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Ethanol Withdrawal Tremor does not Interact With Physostigmine-Induced Tremor in Rat

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GOTHONI, P. *Ethanol withdrawal tremor does not interact with physostigmine-induced tremor in rat.* PHARMACOL BIOCHEM BEHAV **23**(3) 339–344, 1985.—Tremor in rats withdrawn from repeated ethanol administration was analyzed using an electronic device. The ethanol withdrawal tremor appeared in bursts during the first and second day of withdrawal and subsided at the third day of withdrawal. The frequency analysis showed that the mean frequency of withdrawal tremor was 6–7 Hz during the 48 hr observation period used. The frequency spectra of tremor induced by physostigmine (0.7 or 0.9 mg/kg) in control rats revealed that the tremoring frequency encompassed only a narrow peak, which temporarily decreased from 13 Hz to 11 Hz during the tremoring period. Arecoline (25 mg/kg) also induced tremor with a peak frequency at 13 Hz, but this tremor did not show any temporary decrease in peak frequency. The frequency analysis of tremor in ethanol withdrawn rats treated with physostigmine showed that the rats trembled at two frequencies, 6–7 Hz and 13 Hz. These two frequencies, each characteristic for one of the treatments, remained separate during the 48 hr observation period. As these two tremors did not interact with each other, it is suggested that these tremors are mediated by different mechanisms in the central nervous system. Thus it seems unlikely that the central muscarinic cholinergic system is involved in the genesis of tremor during ethanol withdrawal.

Ethanol withdrawal tremor Physostigmine Arecoline Frequency analysis

TREMOR is a well-known sign of the withdrawal state in man following repeated or prolonged ingestion of significant amounts of ethanol. Most of the available animal models for inducing physical dependence on ethanol have shown tremor to occur also in rats on abrupt cessation of repeated ethanol administration [1, 5, 9, 12, 13, 16]. However, in these reports it is only mentioned that tremor is one sign of ethanol withdrawal without any detailed analysis of it. Volicer and Hurter have evaluated the ethanol withdrawal tremor by counting the number of rats showing tremor on an all-or-nothing basis [15].

The present experiments were designed to analyze in more detail the ethanol-withdrawal tremor by using an electronic device for measuring tremor in rats [6]. Physostigmine was used in part of the experiments because central cholinergic mechanisms have been shown to be involved in the genesis of different types of tremor [2,3].

METHOD

Animals

The experiments were performed on male Wistar rats (body weight 270–350 g), which were given standard laboratory pellets and water ad lib until used. The animals were kept on a 12 hr light, 12 hr dark cycle at 22–24°C.

Measurement of Tremor

Tremor activity was measured with an electronic device as described by Gothóni *et al.* [6]. Power spectra of the tremor frequencies were obtained by processing the amplified signal with a microcomputer on-line system [11]. The analog to digital conversion was carried out by an 8-bit A/D-converter and the microcomputer (Rockwell AIM-65 with a memory size of 36 Kb) was programmed to perform spectral analysis in real-time. Tremor activity was sampled at the rate 128/sec for sample period of two-second duration. Thus the available frequency range is 0–64 Hz of which a range of 0–40 Hz was used. The power spectrum was computed for each sample period of 256 points by squaring the components of its fast Fourier transform. Spectra for consecutive two-second sample periods were summed continuously for 40 seconds to produce the final average power spectrum with a resolution of 0.5 Hz. The tremor intensity is given in units of milliwatt-gram (mWg) of the cumulative tremor power during an 8-min period. The means±SEM and the procentual distribution of the peak frequencies (i.e., the frequencies with the highest tremor power) in the spectra were determined for each treatment.

Ethanol Withdrawal Experiments

In order to induce physical dependence upon ethanol the rats were intubated intragastrically with 10% (w/v) ethanol solution 3 times daily, at 8 a.m., 3 p.m. and 8 p.m., for 6–9 days [16]. The rats were weighed daily and the ethanol dose was calculated according to the body weight. The daily dose of ethanol was 6–9 g/kg. Control rats were intubated with similar vol-

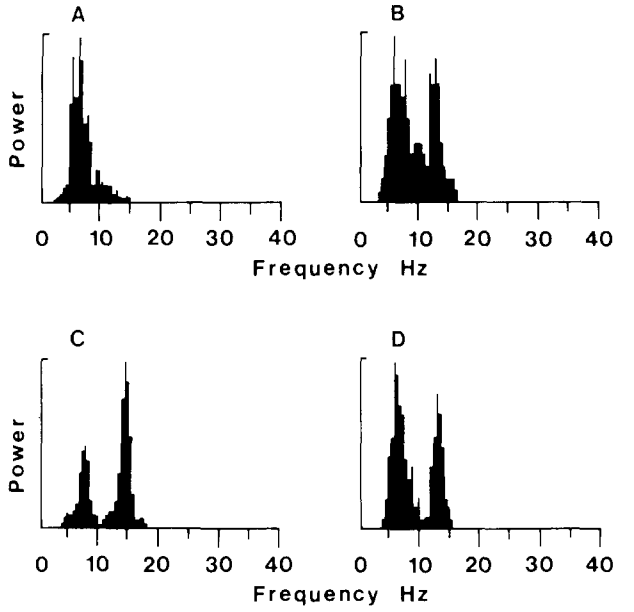

FIG. 1. Frequency spectra of different tremors as characteristic sample recordings of a 40 sec measuring period. A. Ethanol withdrawal tremor (16 hr after the last administration of ethanol); B. Physostigmine 0.7 mg/kg administered in the morning on the 6th day of the intubation period before the next ethanol dose was administered (12 hr after the previous ethanol intubation); C. Physostigmine 0.7 mg/kg at 24 hr after ethanol withdrawal; and D. Physostigmine 0.7 mg/kg at 48 hr after ethanol withdrawal. The samples of physostigmine treatments were recorded 4 min after the injection of physostigmine.

umes of tap water. The rats were housed one to a cage (similar to the transducer cage) on a standard laboratory diet and water ad lib under a lighting schedule with lights on from 7 a.m. to 7 p.m.

The withdrawal tremor was measured during 16–48 hr after the last administration of ethanol (at 8 p.m., 3–4 g/kg). Each measurement period lasted 20–30 min and was repeated 3 to 4 times (see below drug experiments and Fig. 2). The rats withdrawn from ethanol were tested with physostigmine as described below (drug experiments) twice with 24 hr intervals.

Drug Experiments

Control rats given tap water repeatedly were randomly injected IP with physostigmine in doses of 0.7 and 0.9 mg/kg, arecoline 25 mg/kg or the corresponding amount of 0.9% NaCl solution. Atropine and methylatropine were administered SC 10 min before the tremorogenic drug.

During the experiments the rats were deprived of food and water. The rats were familiarized with the new environment (transducer cage) for a period of 5 min before the tremorogenic drug was injected. The recordings started 2 min after the injection of the tremorogenic drug. Each control rat was used 2–3 times in the experiments with 24 hr intervals. The tremor apparatus was placed in a quiet, artificially lighted room at 22–24°C. The experiments were started at 8.30–9.00 a.m. and continued for 6 hr.

Statistical significance of difference between the means was calculated by Student's *t*-test.

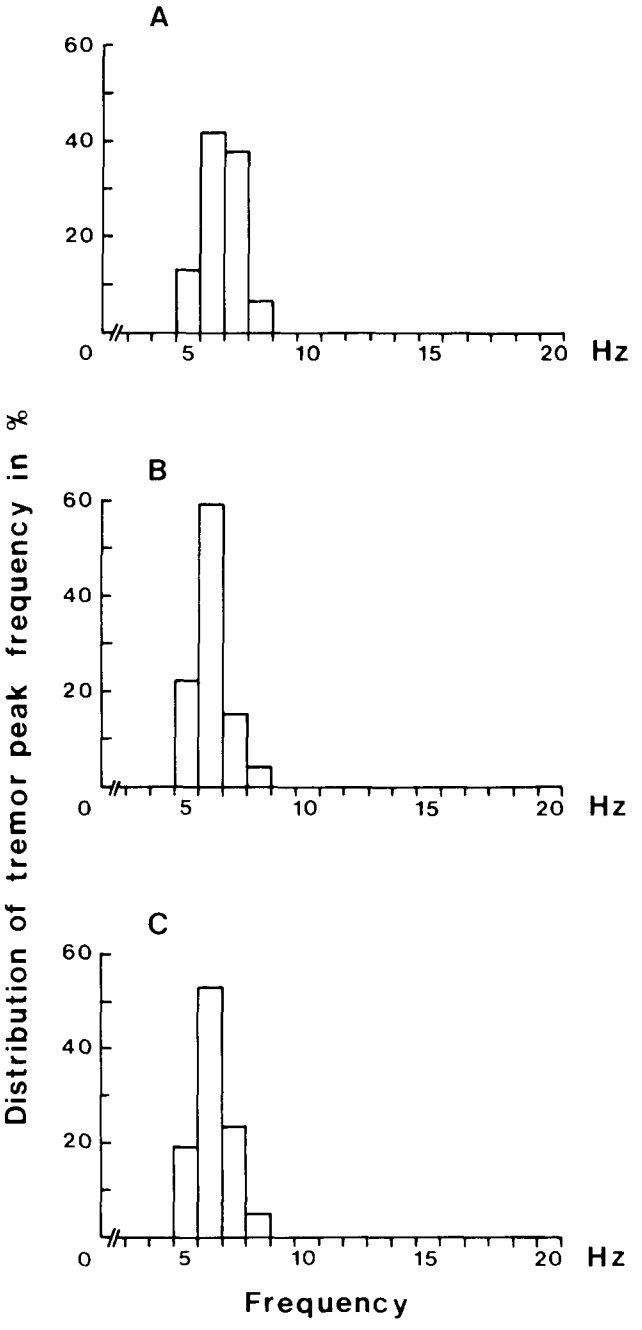

FIG. 2. Distribution of the peak frequencies in the frequency spectra of ethanol withdrawal tremor. A. 16–24 hr after the last administration of ethanol; B. 36–48 hr after the last administration of ethanol and C. 16–48 hr after the last administration of ethanol. The distributions were calculated on the basis of 123 spectra, collected from 20 rats.

Drugs

Physostigmine salicylate (Ph.Nord.), arecoline hydrobromide (Sigma, USA), atropine sulphate (Ph.Nord.) and methylatropine bromide (Ph.Nord.) were dissolved in 0.9% NaCl solution and administered in a volume of 1 ml/kg. Drugs doses are given as the base.

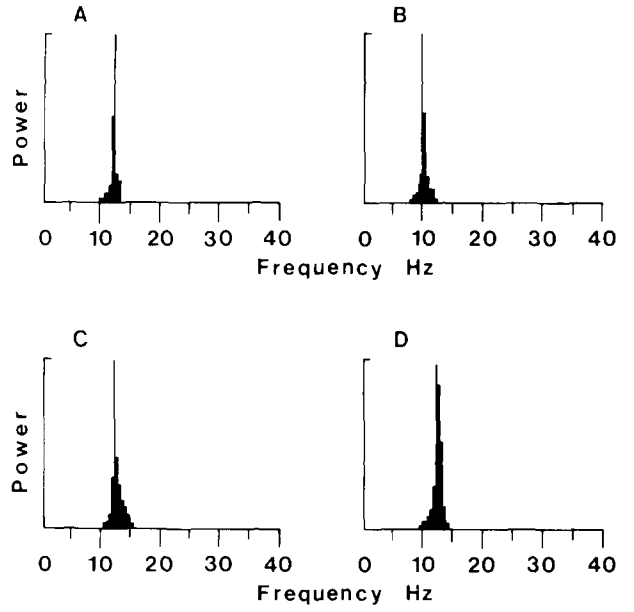

FIG. 3. Frequency spectra of physostigmine-induced tremors as characteristic sample recordings of a 40 sec measuring period. A. Physostigmine 0.9 mg/kg, recorded 4 min after injection; B. Physostigmine 0.9 mg/kg, recorded 6 min after injection; C. Atropine 1.2 mg/kg 10 min before physostigmine 2.5 mg/kg, recorded 4 min after physostigmine injection and D. methylatropine 0.3 mg/kg 10 min before physostigmine 0.9 mg/kg, recorded 4 min after physostigmine injection.

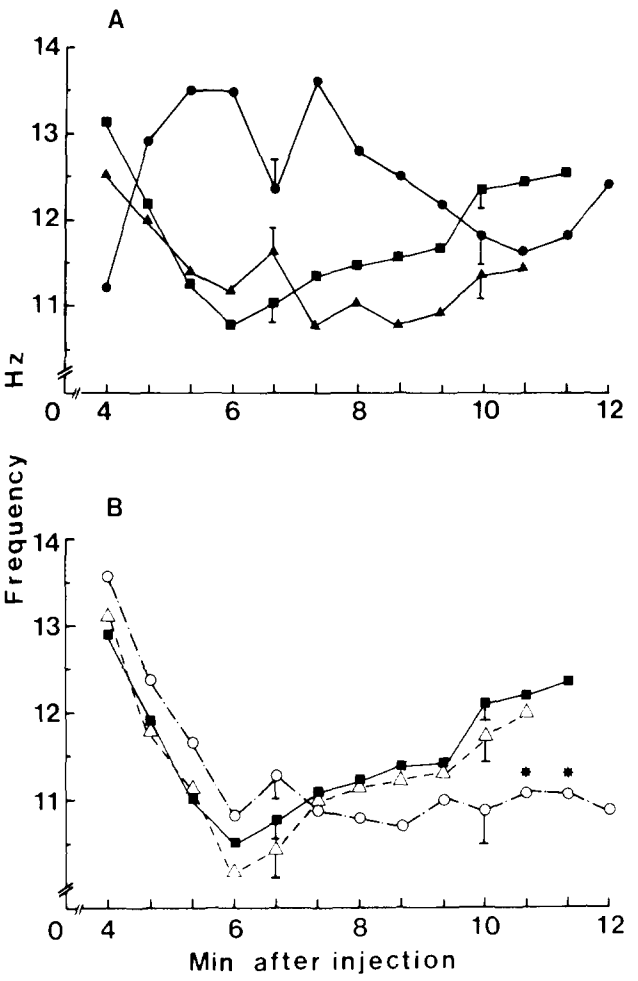

FIG. 4. Time course of the physostigmine-induced tremoring frequency. A. Closed squares: physostigmine 0.9 mg/kg; closed triangles: physostigmine 0.7 mg/kg and closed circles: methylatropine 0.3 mg/kg 10 min before arecoline 25 mg/kg; B. Closed squares: physostigmine 0.9 mg/kg; open triangles: atropine 1.2 mg/kg 10 min before physostigmine 2.5 mg/kg and open circles: methylatropine 0.3 mg/kg 10 min before physostigmine 0.9 mg/kg. The vertical bars show the SEM from 9–11 rats. The asterisks in B indicate significant differences ($*p<0.05$) from physostigmine control values.

RESULTS

Ethanol Withdrawal Tremor

Already after five days of repeated ethanol administration, the rats showed withdrawal signs after the longest interval before the next ethanol administration (12 hr, between 8 p.m. and 8 a.m.). The earliest signs of withdrawal consisted of tremor and stiffness of the tail. When the rats were withdrawn from ethanol, signs as general tremor, spasticity, aggressive behaviour and squealing on touching appeared. Rats with a fully developed withdrawal syndrome remained in a characteristic posture with paws spread out and with tremor in bursts.

The rats withdrawn from ethanol started to tremble in bursts at about 16 hours after the last administration of ethanol. These bursts of tremor continued to appear on the second day of withdrawal, but subsided on the third day of withdrawal. Thus the observation period of 16–48 hr after the last administration of ethanol was chosen for the tremor experiments.

Figure 1A shows a typical sample recording of tremor frequency spectra from one rat withdrawn from ethanol. The withdrawal tremor shows a relatively broad peak frequency at about 6–7 Hz. This peak frequency was the same during the first and second day of withdrawal from ethanol (Fig. 2) and was the same in all rats. The intensity of ethanol withdrawal tremor could not be determined, since the tremor was not continuous but appeared in bursts.

The ethanol treated rats lost about 20% of their weight during the treatment, whereas the weights of the control rats did not change or slightly increased. During the repeated ethanol treatment 15–20% of the rats died. The deaths were most often due to overdosage of ethanol, because those rats which died, were still heavily intoxicated during the last ethanol intubation. On the other hand, large enough ethanol doses had to be given in order to induce physical dependence and withdrawal tremor. Therefore these deaths could not be avoided.

Physostigmine-Induced Tremor

At the beginning of the tremor period the physostigmine-induced tremor consisted characteristically of a narrow peak frequency at about 13 Hz (Fig. 3A). However, this peak frequency was dependent on the duration of time. It decreased significantly ($p<0.01$) from 13 Hz to 11 Hz causing a parabolic shape to the curve (Fig. 4A). Although the peak frequency changed, the peak was very narrow (Fig. 3B). The change in peak frequency was most clear with the dose 0.9

mg/kg of physostigmine (Fig. 4A). Atropine in a dose (1.2 mg/kg) which clearly antagonized the intensity of the physostigmine-induced tremor neither broadened the peak frequency (Fig. 3C) nor changed the parabolic shape of the curve (Fig. 4B), when the physostigmine dose was increased to 2.5 mg/kg in order to induce tremor in the rats. Methylatropine in a dose (0.3 mg/kg) which did not antagonize the physostigmine-induced tremor did not either change the peak frequency of physostigmine-induced tremor, when the tremor started (Fig. 3D). But it did change the parabolic shape of the frequency-time curve by significantly inhibiting the increase in peak frequency 10 min after physostigmine injection (Fig. 4B).

Arecoline, 25 mg/kg, produced clear parasympathetic signs but not tremor. However, if the rats were pretreated with methylatropine 0.3 mg/kg before arecoline (25 mg/kg), these parasympathetic signs were clearly less severe and the rats trembled. This arecoline-induced tremor showed in the frequency spectra a peak frequency at about 13 Hz, which did not temporarily decrease as that of physostigmine-induced tremor (Fig. 4A).

Physostigmine to Ethanol-Withdrawn Rats

When physostigmine was administered IP to rats withdrawn from ethanol, there appeared two different peak frequencies in the frequency spectra (Fig. 1C and D). The lower peak frequency at about 6 Hz is similar to that of ethanol withdrawal tremor and the higher peak frequency at 13–14 Hz is similar to that of physostigmine-induced tremor. These two peak frequencies appeared in the frequency spectra as soon as the ethanol withdrawal tremor was observed. Thus already when the rats treated five days with ethanol were tested with physostigmine in the morning 12 hr after the previous ethanol intubation, the two peak frequencies were observed (Fig. 1B). These two peak frequencies appeared also on the second day of withdrawal, when the rats began to recover from the withdrawal syndrome (Fig. 1D).

By observing the rats the ethanol-withdrawal and the physostigmine-induced tremors could not be differentiated. Furthermore, when the frequency spectra revealed the two different peak frequencies these could not be verified visually.

Similar to the peak frequency of tremor induced by physostigmine (0.7 mg/kg) alone the higher of the two tremor peak frequencies induced in ethanol-withdrawn rats decreased with time from 14 Hz (at 4 min) to 12.5 Hz (at 8 min), but this frequency was significantly higher than that induced by physostigmine alone (Fig. 5A). The peak frequency of tremor induced by 0.9 mg/kg physostigmine in ethanol-withdrawn rats was also significantly higher than that of the tremor induced by physostigmine alone (Fig. 5B). However, when the physostigmine dose was 0.9 mg/kg, the higher peak frequency did not follow a parabolic shape (Fig. 5B). Furthermore, 0.9 mg/kg of physostigmine was too large a dose to administer to rats withdrawn from ethanol because 8 out of 13 rats died during this treatment.

Atropine, 1.2 mg/kg, and methylatropine, 0.3 mg/kg, did not change the peak frequency of ethanol withdrawal tremor (data not shown). In rats withdrawn from ethanol atropine, 1.2 mg/kg, neither changed the frequency of physostigmine-induced tremor (Fig. 5B) nor reduced its intensity (Fig. 6) but atropine protected the rats from death. Methylatropine, 0.3 mg/kg, did not change the tremor frequency response of the ethanol-withdrawn rats to physostigmine. So that, this fre-

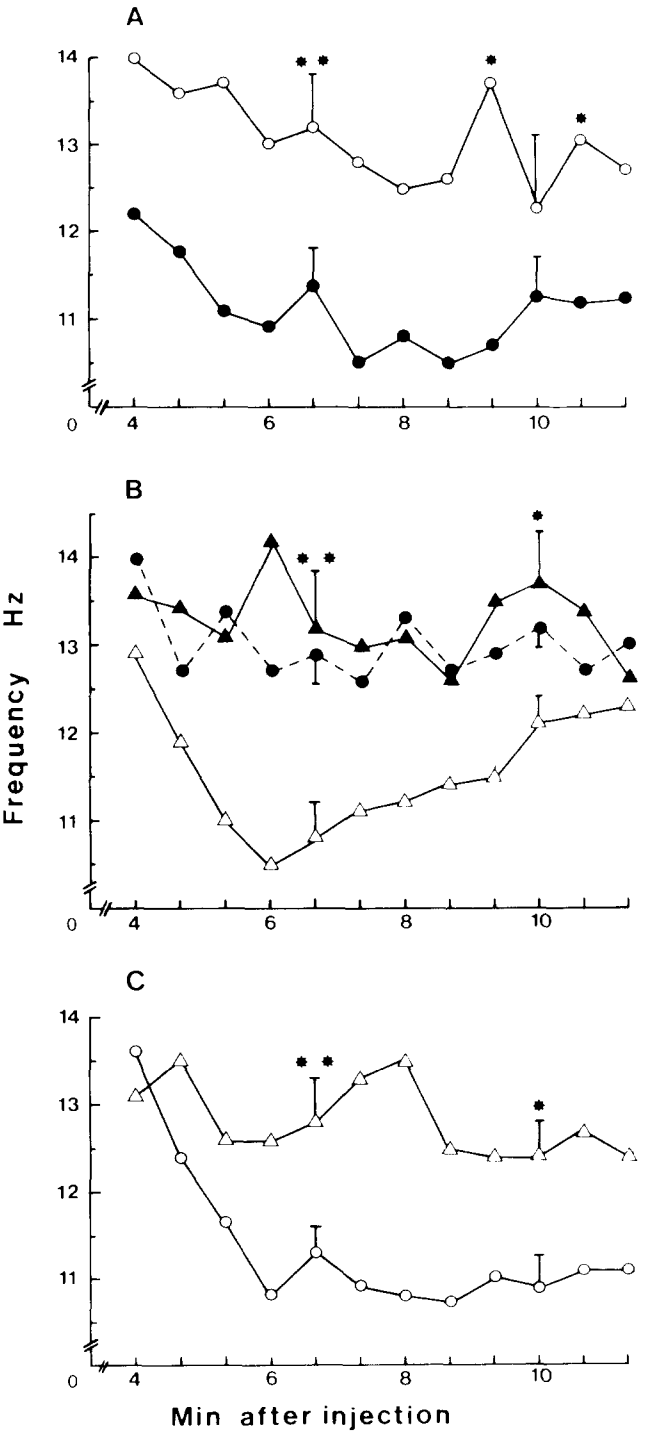

FIG. 5. Time course of the physostigmine-induced tremoring frequency in ethanol withdrawn rats. A. Closed circles: physostigmine 0.7 mg/kg and open circles: physostigmine 0.7 mg/kg during ethanol withdrawal; B. Open triangles: physostigmine 0.9 mg/kg; closed triangles: physostigmine 0.9 mg/kg during ethanol withdrawal and closed circles: atropine 1.2 mg/kg 10 min before physostigmine 0.9 mg/kg during ethanol withdrawal; C. Open circles: methylatropine 0.3 mg/kg 10 min before physostigmine 0.9 mg/kg and open triangles: methylatropine 0.3 mg/kg 10 min before physostigmine 0.9 mg/kg during ethanol withdrawal. The vertical bars show the SEM from 9–14 rats. The asterisks indicate significant differences ($*p<0.05$, $**p<0.01$) from physostigmine control values.

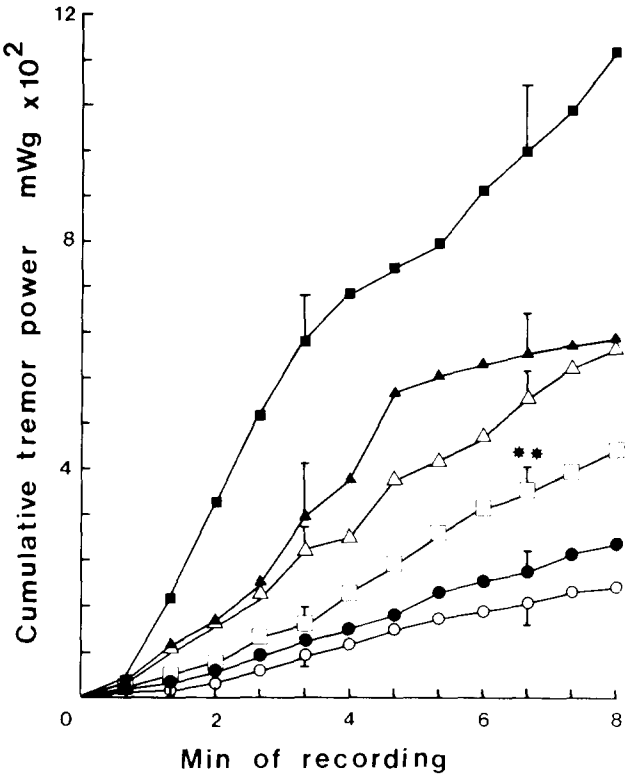

FIG. 6. Tremor intensities in rats after ethanol withdrawal and physostigmine treatments. Closed squares: physostigmine 0.9 mg/kg; closed triangles: physostigmine 0.7 mg/kg; open triangles: physostigmine 0.7 mg/kg during ethanol withdrawal; open squares: methylatropine 0.3 mg/kg 10 min before physostigmine 0.9 mg/kg during ethanol withdrawal; closed circles; atropine 1.2 mg/kg 10 min before physostigmine 0.9 mg/kg during ethanol withdrawal and open circles: physostigmine 0.9 mg/kg during ethanol withdrawal. The vertical bars show the SEM from 9–14 rats. The asterisks indicate significant differences (**$p<0.01$) from physostigmine 0.9 mg/kg during ethanol withdrawal.

quency was significantly higher in ethanol-withdrawn rats treated with physostigmine + methylatropine than in correspondingly treated control rats (Fig. 5C).

The cumulative tremor powers for different physostigmine treatments are shown in Fig. 6. Ethanol withdrawal did not change the intensity of 0.7 mg/kg of physostigmine-induced tremor, but tended to prolong its duration from about 6 min to about 10 min. However, ethanol withdrawal decreased significantly the intensity of the tremor induced by 0.9 mg/kg of physostigmine. Atropine, 1.2 mg/kg, did not alter the intensity of this tremor. However, methylatropine, 0.3 mg/kg, significantly ($p<0.01$) increased it.

DISCUSSION

Rats withdrawn from 6–9-day repeated ethanol administration showed withdrawal signs during 16–48 hr after the last administration of ethanol, which is in accordance with the findings of Majchrowicz [12]. Tremor was not continuous but appeared in bursts and was the first sign to emerge and the last to disappear when the rat recovered from the withdrawal syndrome. The frequency analysis of tremor during ethanol withdrawal shows that the tremor power was typically concentrated in the frequency spectrum of 6–7 Hz in all 20 rats studied. This frequency range remained the same during the withdrawal period studied. Interestingly, Zilm *et al.* [17] have reported a similar frequency range for the tremor of chronic alcoholic patients in withdrawal state. Thus, the rat seems to be a suitable animal for studying the mechanism of tremor during ethanol withdrawal.

The frequency analysis of physostigmine-induced tremor shows that the tremoring frequency encompassed only a narrow peak, which temporarily decreased from 13 Hz to 11 Hz during the tremoring period. This temporary diminution of the peak frequency was not altered by atropine, although atropine significantly decreases the intensity of physostigmine-induced tremor [6,7]. However, methylatropine inhibited the late increase of the peak frequency from 11 Hz to 13 Hz, and we earlier reported that methylatropine significantly potentiates physostigmine-induced tremor [6]. Arecoline-induced tremor could be observed only in rats pretreated with methylatropine, which lessened peripheral parasympathetic signs. This finding indicates that the arecoline-induced tremor is of central origin and is unmasked by prevention of peripheral parasympathetic signs. Moreover, the arecoline-induced tremor encompassed a peak frequency at 13 Hz, which did not temporarily decrease.

The temporary decrease in peak frequency of physostigmine-induced tremor occurred also in rats withdrawn from ethanol when the physostigmine dose was 0.7 mg/kg and ethanol withdrawal did not change the intensity of the tremor induced by this physostigmine dose. However, ethanol withdrawal decreased the intensity and prevented the temporary decrease in frequency of tremor induced by 0.9 mg/kg physostigmine—a dose which was clearly toxic to ethanol-withdrawn rats. Atropine protected the rats from death but it neither changed the intensity nor the frequency of the tremor induced by 0.9 mg/kg of physostigmine in ethanol-withdrawn rats. Thus these changes of intensity and frequency are most probably unspecific. Methylatropine did not either change the frequency of the tremor induced by 0.9 mg/kg of physostigmine in ethanol-withdrawn rats, although the frequency was at a significantly higher level than that in correspondingly treated control rats. However, methylatropine significantly increased the intensity of this tremor, which is in accordance with our earlier report that methylatropine significantly potentiates physostigmine-induced tremor possibly by preventing peripheral parasympathetic effects of physostigmine [6].

The frequency analysis of physostigmine-induced tremor during ethanol withdrawal showed that the characteristic tremor frequencies of the two treatments remained separate in the frequency spectrum. The only difference between control and ethanol-withdrawn rats treated with physostigmine was an increase in tremoring frequency in ethanol-withdrawn rats as compared to control rats. Thus it seems that the physostigmine-induced tremor does not interact with the ethanol withdrawal tremor. Furthermore, Goldstein [4] reported that physostigmine and atropine have no effect on the ethanol-withdrawal reaction in mice. Although Rawat [14] reported that cerebral acetylcholine concentration is decreased in mice during ethanol withdrawal, Hunt and Dalton [8] did not observe any changes in brain acetylcholine concentration of ethanol-withdrawn rats. Physostigmine-induced tremor has been proposed to be mediated mainly by central muscarinic cholinergic receptors [6,10]. Thus, on the basis of present results it seems unlikely that the central muscarinic cholinergic system is involved in the tremor of ethanol withdrawal.

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