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EFFECT OF ETHANOL ON NEUROMUSCULAR FUNCTION IN RATS. ITS INTERACTION WITH ALCURONIUM

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Abstract—1. The effect of chronic ethanol intake on neuromuscular function has been analyzed by using a rat tibial muscle preparation.

2. The time-course of single twitches, trains-of-four, tetanus and post-tetanic facilitation with and without blockade with alcuronium was evaluated.

3. A decrease in these parameters was observed, being more pronounced in ethanol fed rats during 10 than 30 days.

4. The twitch was the most affected parameter.

5. After recovery of alcuronium blockade, the depressant effects of ethanol were completely reversed.

6. These data suggest that low but sustained ethanol blood levels causes evident alterations of neuromuscular function due, probably, to a postjunctional action.

INTRODUCTION

Ethanol causes both, *in vivo* and *in vitro* alterations in neuromuscular function (see reviews by Israel and Mardones, 1971; Goldstein, 1983) however, its precise effect on neuromuscular transmission is not known. It has been well established that the frequency of miniature end plate potentials (m.e.p.p.) rises in the presence of over 0.16 M ethanol (Gage, 1965). Moreover, an increase in the amplitude and duration of m.e.p.p. was observed by other authors (Gage *et al.*, 1975). Other effects concerned with the activity of ethanol are: a fluidificant effect on the presynaptic membrane of the rat phrenic nerve-diaphragm followed by the development of tolerance (Curran and Seeman, 1977), a reduction in the number of cholinoceptive sites and a decrease in the apparent dissociation constant for acetylcholine at high and low concentrations, respectively (Bradley *et al.*, 1984). Finally, a depressant effect on the muscular contractility has also been observed (Gage, 1965).

In the majority of such studies ethanol was used at doses reaching blood levels of 0.5–4 mg/ml *in vivo*, or concentrations of 0.01–2 M *in vitro*. Under these circumstances toxic effects ranging from slight intoxication to ataxia, or even lethality, would be expected. It is not clear, therefore, whether or not the long term administration of lower doses of ethanol are able to induce significant changes in the neuromuscular function. In our present study, the effect of low but sustained (10–30 days) ethanol blood levels on the rat peroneal nerve-tibial muscle preparation has been explored. In such preparation we have studied several parameters (twitch, train-of-four, tetanus and post-tetanic facilitation) each of them having a special significance in the evaluation of the neuromuscular function. In order to assess the possible involvement of postsynaptic cholinoceptors, the neuromuscular blocking activity of alcuronium (Aloferin, Roche), a short acting and negligibly metabolized compound (Raaflaub and Frey, 1972), was also tested in the presence of ethanol.

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MATERIAL AND METHODS

Animal treatments

Forty-two male Sprague–Dawley rats weighing 259 (SD 30.6) g were distributed in six groups as follows:

—Control (water as the only source of liquid).
—Et10 (ethanol 15% for 10 days, as the only source of liquid).
—Et30 (ethanol 15% for 30 days, as the only source of liquid).
—Alcuronium (as in control group, but treated with alcuronium).
—Al-Et10 (as in Et10, but treated with alcuronium).
—Al-Et30 (as in Et30, but treated with alcuronium).

Alcuronium (0.1 mg/ml) was injected through i.v., so as to block the twitch response at 25%, (0.18–0.24 mg/kg).

The 15% v/v concentration of ethanol used is the maximum tolerated by the animals without causing a significant decrease in the volume of fluid ingested (Thorpe and Shorey, 1966). Animals were placed in individual cages and in groups Et10, Et30, Al-Et10 and Al-Et30 the intake of ethanol was measured. Ethanol solutions were prepared daily.

Experiment set up.

Under halothane (Halothan, Hoechst®) anaesthesia, the trachea, one common carotid and one external jugular vein were cannulated. The tracheal cannula was used for both maintenance of anesthesia and ventilation of the animals by means of a Harvard rodent respiration pump which delivered 2 ml of 1% halothane in O_2 mixture per breath, at a frequency of 70 beats $min.^{-1}$ The arterial catheter was employed for monitoring blood pressure, heart rate and extraction of blood samples for analytical determinations. The vein cathether was used for injecting alcuronium. The rectal temperature was maintained at a constant level (37.5 ± 0.5°C) by radiant heat.

The peroneal nerve was dissected, severed and stimulated distally with supramaximal square pulses of 0.2 msec duration, generated by a Grass S48 stimulator. A SIU5

stimulus isolation unit and a bipolar platinum electrode were used. The tibialis anterior muscle was detached from its insertion, freed from the surrounding tendinous sheath and attached to a force displacement transducer by silk thread. A baseline tension of 10 g was applied and the contraction force was recorded on a Beckman multichannel recorder.

Experimental design

Individual muscle contractions were evoked by stimulating the nerve at 0.1 Hz continously, trains of four twitches (T_4) were elicited at 2 Hz for 2 sec. Tetanus was elicited by trains of stimuli at 50 Hz for 5 sec. Approximately 10 sec elapsed before the stimulation frequency was changed from twitch to T_4 to tetanus and from tetanus to post-tetanic twitches.

A tetanus was first applied to set the preparation and it was allowed to stabilize for 15 min. Each experiment consisted of 5 runs of tests performed at 0, 15, 30, 45 and 60 min after stabilization. Each run consisted of individual twitches, T_4, tetanus and post-tetanic twitches. Twitching was continued at 0.1 Hz throughout the whole of the experiment, and interrupted only for the runs.

Blood gases were determined (Radiometer, ABL2) just after stabilization as well as at the end of the experiments. Likewise, ethanol and halothane blood levels were determined by gas chromatography at the end of the experiments.

Six habitually used parameters (see Lee *et al.*, 1976) were obtained and defined as follows:

(A) Twitch: the twitch height.

(B) Train-of-four fade: the difference between the fourth twitch and the first twitch. It was introduced in clinical practice by Ali *et al.*, 1970, 1971a, b) and measures the effect of 3 previous conditioning twitches on a fourth one.

(C) Tetanic peak: the instantaneous tetanic force before fade. It represents the muscle power which can be generated instantaneously on initiation of a tetanic train of shocks to the nerve.

(D) Sustained tetanus: the residual tetanic force exhibited by the muscle at the end of 5 sec. It measures the force sustainable by the neuromuscular system.

(E) Tetanic fade: the difference between the peak of the tetanus and the sustained residue.

(F) Post-tetanic facilitation: the ratio of the first post-tetanic twitch to the pre-tetanic one. It measures the residual effect of the tetanizing shocks.

Parmeters A, C and D were expressed as % variations as compared to the last stabilization twitch. Parameter B was expressed as % decrease of the fourth twitch as compared to the first within each run. Parameter E was expressed as % variation of the sustained tetanus to the tetanic peak within each run. Finally, parameter F was expressed as a ratio (the post/pre-tetanic ratio within each run). Figure 1 illustrates diagramatically 2 tests carried out in control and alcuronium-blocked conditions.

Statistical analysis

Analysis of variance and Student's t-test of the results were carried out. Differences with $P \leqslant 0.05$ were accepted as significant.

RESULTS

The ethanol consumption on the 10th and 30th days (just at the end of the ethanol exposure periods) was 7.1 g/kg and 8.5 g/kg for the 10 and 30 days groups, respectively. The ethanol blood levels were (means $\pm$ SE) 3610 ± 467 μg/dl in Et10 and Al-Et10 groups, and 76 ± 11 μg/dl in Et30 and Al-Et30 groups. The halothane blood levels were 23.58 ± 1.71 mg/dl. Blood gas levels stayed within

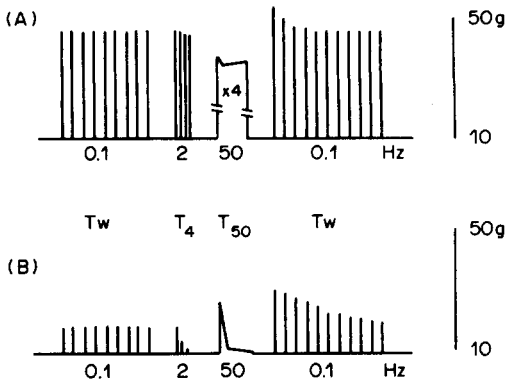

Fig. 1. Diagrammatic representation of muscle contractions of the rat peroneal nerve-tibial muscle preparation evoked by 2 runs of electrical stimulation tests, in control (A) and alcuronium blocked (B) conditions. Tw: Twitch. T_4: Train-of-four. T_{50}: Tetanus.

normal limits through the experiment and the same can be said for arterial pressure and heart rate, except for a slight and short-lasting hypotension and bradycardia observed after alcuronium administration.

Control rats

In Fig. 2 the time-course of the parameters evaluated in the different groups is shown. A remarkable stability in the values of all parameters in control group (without ethanol and alcuronium) was noticeable (closed circles in A–F, Fig. 2). By considering the time course of the parameters in the alcuronium group, it can be observed that after blockade the recovery was faster in twitch than T_4 and T_4 faster than tetanic peak and sustained tetanus (closed circles in A′, B′, C′ and D′, Fig. 2).

The time-course of the post-tetanic facilitation (closed circles in F′, Fig. 2) deserve further commentary, as after the initial blockade this parameter fell by 15 min and then a progresive recovery was observed. This fact was due to the fast recovery seen in the twitch that mask the time-course of the post-tetanic facilitation.

Ethanol fed rats

As shown in Fig. 2, in ethanol fed rats a steady decrease in the twitch (A), tetanic peak (C) and sustained tetanus (D) was evident throughout the experiments. Such effect was greater in Et10 group (closed triangles in Fig. 2) than in Et30 group (closed squares in Fig. 2). Slight differences in train-of-four fade were observed between control animals and those feeding on ethanol. No differences were noticeable in tetanic fade.

A progressive increase throughout the experiment in the post-tetanic facilitation was evident in Et30 (closed squares in F, Fig. 2).

Alcuronium treated ethanol fed rats

After alcuronium blockade the time course of the twitch (A′), train-of-four fade (B′), tetanic peak (C′) and sustained tetanus (D′) recovery was similar in all groups (Fig. 2), although it was somewhat faster in Al-Et10 and Al-Et30 groups, being specially evident in the recovery of sustained tetanus in Al-Et30 group

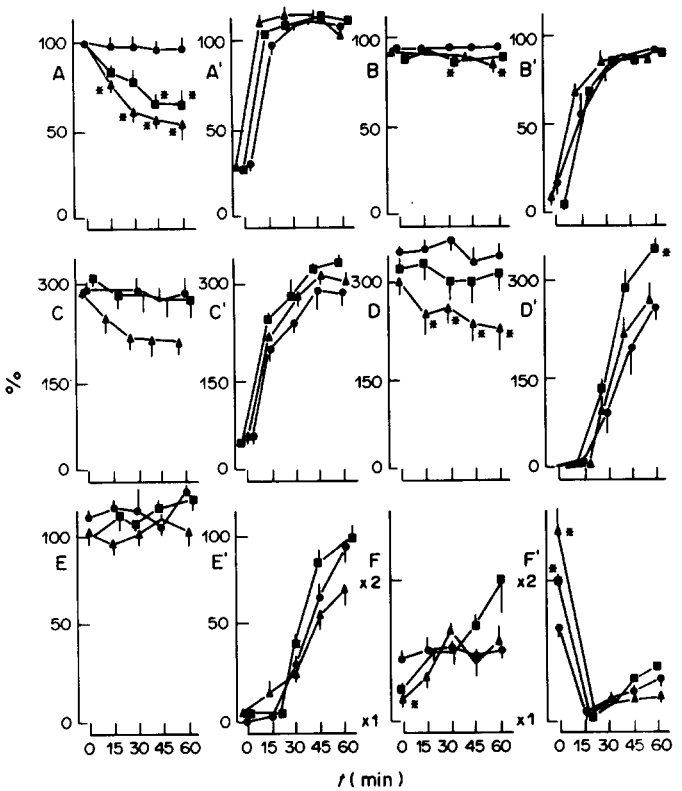

Fig. 2. Time-course of the parameters used to evaluate the neuromuscular function, corresponding A–F to groups control, Et10 and Et30, and A′–F′ to groups alcuronium, Al-Et10 and Al-Et30. (●) ethanol free, (▲) ethanol fed for 10 days and ethanol (■) fed for 30 days. Each point is expressed as mean ± SE $n = 7$. $^*P \leqslant 0.05$ as compared to control values in groups control and alcuronium.

(closed squares in D′, Fig. 2). Post-tetanic facilitation at O time was higher in both ethanol groups (closed triangles and squares in F′, Fig. 2) as compared to the alcuronium group (closed circles in F′, Fig. 2).

Effect of ethanol on absolute twitch tension

Focusing on more relevant results, a summary of the absolute twitch tensions is shown in Fig. 3, in which a significant difference between the initial and final values in ethanol fed rats may be seen. Such a difference was not observed, however, when alcuronium was administered in spite of the presence of ethanol (Al-Et10 and Al-Et30 groups).

DISCUSSION

This study shows 2 main observations: first, a progresive depression in all the parameters, except for tetanic fade and post tetanic facilitation, was observed in animals feeding on ethanol during 10 and 30 days; and second, after alcuronium blockade recovery the depressant effects observed in ethanol fed rats were reversed. Focusing on the first observation, the effect was more marked in animals feeding on ethanol for 10 than 30 days. This fact seems to point towards the development of a certain grade of tolerance to the ethanol depressant effects. In fact, by considering the ethanol blood levels, a metabolic tolerance may be seen.

Although aparently there are great differences between ethanol levels in both groups 10 and 30 days they are not correlated with the depressent effect of ethanol on neuromuscular function.

The progressive depression observed throughout the experiments in twitch and tetanus suggest a postjunctional effect of ethanol. So, according to

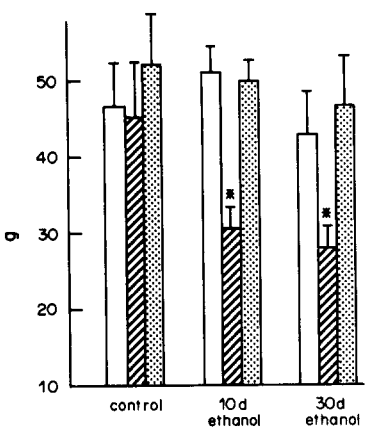

Fig. 3. Absolute twitch tensions expressed in grams, at the start and at the end of the experiments in the different groups. White bars: start values. Black bars: final values. Pointed bars: final values in alcuronium treated groups. Each bar represents the mean ± SE $n = 7$. $^*P \leqslant 0.05$ as compared to starting values in control, Et10 and Et30 groups.

Bowman and Webb (1976), the depression of both twitch and peak tetanic tensions observed during the neuromuscular block is a consequence of blockade of postjunctional cholinoceptors. Our results show that these 2 parameters were depressed in animals feeding on ethanol during 10 days. The absence of effect upon tetanic fade would rule out a prejunctional effect of ethanol since tetanic fade can be interpreted as a failure of the motor ending to maintain supply of acetylcholine to sustain a tetanic contraction. On the other hand, the effects observed could also be due to a decrease in muscle contractility, as pointed out by Gage (1965), who found, as in our case, a greater depression in twitch than in tetanic peak tensions in curarized and ethanol-treated preparation in which the muscle was directly stimulated. This author explained his findings by a reduction of the degree of activation of individual muscle fibers following a single stimulus.

The slight decrease of train-of-four fade and the increase of post-tetanic facilitation observed throughout the experiments in animals taking ethanol shows the development of a slight non-depolarizing block. Nevertheless, taken as a whole, the effects observed cannot be explained by a non-depolarizing blockade, since the parameters with a lower safety factor (tetanus and train-of-four fade) should be effected earlier. A reduction either in the number of cholinoceptors or in the apparent dissociation constant for acetylcholine, as has been shown by Bradley *et al.* (1984) for high and low ethanol concentrations, respectively, is not a satisfactory explanation for the larger depression in twitch than in tetanic peak. Furthermore, the alcuronium doses necessary for blocking the twitch by 75% were not different in animals taking ethanol or not, which seems to exclude a receptor change produced by low ethanol blood levels.

By considering the time-course of the parameters after alcuronium block (A′–F′ in Fig. 2) we observed that their recovery was similar in all groups. This means that low and sustained ethanol blood levels do not affect the recovery from alcuronium block. But, on the other hand, this is an unexpected finding since the recovery of each parameter after neuromuscular block in ethanol fed rats should not have overtaken the values obtained in those groups without alcuronium block (A–F in Fig. 2 and Fig. 3). One could think that this antagonizing effect was due to the transitory resting period imposed on the preparation as a consequence of the neuromuscular block. In this case, however, a further decrease of the parameters would be expected once the alcuronium block had ended, a fact which was not observed. The reversal after alcuronium block of the depressant effects observed in ethanol-fed rats seems to point towards an interaction between these 2 very dissimilar drugs, a phenomenon that cannot, at this moment, be satisfactorily explained.

In conclusion, our results show that low and sustained blood levels of ethanol cause a depression of neuromuscular function in rats. Such depression, elicited by continued nerve stimulation, could be related to an action of ethanol at a postjunctional non-receptorial site.

SUMMARY

The effect of ethanol intake that produced low (4 mg/dl) but sustained ethanol blood levels during 10 and 30 days, on neuromuscular function has been studied by using a rat tibial muscle preparation. The time-course of single twitches, trains-of-four, tetanus and post-tetanic facilitation was evaluated either in the presence or in the absence of alcuronium. In ethanol-fed rats, a progressive decrease in the majority of the parameters studied was observed; this decrease was more pronounced in rats fed on ethanol during 10 days than those fed during 30 days, the twitch being more affected than other parameters. After alcuronium-block recovery the depressant effects observed in ethanol fed rats were reversed. It is concluded that ethanol, even at low but sustained blood levels, causes alterations in neuromuscular function in rats. Such effects may be due to an action of ethanol at postjunctional site.

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