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Changes in seizure susceptibility after successive treatments of mice with tryptophol and ethanol

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Changes in leptazol (pentetrazol) seizure susceptibility after successive treatments of mice with tryptophol, a neutral metabolite of indoleamine, in combination with ethanol have been examined. Mice treated with tryptophol plus ethanol became highly susceptible to convulsions. There was little or no difference in seizure susceptibility in mice treated with tryptophol or ethanol alone, compared with the corresponding controls. In the mice treated with tryptophol plus ethanol, a much higher brain tryptophol level was observed, compared with that in mice treated with tryptophol alone. There appeared to be a good correlation between the reduction of the length of the seizure latency time and the time for which the brains were exposed to high levels of tryptophol. These results suggest that elevation of the levels of neutral indoleamine metabolites in the brain may have resulted in the increase in the seizure susceptibility.

Acetaldehyde generated during ethanol metabolism is capable of competitively inhibiting biogenic aldehyde oxidation (Lahti & Majchrowicz 1967), which results in an increment of the steady-state levels of biogenic aldehydes and the formation of biogenic alcohols (Davis et al 1967). Such an aberrant biogenic aldehyde metabolism has been implicated in the addictive and dependent state caused by ethanol (Deitrich & Erwin 1975). When administered acutely, the neutral metabolites of indoleamines (biogenic alcohols such as tryptophols and biogenic aldehydes such as indoleacetaldehydes) have been reported to produce ethanol-like effects, such as sleep-inducing (Feldstein & Kurcharski 1971; Sabelli et al 1969; Taborsky 1971) and hypothermic (Seed & Sechelski 1977) ones. In addition, 5-hydroxytryptophol has been shown to promote ethanol drinking behaviour when infused centrally into rats (Myers et al 1972). In the present study, we examined changes in leptazol seizure susceptibility after successive treatments of mice with tryptophol in combination with ethanol on the assumption that neutral metabolites of indoleamines may be involved in the development of physical dependence on ethanol.

MATERIALS AND METHODS

Materials

Tryptophol was obtained from Sigma Chemical Co., St Louis, Missouri, and PTZ from Tokyo Kasei Co., Tokyo. All other chemicals were of the highest quality commercially available. Tryptophol was dissolved in 15 or 25% ethanol solution or sesame oil and administered 0·05 ml per 10 g weight.

Treatments

Male ICR mice (Charles River, Japan) were maintained in our laboratory for at least 7 days before being used. They were housed in an air conditioned room (23 °C) with free access to food and water. The mice, 25–30 g at the beginning of the experiments, were treated intraperitoneally twice daily (every 12 h) for 3 days with tryptophol, ethanol or both. Animals treated with 0·9% NaCl (saline) solution or sesame oil served as controls.

Estimation of seizure susceptibility

To test the effects of the successive drug treatments on leptazol seizure susceptibility, mice were injected with PTZ (100 mg kg^{-1} s.c.) 18 h after the last dose of the treatment and latencies were recorded to the onset of (1) the first myoclonic jerk of the neck and head musculature; and (2) clonic convulsion with loss of righting reflex.

Determination of tryptophol

Mice were decapitated at definite times after administration of tryptophol. The brains were removed quickly, frozen on dry ice and stored at −25 °C until analysed. Tryptophol was assayed by the method of Seed & Sechelski (1977) with minor modifications (Satoh et al 1979).

The results were subjected to statistical evaluation by Student's *t*-test and significant differences between

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the means (calculated as P values) are shown. Statistical significance is indicated when the P value was $< 0{\cdot}05$.

RESULTS

Effect of combined administration of tryptophol and ethanol

Fig. 1 shows changes in the latencies to the onset of the leptazol-induced myoclonic and clonic seizure after successive treatments with tryptophol, ethanol or both. The myoclonic latency was slightly but significantly reduced in both ethanol groups (0·75 and 1·25 g kg^{-1}). No difference in the latency time was noted between mice treated with 0·75 g kg^{-1} of ethanol plus 50 mg kg^{-1} of tryptophol and those treated with the same dose of ethanol alone. However, mice treated with 0·75 g kg^{-1} of ethanol plus 100 mg kg^{-1} of tryptophol and those treated with 1·25 g kg^{-1} of ethanol plus 50 mg kg^{-1} of tryptophol showed significantly shorter myoclonic latency, compared with those treated with corresponding doses of ethanol alone. There was no difference in clonic latency between mice treated with saline and those treated with either dose of ethanol alone. However, the subjects treated with tryptophol plus ethanol showed significantly shorter clonic latencies, compared with those treated with corresponding doses of ethanol alone.

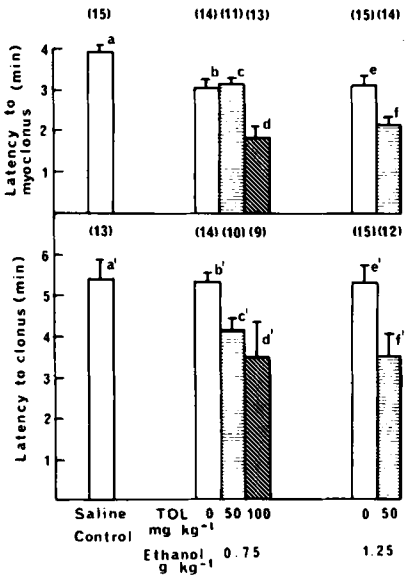

FIG. 1. Effects of successive combined administration of tryptophol and ethanol on leptazol seizure susceptibility. Mice were injected with leptazol (100 mg kg^{-1} i.p.) 18 h after the last dose of the successive treatment. Mice treated with saline served as a control. Drugs and saline were injected intraperitoneally twice daily for 3 days. Tryptophol (TOL) was dissolved in ethanol solution. Figures in parentheses represent numbers of animals used. s.e. values are shown on each column (vertical bars). Statistical significance: a vs b, b vs d, e vs f and b′ vs c′ are $P < 0{\cdot}01$; a vs e and e′ vs f′ are $P < 0{\cdot}02$; b′ vs d′ is $P < 0{\cdot}05$.

Effect of tryptophol alone

Fig. 2 shows changes in the latencies to both forms of leptazol seizure after successive treatments with tryptophol alone. There was no significant difference between the control and subjects treated with tryptophol alone in the latency to either form of seizure.

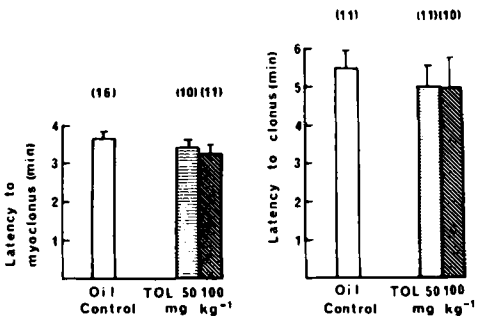

FIG. 2. Effects of successive administration of tryptophol alone on leptazol seizure susceptibility. Tryptophol was dissolved in sesame oil. Mice treated with sesame oil alone served as a control. For details, see the legend to Fig. 1. Statistically, there was no difference between the control and either treated group in the latency to either form of seizure.

Time course of brain tryptophol levels

Fig. 3 shows the time course of changes in brain tryptophol concentrations after tryptophol injection. In the subjects treated with tryptophol plus ethanol, the maximal tryptophol level in the brain was much higher and the disappearance of tryptophol from the brain was much slower, compared with those in the subjects treated with tryptophol alone.

DISCUSSION

The mice treated successively with tryptophol plus ethanol became highly susceptible to convulsions. In addition, there appeared to be a good correlation between the reduction of the length of the seizure latency time and the time for which the brains were exposed to tryptophol. Ethanol may block tryptophol metabolism by competing with tryptophol for the alcohol dehydrogenase receptor sites in the liver, leading to an increase in the brain tryptophol level.

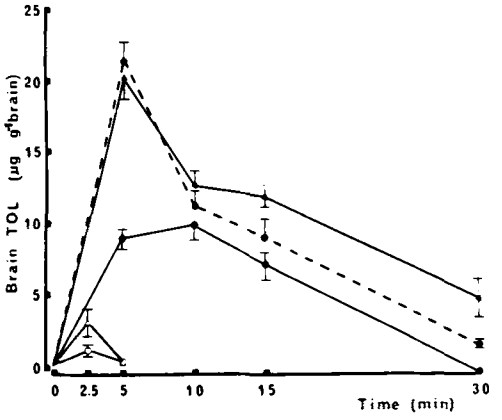

FIG. 3. Time course of changes in brain tryptophol concentration after tryptophol injection. ○—○ Mice injected with tryptophol alone (50 mg kg^{-1}). △—△ Mice injected with tryptophol alone (100 mg kg^{-1}). ●—● Mice injected with tryptophol (50 mg kg^{-1}) plus ethanol (0·75 g kg^{-1}). ▲—▲ Mice injected with tryptophol (50 mg kg^{-1}) plus ethanol (1·25 g kg^{-1}). ●- - -● Mice injected with tryptophol (100 mg kg^{-1}) plus ethanol (0·75 g kg^{-1}). Each point is the mean value of 4–6 animals. s.e. values are shown on each curve (vertical bars).

However, in the brain, tryptophol is converted to indoleacetaldehyde by way of NADPH-dependent aldehyde reductase (Satoh et al 1979), which does not catalyse the oxidation of low molecular aliphatic alcohols such as ethanol (Tabakoff & Erwin 1970). Therefore, there is no such metabolic competition between these alcohols. Indoleacetaldehyde is further oxidized to indoleacetic acid by aldehyde dehydrogenase. However, the oxidative metabolite of ethanol, acetaldehyde, may block the metabolism of indoleacetaldehyde to indoleacetic acid by competing with it for brain aldehyde dehydrogenase (Fig. 4), which may lead to an elevation of indoleacetaldehyde in the brain. Thus the simultaneous administration

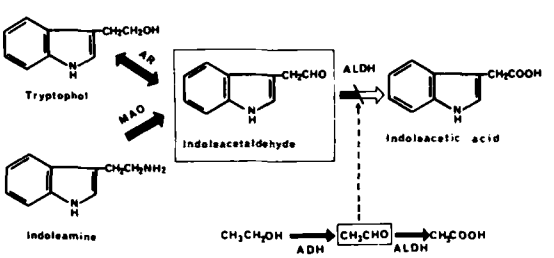

FIG. 4. Inhibition of the metabolism of indoleacetaldehyde derived from exogenous tryptophol and endogenous indoleamine by acetaldehyde. AR = aldehyde reductase, ALDH = aldehyde dehydrogenase, ADH = alcohol dehydrogenase, MAO = monoamine oxidase.

of ethanol and tryptophol may increase not only the brain level of tryptophol itself but also that of indoleacetaldehyde. At present, it is not clear whether tryptophol itself may cause a reduction of seizure threshold or whether its metabolite indoleacetaldehyde may be active. In a previous report we suggested that the acute action of tryptophol on the central nervous system may be mainly due to its metabolite, indoleacetaldehyde (Satoh et al 1979). In addition, indoleacetaldehydes arising from deamination of indoleamines are demonstrated to be biologically active (Sabelli et al 1969) and postulated to be involved in the sleep mechanism (Jouvet 1969) and the hypnotic action of barbiturates (Fukumori et al 1980a). Therefore it is likely that the enhancement of the seizure susceptibility after successive treatments with tryptophol may be also caused by its active metabolite, indoleacetaldehyde.

It is conceivable that the intervention of acetaldehyde generated during ethanol metabolism may, to some extent, evoke aberrant metabolism of indoleamines (Davis et al 1967; Huff et al 1971; Fukumori et al 1980b) (Fig. 4). There is clinical evidence that during ethanol intoxication, the 5-hydroxytryptophol level in the cerebrospinal fluid of alcoholic inpatients is increased (Beck et al 1980). In addition, it is reported that a significant quantity of tryptophol is present in alcoholic beverages (Nykanen et al 1966). Exogenously administered tryptophol potentiates the hypnotic and hypothermic effects of ethanol (Seed & Sechelski 1977; Feldstein & Kurcharski 1971). Seed & Sechelski (1977) postulated that the contaminating tryptophol may be partially responsible for the sedation observed following alcoholic beverage consumption.

One of the chief characteristics of the ethanol withdrawal syndrome is an increased susceptibility to seizure (Goldstein & Pal 1971; Hunter et al 1973; Majchrowicz 1975). The neutral metabolites of indoleamines have been suggested to play an important role in the modulation of seizure threshold (Fukumori et al 1980c; Minegishi et al 1981). Although indirect, the evidence obtained in the previous and present investigations suggests that alteration of biogenic amine metabolism, resulting in increases in the steady-state levels of neutral metabolites of indoleamines (presumably indoleacetaldehydes), may be partly responsible for the development of physical dependence on ethanol.

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