.

Brain NE levels at 90 min after ethanol in rats and rabbits

Table 3 shows brain NE levels for a number of different treatments measured 90 min after the ethanol (or control vehicle) injection. It is evident in the rat that the decreases produced by ethanol alone are not greater than the effect of a similar volume of saline. However, the administration of ethanol to TETD-pretreated animals

TABLE 2. BRAIN LEVELS OF ACETALDEHYDE AND ETHANOL IN RATS PRETREATED WITH TETD AT VARIOUS TIMES AFTER ETHANOL (2 g/kg i.p.)

	No. of rats	Time after ethanol (min)			
		30	90	270	540
AcH μg/ml (± S.E.)	12	7·75 (0·48)	9·51 (0·43)	5·93 (0·68)	3·54 (0·32)
EtOH μg/ml (± S.E.)	12	976 (46)	1220 (38)	250 (50)	62 (20)

TABLE 3. EFFECTS OF ETHANOL (EtOH) ALONE AND AFTER DISULFIRAM (TETD) AND CHLORPROMAZINE (CPZ) PRETREATMENT ON RAT BRAIN AND RABBIT BRAIN STEM NOREPINEPHRINE (NE) 90 MIN AFTER ETHANOL I.P.

Treatment	Dose (g/kg)	Rats		Rabbits	
		No. of determ.	Per cent of untreated controls (± S.E.)	No. of determ.	Per cent of untreated controls (± S.E.)
Untreated controls		19	100 ± 0·71 (0·42 ± 0·003 μg/g)	15	100 ± 1·54 (0·54 ± 0·008 μg/g)
Saline control	11·25*	8	86·2 ± 1·20		
EtOH	2·0	10	88·4 ± 1·58†	8	106·1 ± 1·76
EtOH	4·0	2	88·9‡	7	100·7 ± 1·63
TETD	0·2§	7	88·5 ± 1·47	4	80·8 ± 0·93†
Saline	11·25*				
TETD	0·2§	11	70·2 ± 1·32¶	6	74·7 ± 2·93
EtOH	2·0				
TETD	0·2§	10	75·0 ± 1·24¶	7	72·7 ± 1·41¶
EtOH	4·0				
TETD	0·2§	9	94·6 ± 1·42		
CPZ	0·01				
Saline	11·25*				
TETD	0·2§	24	97·4 ± 1·82		
CPZ	0·01				
EtOH	2·0				

* Saline, 11·25 ml/kg ≏ EtOH 2 g/kg as 20% solution.

† $P = <0·01$ vs. untreated controls only.

‡ Insufficient data.

§ TETD, 0·2 g/kg p.o. for 3 days, 1 hr prior to EtOH on 3rd day.

¶ $P = <0·01$ vs. untreated controls, saline controls, TETD-saline and EtOH 2·0 g/kg.

caused a further decline in brain NE which was statistically significant ($P = <0·01$). The similar decreases in rat brain NE for saline controls, TETD-saline control, and for two ethanol doses suggest that these may be nonspecific nervous system effects from the intraperitoneal injection and handling. A similar increased effect was found in the rabbit for TETD and ethanol together when compared to ethanol alone.

However, ethanol alone had little apparent effect relative to untreated controls and produced an increase when compared to TETD–saline treatment.

Table 3 also shows that chlorpromazine (CPZ) administration prior to the injection of ethanol in TETD-pretreated rats prevents the decline in NE noted previously when ethanol is given to these animals. Table 5 shows that the administration of CPZ does not accomplish this by altering the blood levels of acetaldehyde or ethanol.

Brain 5-HT levels at 90 min after ethanol in rats and rabbits

Table 4 shows the effects of EtOH alone and in combination with TETD-pretreatment on rat and rabbit brain 5-HT 90 min after ethanol administration. No

TABLE 4. EFFECTS OF ETHANOL (EtOH) ALONE AND AFTER DISULFIRAM (TETD) AND CHLORPROMAZINE (CPZ) PRETREATMENT ON RAT BRAIN AND RABBIT BRAIN STEM SEROTONIN (5-HT) 90 MIN AFTER ETHANOL DOSE I.P.

Treatment	Dose (g/kg)	Rats		Rabbits	
		No. of determ.	Per cent of untreated controls (± S.E.)	No. of determ.	Per cent of untreated controls (± S.E.)
Untreated controls		18	100 ± 0·67 (0·45 ± 0·003 μg/g)	14	100 ± 2·89 (0·49 ± 0·004 μg/g)
Saline control	11·25*	9	86·1 ± 2·00		
EtOH	2·0	10	88·4 ± 2·70†	8	89·0 ± 3·30
EtOH	4·0	3	88·4‡	7	94·8 ± 0·71
TETD	0·2§	7	97·4 ± 4·03	4	87·3‡
Saline	11·25*				
TETD	0·2	6	87·6 ± 1·47‡	2	105·5‡
EtOH	2·0				
TETD	0·2§	10	87·4 ± 4·37§	2	96·4‡
EtOH	4·0				
TETD	0·2§	7	102·0 ± 1·29		
CPZ	0·01				
Saline	11·25*				
TETD	0·2§	20	104·5 ± 6·73		
CPZ	0·01				
EtOH	2·0				

* Saline, 11·25 ml/kg ≏ EtOH 2·0 g/kg as 20% solution.
† $P = <0·01$ vs. untreated control only.
‡ Insufficient data.
§ TETD, 0·2 g/kg p.o. for 3 days, 1 hr prior to EtOH on 3rd day.

significant decreases are seen in brain 5-HT with any of the drugs or drug combinations used when compared to saline-treated controls. Again the decreases in 5-HT that are evident are probably due to nonspecific nervous system influences as evidenced by the equivalent decrease in 5-HT caused by saline controls.

Table 4 also shows that the decreases in brain 5-HT attributed to nonspecific nervous system influenced are prevented by CPZ. The 5-HT content in the brains of CPZ-treated animals does not significantly differ from untreated controls.

Blood levels of acetaldehyde and ethanol 90 min after ethanol administration to rats and rabbits

Table 5 shows that blood ethanol levels do not greatly differ when ethanol is given alone or to TETD- and CPZ-pretreated animals. In sharp contrast, ethanol

TABLE 5. BLOODLEVELS OF ACETALDEHYDE (AcH) AND ETHANOL (EtOH) IN RATS AND RABBITS 90 MIN AFTER I.P. DOSE OF EtOH ALONE OR AFTER DISULFIRAM (TETD) AND CHLORPROMAZINE (CPZ) PRETREATMENT*

Treatment	Dose (g/kg)	Rats			Rabbits		
		No. of determ.	AcH μg/ml (± S.E.)	EtOH μg/ml (± S.E.)	No. of determ.	AcH μg/ml (± S.E.)	EtOH μg/ml (± S.E.)
EtOH	2·0	27	4·11 (0·27)	1560 (34·4)	8	4·03 (0·08)	1840 (52·3)
EtOH	4·0	34	1·60 (0·09)	3410 (51·9)	7	3·40 (0·15)	3810 (72·1)
TETD EtOH	0·2 2·0	18	20·5 (0·69)	1570 (38·2)	8	12·5 (0·24)	1810 (34·4)
TETD EtOH	0·2 4·0	30	19·5 (0·85)	3100 (116)	4	15·4 (0·41)	3620 (8·7)
TETD EtOH CPZ	0·2 2·0 0·01	22	22·4 (0·73)	1480 (101·0)			

* TETD (0·2 g/kg) p.o. for 3 days; CPZ (0·01 g/kg) i.p. 30 min and EtOH (2 g/kg) i.p. 60 min after TETD on 3rd day.

given to TETD-pretreated rats produces a five- to twelve-fold increase in blood acetaldehyde when compared to the acetaldehyde levels observed when ethanol was given alone. A three- to five-fold increase in blood acetaldehyde is noted when this combination is administered to rabbits. This substantial increase in blood acetaldehyde after the TETD–ethanol reaction has been questioned by both Wagner[32] and Casier and Polet,[33] none of whom could find appreciable amounts of acetaldehyde. The reasons for these discrepancies will be discussed later.

There is no significant change seen in blood ethanol levels when the effect of ethanol alone is compared to TETD-pretreatment plus ethanol with respect to the 2 g/kg dose. However, the TETD–ethanol-treated animals (4 g/kg) have a lower blood ethanol level than their ethanol (4 g/kg) counterparts. An unexpected finding was the lower acetaldehyde level of the animals treated with 4 g ethanol/kg when compared with those given 2 g/kg. The animals given the higher dose were very lethargic throughout the course of the experiment, and their metabolic rates could have been depressed to an extent that effected lower acetaldehyde levels. Table 5 also shows that CPZ administration in the manner described in Methods does not significantly alter blood acetaldehyde or ethanol levels.

Studies with repeated administration of acetaldehyde

In order to determine the effects of acetaldehyde on brain amines without using ethanol and TETD, a method of repeated intraperitoneal injection was employed which simulates partially the continuous production of acetaldehyde from ethanol. Table 5 shows that there is no accumulation in blood acetaldehyde levels after two or after three injections of 300 mg/kg.

Table 5 also shows that two or three injections of acetaldehyde cause significant decreases in rat brain NE, both series of injections causing decreases to about 77 per cent of untreated controls. There is no significant difference between the two treatments with respect to their effect on brain NE. The very potent effect of acetaldehyde on brain NE is indicated by the fact that these amounts of acetaldehyde, equivalent to the administration of ethanol at doses of 0·6 and 0·9 g/kg, can cause significant decreases in brain NE similar to that when ethanol (2 g/kg) is given to TETD-pretreated animals.

Neither the two- nor the three-injection schedule has a significant effect on brain 5-HT when compared to saline controls. Although triple administration of acetaldehyde causes a decrease in 5-HT to 84 per cent, which is highly significant when compared to untreated control values, this change is probably due to nonspecific influences, if one considers the equivalent decreases in saline-treated controls.

DISCUSSION

The primary aim of this study has been an attempt to explain the contradictory reports pertaining to a possible effect of alcohol on brain biogenic amines. Brain levels of 5-HT and NE have been examined by many investigators at various times after single and multiple doses of alcohol and in three species of laboratory animals.[14–20] Only in the data from one group[14, 15] were the changes rated as statistically significant and both NE and 5-HT were decreased.

The most apparent explanation for this discrepancy from the data reported here is that significant changes in brain amines occur only for NE and appear to be related to

the effects of acetaldehyde formed in the oxidation of ethanol. In this respect the action could be proportional to the blood or brain level of acetaldehyde, or its rate of oxidation. The evidence for this is based on the observations that the relative amount of acetaldehyde must be increased by slowing its oxidation with TETD or by injection of acetaldehyde itself in order to achieve significant decreases in NE greater than those that follow isotonic saline or ethanol. The peak effect in a time-course study coincided with periods of elevated blood and brain acetaldehyde, although recovery of NE levels occurred before the decline of blood acetaldehyde levels. The degree of depletion was not proportional to the dose of ethanol when given to TETD-pretreated animals, since the rate of ethanol oxidation was probably maximal with each dose. Effects from acetaldehyde alone required repeated doses because of its rapid metabolism.

The lack of effect of ethanol, acetaldehyde, and formaldehyde on the butanol extraction efficiency for NE and 5-HT precludes an explanation of the observed effects as an artifact resulting from inebriating levels of these substances. Corrodi and Hillarp[34] developed fluorescence with primary and secondary catecholamines in a dried protein layer, using formaldehyde gas for the histochemical identification of these amines. However, Falck and Owman[35] noted that the final step in the formation of the fluorophore upon treatment with formaldehyde does not occur if the reaction is performed in a solvent system.

Species and strain differences in the activity of alcohol dehydrogenase and a resultant variation in the relative production of acetaldehyde can exist, since the enzyme is heterogenous,[36] thus accounting for the varied results from different laboratories.[14–20] Similar animal differences in the lability of the brain amines are also possible. The blood level data for acetaldehyde and ethanol reported here show that few of the published studies selected the peak time of effect for rats found at 90 min in this study. The inconsistencies in the listed control values for NE and 5-HT reflect the difficulties in comparing data for these determinations between laboratories.[37]

If acetaldehyde is accepted as the releasing factor for brain NE after ethanol, some comparisons can be made to compounds with similar effects. The action is most like that of tyramine, which releases mainly NE and acts upon the loosely bound granular stores of the neurohormone at nerve endings. Both acetaldehyde and tyramine have a vasopressor action by release of peripheral NE that is prevented by reserpine pretreatment.[27, 38] The blocking of the acetaldehyde depletion of NE by CPZ shown here is also paralleled by a similar action of CPZ in inhibiting brain amine depletion by reserpine.[39] Thus the results reported here coupled with the observations of others establishes acetaldehyde as a NE-releasing sympathomimetic substance because of its ability to release catecholamines from the brain, cardiovascular system,[27, 40] and adrenal gland.[41]

A similar pattern and degree of NE depletion was found by Maynert and Levi[42] in rats receiving repeated floor grid shocks or cold stress. The maximal decrease was to 60–70 per cent of control NE levels, and the repletion to normal levels was quite rapid. No significant changes were noted in brain 5-HT or acetylcholine concentrations. Interestingly, this stress-induced decrease was also prevented by CPZ and phenobarbital.

It is perhaps more difficult to reconcile the multiple failures to corroborate either a decrease in brain 5-HT,[14, 15] an increase in brain levels,[16] or changes in 5-HT