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ETHANOL PROTECTION AGAINST HEMICHOLINIUM TOXICITY IN MICE

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Abstract—The minimum lethal dose of hemicholinium-3 (HC-3) (intra-peritoneal) was determined in groups of male albino mice. Death occurred in the majority of cases within 10 min after injection and was attributed to the "curare-like" action of HC-3. However, pretreatment with ethanol (i.p.) was found to afford protection against these toxic effects. The site of action of ethanol protection appeared to be peripheral since ethanol (i.p.) also protected against a lethal dose of the post-synaptic neuromuscular blocking agent, *d*-tubocurarine (dtc) (intravenous). The time course of the protection suggested that a metabolite of ethanol might be involved. The phrenic nerve-diaphragm preparation was used to demonstrate the "curare-like" action of HC-3 and to investigate the effect of ethanol on this. Ethanol added to the bathing medium produced a transient reversal of the fall in twitch height produced by either HC-3 or dtc. This reversal may be primarily explained by depolarisation of the post-synaptic membrane by ethanol since this potentiated the blockade due to decamethonium and caused an increase in resting tension of the muscle.

Hemicholinium-3 (HC-3), first described by Long and Schueler [1], has been shown to interfere with cholinergic transmission at several sites both in the central nervous system and in the periphery [2]. At the neuromuscular junction it depressed transmission by: (1) inhibition of acetylcholine (ACh) synthesis in the nerve ending [3–10], (2) depression of the sensitivity of the motor end plate to ACh [11–13], (3) blockade of ACh release from the nerve [14, 15] and (4) reduction in the number of synaptic vesicles [16].

In the whole animal the primary manifestation of HC-3 toxicity is respiratory failure [1, 6, 17, 18]. Since ethanol has been shown to facilitate neuromuscular transmission [19–22], the possibility that it might afford some protection against HC-3 toxicity in mice was investigated. In addition, the site and mechanism of action of any protective effect was studied. The possibility that the site of action was the peripheral neuromuscular junction was investigated (a) by comparing the effect of ethanol on HC-3 toxicity with its effects on the toxicity of *d*-tubocurarine (dtc) which produces respiratory failure due to a purely peripheral action and (b) by examining the effects of ethanol on the isolated phrenic nerve-diaphragm preparation poisoned by HC-3 or by the neuromuscular blocking agents dtc or decamethonium (C_{10}).

METHODS

In all *in vivo* experiments the volume of each injection was 0·1 ml. HC-3 (i.p.) or dtc (i.v.) was injected into groups of ten albino mice (20–30 g) and the toxic range of doses determined.

Ethanol pretreatment. To determine the effect of varying concentrations of ethanol, various doses of ethanol (in 0·9% saline) were injected (i.p.) into groups of 20 mice 1 hr prior to administration of a lethal dose of HC-3 (i.p.). The times of death were recorded up to 2 hr after HC-3 administration.

In order to investigate whether the rate of ethanol metabolism was important, the effect of varying the pretreatment time was determined. A standard dose of ethanol was administered at varying times from 60 min before to 8 min after a lethal dose of HC-3 and the times of death recorded.

The effect of ethanol pretreatment on dtc toxicity was investigated by administering ethanol 15–30 min before a lethal dose of dtc (i.v.).

Phrenic nerve-diaphragm preparation. Male Wistar rats (180–260 g) were killed by stunning followed by exsanguination. The left hemidiaphragm with phrenic nerve attached was removed and placed in a 100 ml organ bath containing Krebs-bicarbonate solution [23] (37°) which was gassed with 95% O_2: 5% CO_2. The phrenic nerve was stimulated supramaximally at 0·1 Hz with a pulse width of 1 msec and contractions of the diaphragm were measured isometrically using a Grass force-displacement transducer and displayed on a Grass polygraph.

RESULTS

Results from 100 mice indicated that the minimum lethal dose of HC-3 lay between 0·100 mg/kg and 0·175 mg/kg while that of dtc was 0·170 mg/kg. For any one batch of mice the respective lethal dose was

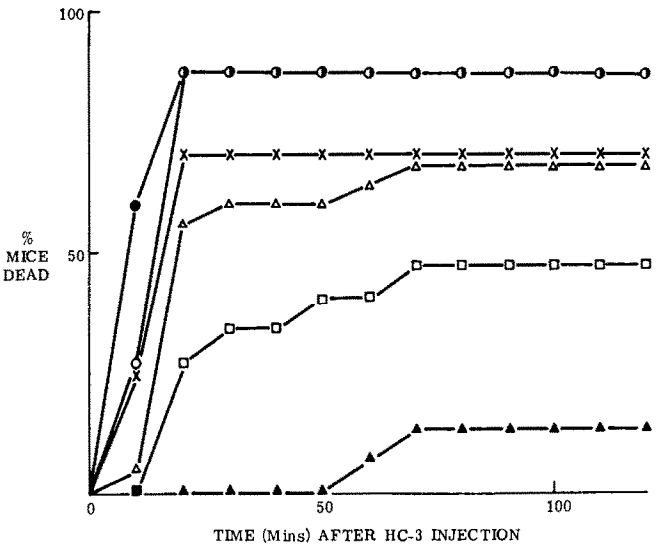

Fig. 1. The effect of varying the concentration of ethanol on the number of mice killed by a lethal dose of HC-3. Each group consisted of twenty mice. A lethal dose of HC-3 (i.p.) was injected 60 min after administration of ethanol (i.p.) in concentrations of 1% (○); 5% (×); 20% (△); 50% (□) and 100% (▲). Control animals (●) received a saline injection.

determined and used in all subsequent experiments on that batch.

Ethanol and HC-3. When injected 1 hr prior to the lethal dose of HC-3, increasing doses of ethanol produced a corresponding increase in protection against HC-3 toxicity (Fig. 1). This was apparent both by the increase in time to death and by the number of mice remaining alive after 2 hr. On no occasion were there any deaths after the 2 hr period.

0·1 ml of 50% ethanol was found to be the dose which produced 50 per cent protection against HC-3 (Fig. 1). Using this dose it was found that the optimum protection was provided when the pretreatment time was reduced to 15 min (Fig. 2).

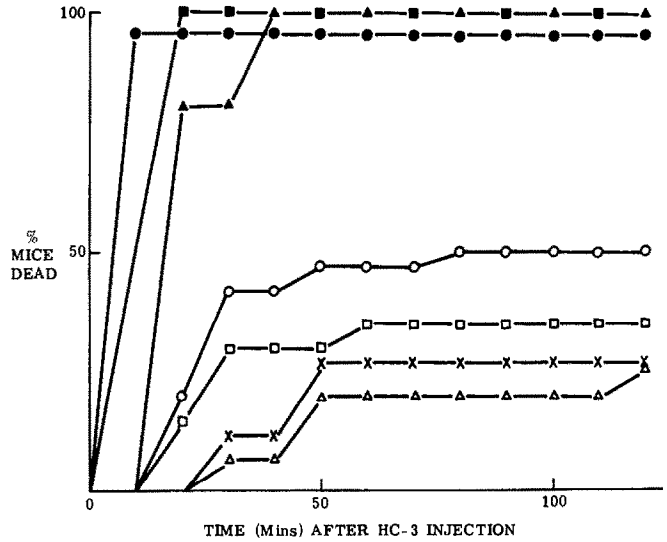

Fig. 2. The effect of varying the pretreatment times of 50% ethanol on the number of mice killed by a lethal dose of HC-3. Each group consisted of twenty mice. A lethal dose of HC-3 (i.p.) was injected 60 min (○), 30 min (×) or 15 min (△) after: simultaneously (□); or 4 min (■) or 8 min (▲) before administration of 50% ethanol (i.p.). Control animals (●) received a saline injection 15 min prior to HC-3.

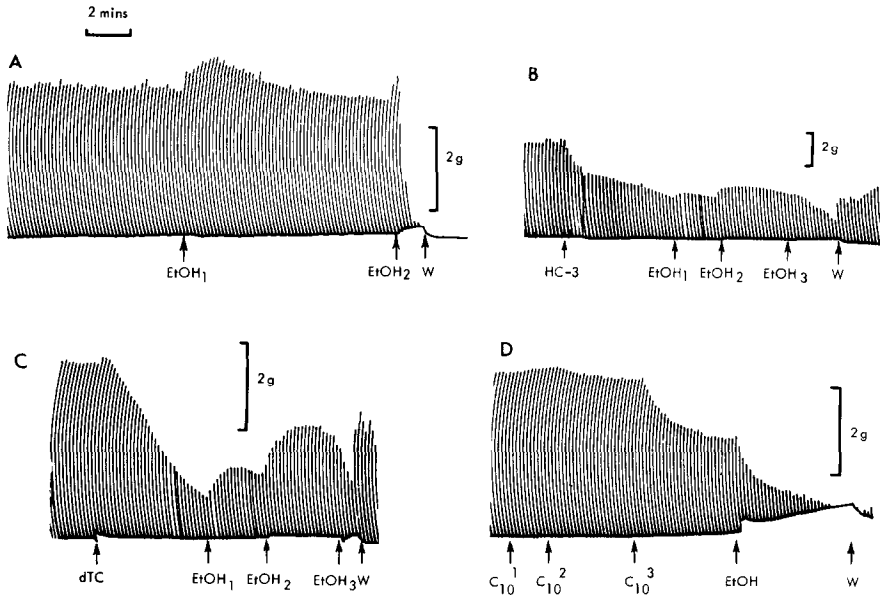

Fig. 3. The effect of various drugs on the responses of the phrenic nerve-diaphragm preparation to supramaximal stimulation. Doses refer to the final bath concentrations. W represents washout of drugs. (a) The effects of adding ethanol to give concentrations of 0·67% ($EtOH_1$) and 2% ($EtOH_2$). (b) The effects of adding ethanol to give concentrations of 0·33% ($EtOH_1$), 0·67% ($EtOH_2$) and 1·33% ($EtOH_3$) on the blockade produced by $6·7 \times 10^{-5}$ g/ml HC-3. (c) The effects of adding ethanol to give concentrations of 0·67% ($EtOH_1$), 1·33% ($EtOH_2$) and 2% ($EtOH_3$) on the blockade produced by $5·3 \times 10^{-7}$ g/ml dtc. (d) The effect of 0·67% ethanol on the blockade produced by C_{10}. C_{10} was added to give concentrations of $5·3 \times 10^{-6}$ g/ml (C_{10}^1), $1·83 \times 10^{-5}$ g/ml (C_{10}^2) and $4·43 \times 10^{-5}$ g/ml (C_{10}^3).

Ethanol and dtc. In a group of 25 mice, the average time until death produced by dtc (0·170 mg/kg, i.v.) was 1·48 ± 0·13 min, all the mice being killed. However, ethanol (0·1 ml of a 50% solution, i.p.) given 15–30 min before dtc completely protected 15 mice in a group of 20. Of the 5 that died, the average time of death was increased to 12·3 ± 6·2 min.

Phrenic nerve-diaphragm preparation. Ethanol alone produced a transient increase in twitch height. However, as the concentration of ethanol was increased from 0·67 to 2·00 per cent, this transient increase preceded a fall to zero in twitch height, both stages being accompanied by a prolonged rise in the resting tone of the muscle, (Fig. 3a). The lower dose of ethanol transiently reversed the neuromuscular block produced by HC-3 (Fig. 3b) or by dtc (Fig. 3c). However, the block produced by C_{10} was enhanced by this dose of ethanol (Fig. 3d).

Twitch height reduced by HC-3, dtc or C_{10} was restored to original levels by washing the preparation with fresh Krebs solution.

DISCUSSION

Ethanol was found to afford marked protection against the toxic effects of HC-3. Furthermore, it was shown to protect against dtc toxicity, suggesting a peripheral site of action for ethanol, since dtc produces blockade of neuromuscular transmission in the respiratory muscles. Indeed, it is probable that HC-3 produces respiratory failure due to a "curare-like" action since reduced ACh synthesis, caused by blockade of choline transport to its site of acetylation, seems unlikely in such a short period before death.

Some insight into the possible peripheral mode of action of ethanol which might protect against both HC-3 and dtc toxicity was provided by use of the phrenic nerve-diaphragm preparation. HC-3 was shown to produce neuromuscular blockade similar in character to that produced by dtc. Since ethanol reversed the blockade produced by dtc or HC-3, but enhanced that produced by C_{10}, it is possible that ethanol has a depolarising effect [21] on the postsynaptic membrane. This suggestion is supported by the finding that ethanol produced a rise in the resting tension of the muscle. Ethanol may also act to increase transmitter release [21, 22], although it is doubtful whether this could explain the rise in tension.

However, Gessner [24] reported that the half-life of ethanol (i.p.) in mice was 3·6 min. Since 0·1 ml of 50% ethanol was found to be most effective when administered 15 min before HC-3, it is possible that some protection may also be afforded by a metabolite. Ethanol, given 4 min after administration of HC-3 (i.e. approxi-

mately 4 min before the predicted time of death) had very little protective ability. However, when administered simultaneously with HC-3, ethanol produced about 70 per cent protection of the mice. This could also be explained by the time dependence of the appearance of sufficient metabolite at the site of HC-3 action.

In conclusion, it would appear that HC-3 may produce respiratory paralysis due to a "curare-like" action and this effect may be antagonised by ethanol and/or a metabolite of ethanol.

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