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The effect of antidepressants and "tranquillizers" on the response of mice to ethanol

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1. The techniques and apparatus used for investigation of the interaction of various psychotropic drugs with ethanol in mice are described. The parameters measured were (*a*) length of loss of righting reflexes; (*b*) continuous coma; (*c*) subjects remaining in coma 12 hr after dosing; (*d*) changes in toxicity.

2. The following drugs were tested: amitriptyline, trimipramine, imipramine, nortriptyline, desipramine, thioridazine, phenelzine, methylphenidate, chlorpromazine, trifluoperazine, phenobarbitone, and diazepam. The total number of mice used was 3,140.

3. Imipramine caused no significant changes in the effects of ethanol. Methylphenidate and desipramine protected the mice against ethanol induced coma. All other drugs induced statistically significant potentiation of the depressant and toxic effects of ethanol in mice.

4. Late (delayed) deaths after a tricyclic antidepressant have been noted in animals and man.

5. It is suggested that the potentiation of alcohol by some psychotropic drugs may add to the hazards of drug overdosage and contribute to traffic accidents. Hence it is necessary to test all psychotropic drugs for interaction with alcohol.

Evidence of the interaction between psychotropic drugs and ethanol is mounting. Soon after the introduction of chlorpromazine, Brodie, Shore & Silver (1955) showed that this drug increased the sedative effects both of hexobarbitone and ethanol in mice. These observations have been confirmed by Halliwell, Quinton & Williams (1964), who also demonstrated that amitriptyline, imipramine and desipramine (in descending order of potency) increased the mean sleeping time of mice under ethanol. Theobald, Buech, Kunz, Morpurgo, Stenger & Wilhelmi (1964) found that imipramine and desipramine caused, however, only a weak and variable enhancement of the toxicity of ethanol. Some tricyclic antidepressants produce central stimulation when examined in appropriate conditions; thus desipramine and, to a lesser extent, imipramine were shown by Sulser, Watts & Brodie (1962) to reduce the duration of sleep caused by the combined actions of reserpine and ethanol in mice.

Animal laboratory investigations can aid the prediction of adverse drug effects resulting from polypharmacy in man. Unfortunately, preliminary laboratory investigation of psychotropic drugs has rarely included tests for significant interaction

with ethanol. There have, for example, been no reports of tests for the interaction of ethanol with such widely prescribed agents as diazepam and thioridazine.

This paper describes a method for studying such interactions in mice and provides data on the interactions of ethanol with twelve psychotropic agents.

Methods

Albino mice, obtained from the Institute of Medical and Veterinary Science, Adelaide, South Australia, were maintained in constant conditions of lighting within the temperature range 20.5°–22.5° C. Free access was given to a standard pellet diet (Westfarmers Ltd., Western Australia) and water. Young adult male mice were used in the main, but in a few experiments each dose group contained an equal proportion of males and females.

Design of experiments

Mice to be used in each experiment (thirty–sixty) were assigned in equal numbers to each treatment group, matching the groups on a weight basis. The tails were coloured to ensure individual identification. Solid food was withdrawn overnight before an experiment. Three treatments were administered at the start of an experiment in the absence of the future observer, so that all recording was " blind ". The three treatments were: (*a*) drug solution and ethanol 25 ml./kg 25% v/v ; (*b*) water (equal in volume to the drug solution) and ethanol ; (*c*) drug solution and 5% glucose in volume equal to the ethanol. All treatments were administered by mouth. The oral dose of ethanol 25 ml./kg of a 25% solution was selected because preliminary tests had shown that this amount caused loss of righting reflexes for 1 to 2 hr but no deaths.

Immediately after treatment, the animals were placed in individual chambers (permitting continuous observation) for a period of 6 hr. Deaths were counted and righting reflexes were tested at 30 min intervals. All animals were returned, at 6 hr, to their holding cages and given access to water and food. Further inspections of all animals used were made at 12, 24 and 36 hr after dosing. Each experiment was repeated several times.

Analysis of observations

Righting reflexes were deemed absent after death for the remainder of the 6 hr period of observation but dead mice were excluded from assessments made at 12 hr. Data obtained in the 6 hr period of continuous observation and in replications of individual experiments were subjected to analysis of variance. Chi-squared tests for statistical significance were applied to data concerning deaths and the numbers of animals in coma.

Special equipment

The individual rodent observation chambers used were a modification of those described by Kerley, Carter & Briscoe (1964). The chambers were constructed of opaque Perspex, only the doors being clear. Thus the mice were more effectively isolated than in the original design. Large numbers of mice could be efficiently observed and deaths were not artificially increased by, for example, a fit in one

mouse precipitating a fit in another, or by other crowding effects and metal cage noises.

Drugs

The drugs used were commercially available solutions for intramuscular or intravenous administration to man and were received as gifts from the various companies. When necessary, the solutions were diluted, but care was taken that the active ingredient was not damaged by, for example, changes in pH. The antidepressants tested were: amitriptyline (" Laroxyl ", Roche), nortriptyline (" Aventyl ", Eli Lilly), imipramine (" Tofranil ", Geigy), desipramine (" Pertofran ", Geigy), trimipramine (" Surmontil ", May & Baker), phenelzine (" Nardil ", Warner), and methylphenidate (" Ritalin ", Ciba). The remaining drugs tested were: thioridazine (" Melleril ", Sandoz), chlorpromazine (" Largactil ", May & Baker), trifluoperazine (" Stelazine ", Smith, Kline & French), phenobarbitone (" Gardinal ", May & Baker), and diazepam (" Valium ", Roche).

Results

(1) *Diazepam* (10 mg/kg) caused no loss of righting reflexes or deaths when given orally, but it was found that at each dose level of 2, 5 and 10 mg/kg, there was a significant interaction between diazepam and ethanol. The duration of sleep caused by ethanol alone was trebled by 5 mg/kg and nearly doubled by 2 mg/kg diazepam. At each of the above dose levels a significant proportion of mice given diazepam and ethanol were still unable to right themselves 12 hr after dosing and were without righting reflexes for more than 5 consecutive hours after dosing. For example, with diazepam 2 mg/kg and ethanol (25 ml./kg 25%), 29% of the mice were without righting reflexes throughout the period of continuous observation ($P<0.001$) and 22% were unable to right themselves 12 hr after dosing ($P<0.001$). Deaths occurred in the group given diazepam and ethanol but not in a statistically significant proportion of subjects. (Total number of mice, $N=210$.)

(2) *Thoridazine* (30 mg/kg) increased the average length of " loss of righting reflexes " (L.R.R.) from 1.5 to 5.1 hr: 79% of the drug and ethanol treated mice were unconscious throughout the period of continuous observation ($P<0.001$); 29% were in coma 12 hr after dosing ($P<0.001$) and all of this last group died.

The average length of L.R.R. was increased from 1.3 to 3.2 hr when thioridazine 5 mg/kg was given in addition to ethanol. Of mice treated with this drug and ethanol, 28% slept through the first 6 hr after dosing; 29% were still in coma after 12 hr ($P<0.001$), but there were no deaths. ($N=330$.)

(3) *Chlorpromazine,* when tested at a dose level of 5 mg/kg, caused no L.R.R., but increased L.R.R. due to ethanol (25 ml./kg 25%) from an average 1.5 to 5 hr ($P<0.001$). Twenty-seven per cent of the combined treatment groups slept throughout the period of continuous observation ($P<0.001$) and 13% were unable to right themselves 12 hr after dosing ($P<0.001$). Seven per cent of mice given chlorpromazine 5 mg/kg plus ethanol died. ($N=260$.)

(4) *Trifluoperazine* significantly potentiated the effects of ethanol at dose levels of 2, 5 and 10 mg/kg, although 10 mg/kg by itself caused no L.R.R. At the low dose level (2 mg/kg) seven of the forty mice which were also given ethanol slept for more than 5 hr ($P<0.05$) and only four were still in coma 12 hr after dosing

(insignificant). At the higher dose levels, a significant number of mice slept for more than 5 hr ($P<0.001$) and were in coma at 12 hr ($P<0.001$). There was a 40% death rate in the group given trifluoperazine 5 mg/kg together with ethanol, but no deaths in the 2 mg/kg plus ethanol group. ($N=160$.)

(5) *Sodium phenobarbitone* caused no L.R.R. when given in a dose of 30 mg/kg, but 10 mg/kg quadrupled the average length of L.R.R. caused by ethanol. There were no deaths at the 10 mg/kg dose, but 48% died when the mice were given phenobarbitone 30 mg/kg plus ethanol ($P<0.001$). ($N=160$.)

(6) *Amitriptyline* (50 mg/kg) trebled the length of L.R.R. caused by ethanol alone in 5 and 6 hr periods of observation, although the drug by itself at this dose caused no L.R.R. ($P<0.001$).

The three other parameters studied were L.R.R. throughout the period of continuous observation, coma at 12 hr, and death. Amitriptyline (50 mg/kg) significantly increased the effects of ethanol on all these parameters. There were seventy deaths among the 270 mice given this dose of amitriptyline and ethanol ($P<0.001$). A third of these deaths were "late" or delayed. Late deaths are defined as those occurring in mice which had recovered from an early drug and ethanol-induced coma (or had never lost their righting reflexes in the early stages of the experiment) but later lost consciousness and died.

TABLE 1. *Potentiation of ethanol (25 ml./kg 25%) by psychotropic drugs, when given orally to mice*

Drug	Dose (mg/kg body weight)	Factor by which sleeping time changed	Mortality in group given drug and alcohol
Diazepam	10	2·3‡	5/30
	5	3·0†	0/20
	2	1·8†	2/40
Thioridazine	30	3·4‡	20/70‡
	5	2·5†	0/60
Chlorpromazine	50	3·3†	17/20‡
	30	2·9†	5/20†
	5	3·3‡	4/60
Trifluoperazine	10	2·1†	4/10
	5	2·4‡	8/20†
	2	1·9†	0/40
Na phenobarbitone	30	1·7†	24/50‡
	10	4·4†	0/20
Amitriptyline	50	3·1‡	70/270‡
	30	1·75†	6/110*
Trimipramine	50	2·1*	12/140†
	30	2·3†	5/60
Imipramine	50	1·3	5/120
Nortriptyline	50	2·3*	33/40‡
	30	1·5	10/60*
Methylphenidate	20	0·2†	0/60
Desipramine	50	0·4†	0/50
Phenelzine	50	1·3†	9/60†

* $P<0·05$. † $P<0·01$. ‡ $P<0·001$.

Amitriptyline in a dose of 30 mg/kg doubled the length of L.R.R. caused by ethanol and significantly increased the proportion of mice without their righting reflexes throughout the period of continuous observation and at 12 hr after dosing. There were six deaths at this dose of drug and ethanol (100 mice treated), five being late. ($N=710$.)

(7) *Trimipramine* (50 and 30 mg/kg) doubled the length of L.R.R. due to ethanol alone, produced significant changes in all other parameters and caused a 9% death rate. ($N=440$.)

(8) *Imipramine* (50 mg/kg) added to the effects of ethanol but not to a statistically significant degree. ($N=280$.)

(9) *Nortriptyline* (50 and 30 mg/kg) significantly potentiated the depressant effects of ethanol. ($N=220$.)

(10) *Methylphenidate* (20 mg/kg) protected mice against drug and ethanol induced coma ; sixty mice given methylphenidate and ethanol showed a total L.R.R. which was only 25% that of the mice in the water and ethanol treated groups ($P<0.01$). ($N=120$.)

(11) *Desipramine* (desmethylimipramine) also protected this strain of mice from ethanol induced L.R.R. Desipramine 50 mg/kg, plus glucose solution, caused no L.R.R. Water and ethanol caused an average length of L.R.R. of 1.4 hr in fifty mice ; desipramine significantly reduced this length of L.R.R. to an average of 0.6 hr per mouse. ($N=120$.)

(12) *Phenelzine* (50 mg/kg) increased by 33% the length of L.R.R. caused by ethanol ($P<0.001$). ($N=130$.)

No significant " between replications " differences were found throughout the experiments.

Discussion

In general the results of this series of experiments on mice confirm and extend the findings of other investigators, for most psychotropic drugs add to the central sedative and toxic effects of ethanol. Diazepam, sodium phenobarbitone, chlorpromazine, thioridazine and trifluoperazine were especially potent in these tests. The tricyclic thymoleptics, which possess both central sedative and excitant properties, showed a weaker and more variable interaction with ethanol than the neuroleptics. Amitriptyline and trimipramine potentiated ethanol to nearly the same degree as thioridazine ; nortriptyline, the desmethylated derivative of amitriptyline, was less powerful than the parent compound.

The individual scores for length of loss of righting reflexes showed a considerable variation, particularly in mice given water and ethanol. Other investigators have reported similar observations ; for example, Halliwell *et al.* (1964): " Values for the duration of sleep in individual mice showed a wide variation, and a percentage difference between the mean sleeping time of 20 test and 20 control mice of less than $\pm 40\%$ was rarely significant ". It is clearly important to use large numbers of mice when testing for the interaction of a drug with ethanol.

Strain and species differences underlie many metabolic factors controlling the duration of drug action. Brodie & Maicel (1962) suggest that studies of strain

differences may explain the extraordinary variability in the rates of metabolism of drugs in man. Even in the one individual, however, a marked personal variation occurs in blood alcohol levels when tested on different occasions under otherwise identical conditions. Enticknap (1967) found, for example, that in one subject tested five times the values were 36, 52, 61, 67 and 87 mg/100 ml.

Halliwell *et al.* also reported that imipramine potentiated the effects of ethanol in mice, but they were using a dose of ethanol (25 ml./kg 20%) that just failed to cause L.R.R. The addition of a drug with even a small degree of sedative activity was bound to tip the balance and hence a small length of L.R.R., compared with a zero score for control mice, proved statistically significant. In the present experiments the dose of ethanol used (25 ml./kg 25%) itself caused an appreciable length of L.R.R. and, though imipramine was not found to add significantly to this, it was possible to note that desipramine antagonized the effects of ethanol. Theobald & Stenger (1962) found that imipramine (30 mg/kg, intravenously) markedly increased the toxicity of ethanol when the latter was given intravenously in doses between 0.25 and 3 ml./kg. Most drugs given intravenously, however, are more potent than when given by mouth (Westermann, 1962).

The interaction of a drug with ethanol can be measured in several ways. A mutual increase in toxicity is related to increased "sleeping time" but is not so sensitive a test. No significant number of deaths was found with diazepam (2, 5 and 10 mg/kg), thioridazine (5 mg/kg), chlorpromazine (5 mg/kg) or trimipramine (30 mg/kg), when given together with ethanol, although an analysis of variance of the lengths of L.R.R. in mice observed over 6 hr did reveal significant potentiation of ethanol (25 ml./kg 25%) by these doses of psychotropic drugs.

The mechanism of psychotropic drug and ethanol interaction is obscure. The increased sleeping time and toxicity may be the result of a summation of sedative effects, but a true potentiation of the effects of ethanol seems more likely. No L.R.R. was caused by any of the drugs tested (other than by chlorpromazine 30 and 50 mg/kg) when given together with glucose (25 ml./kg 5%). Moreover the ethanol, when given with water, caused no deaths and an average length of L.R.R. of only 1–2 hr. The results described indicate a multiplication and prolongation of drug-ethanol effects—potentiation rather than synergism.

The late deaths observed when amitriptyline was given with ethanol to mice may be due to severe fatty change of the liver (Milner & Kakulas, 1968). These fatty changes are most severe in mice which have recovered from an early amitriptyline-and-ethanol induced coma but subsequently die. Delayed deaths have been reported in man after taking tricyclic antidepressants and after apparent recovery from the initial effects of the drug (Fuge, 1967; Masters, 1967; Steel, O'Duffy & Brown, 1967). These deaths recall the late encephalopathy which may follow carbon monoxide intoxication and late liver damage after carbon tetrachloride poisoning.

A recent survey of patients showed that the majority of men and many women might possibly drink and drive while taking a psychotropic drug; drug-alcohol interaction may increase the hazards involved (Milner, 1968).

The methods of testing for drug-ethanol described in this paper may help in the preliminary evaluation of new psychotropic agents. "Toxicity studies in animals may enable one to predict adverse reactions in clinical practice to a drug and to alert the investigator to the possibility of their occurrence" (Cahal, 1967).

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