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Drugs for Parkinson's Disease Reduce Tremor Induced by Physostigmine

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Summary. The effects of anticholinergic and dopaminergic drugs used for Parkinson's disease were studied on the tremor induced by physostigmine (0.3–3.0 mg/kg) in rats. For the measurement of tremor a new electronic device was employed. Atropine (0.3–1.2 mg/kg) and biperiden (0.01–1.0 mg/kg) reduced the physostigmine-induced tremor in a dose-related manner and could abolish it. Biperiden was less potent than atropine. Methylatropine in a dose of 1.2 mg/kg slightly inhibited the tremor. Amantadine (0.3–3.0 mg/kg) reduced the tremor but only to a certain degree. Bromocriptine (0.1–10.0 mg/kg) reduced it in a manner that was not dose-related. Pimozide potentiated the tremor in the dose of 0.2 mg/kg but not in larger doses.

At the onset of the tremor, a small decrease in rectal temperature occurred. The hypothermia lasted significantly longer than the tremor. Neither the anticholinergic nor the dopaminergic anti-Parkinson drugs altered the hypothermic effect of physostigmine.

The results show that those anti-Parkinson drugs, which act by increasing the dopaminergic activity can counteract the tremor induced by physostigmine. However, these drugs are clearly less active than the anticholinergic anti-Parkinson drugs.

Key words: Tremor – Physostigmine – Anti-Parkinson drugs – Hypothermia

Introduction

In the regulation of extrapyramidal function there is presumed to exist a sensitive balance between inhibitory dopaminergic and excitatory cholinergic neurones (eg. Duvoisin 1967; Hornykiewicz 1973). In Parkinson's disease, the striatal dopamine content is reduced; this results in a predominance of cholinergic activity (Hornykiewicz 1966). Accordingly, Parkinson's disease is treated either by suppressing cholinergic hyperactivity or enhancing dopaminergic transmission.

Physostigmine produces transient tremor in rats by increasing cholinergic activity in the brain (eg. Slater and Rogers 1968; Sethy and Van Woert 1973a). In patients with Parkinson's disease physostigmine aggravates tremor, rigidity and hypokinesia at a dose which has no neurological effect on normal subjects (Duvoisin 1967; Weintraub and Van Woert 1971). This aggravation of the symptoms is decreased or blocked by pretreatment with L-dihydroxyphenylalanine (L-dopa) (eg. Weintraub and Van Woert 1971). Moreover, in the spinal cord of rats L-dopa has been shown to antagonize the motor disturbance induced by physostigmine (Jurna et al. 1969). However, Sethy and Van Woert (1973b) found that L-dopa does not prevent physostigmine-induced tremor in rats. For these reasons it seemed appropiate to study in more detail the antitremor activity of certain anti-Parkinson drugs. A new electronic device was constructed in order to improve the recording of tremor.

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Methods

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

Measurement of Tremor. The intensity of tremor is interpreted as the total mechanical power required to maintain the vibrational motion, inferred from simple considerations of a vibrating system. The mechanical energy E is the sum of the momentary kinetic energy of the motion and the potential energy stored in the elastic muscles. During each cycle a proportion K of the energy is lost to friction. Power is lost by the rate $P_d = KfE$ during the vibrational motion of frequency f. This power must be supplied by the metabolic system of the animal to maintain the tremor at a constant energy (so-called loss power).

For sinusoidal motion with amplitude A, frequency f and vibrating mass m the loss power is given by

$$P_d = 2\pi^2 KmA^2 f^3. \quad (1)$$

Generally, vibrational motion is not sinusoidal, but it is possible to consider it as many sinusoids with different amplitudes and frequencies superimposed. The distribution of the tremor energy between different frequencies is described by a spectral density function $S(f)$ (Bendat and Piersol 1971; Blackman and Tukey 1958). In this case the formula (1) may be generalized to

$$P_d = 2\pi^2 K \int_{-\infty}^{\infty} m(f) f^3 \, S(f) df. \quad (2)$$

The objective of the measuring system is to yield values for the loss power obeying the formula (2) as closely as possible. For this purpose, the electronic device for measuring tremor in rats described by Gothóni et al. (1981) was modified. A piezoelectric acceleration sensor was chosen to measure the vibrations induced in an elastically supported animal cage. The output of the acceleration sensor was electrically amplified and filtered to get the proper overall frequency

response as expressed by the formula (2). The filtered signal was fed into a square-law detector, whose output was integrated over pre-set time intervals and recorded by a specially programmed tabletop computer capable to handle data from several channels simultaneously.

The output of the measuring system is given by the formula

$$o = \int_{-\infty}^{\infty} |H(f)|^2 \, S(f) \, df, \tag{3}$$

where $H(f)$ is the frequency response function of the system (Bendat and Piersol 1971; Blackman and Tukey 1958). The low-frequency slope of the H function mechanical resonance peak was adjusted to obey the formula

$$|H(f)|^2 = \text{constant} \cdot \left(\frac{m}{M}\right)^2 |f|^3 \tag{4}$$

in the frequency range of experimental tremor. By comparing the formulas (2) and (3) and supposing that a) the trembling mass m is approximately constant and b) the deviations of the total masses M in different animals can be neglected, the relation is obtained between the output of the measuring system and the loss power

$$o = QmP_d/K \quad \text{or} \quad P_d = \frac{1}{Q} Ko/m,$$

where Q is the system constant determined by calibration with help of a vibrating device whose characteristics are known. Thus, the mass of the trembling part of the animal could be established in relation to the output and the loss power of the system. This is why we express our results in units of one milliwatt-gram (mWg), and our figures actually represent the quantity mP_d/K, which we (erroneously, but for the sake of simplicity) call the tremor power.

Tremor Experiments. Double-blind crossover experiments were performed in which all doses of the drugs were randomly injected. The test substances were administered either 10 min (atropine, methylatropine, amantadine and biperiden), 15 min (bromocriptine) or 60 min (pimozide) before physostigmine. Atropine and methylatropine were injected s.c., while the other drugs were injected i.p. In control experiments, 0.9 % NaCl solution was administered 10, 15 or 60 min before physostigmine. In the figures the control curves are drawn as broken lines.

Between the tremor recordings the rats were kept in opaque plastic cages (20 · 35 · 15 cm, 2 rats in each) which were similar to the transducer cages. The rats were familiarized with the new environment (transducer cage) for a period of 5 min before physostigmine was injected. The intensity of tremor in Figs. 1 – 2 is given as the mean of the tremor power measured during a 20 min period starting from the injection of physostigmine. An example of the time course of the development of the tremor power is shown in Fig. 3A and/or B.

During the experiments the rats were deprived of food and water. Each rat was used 5 – 10 times in the experiments. The rats were allowed to rest at least 3 days between two experiments. The tremor apparatus was placed in a quiet, artificially lighted room at 22 – 24° C. The experiments were started at 8.30 – 9.00 AM and continued for 6 h.

Statistical significance of difference between the means was calculated by Student's t-test. The dose-response curves were tested by analysis of regression and correlation.

Body temperature was measured with an electric thermocouple (Ellab Instruments, Copenhagen, Denmark) inserted 4 cm into the rectum.

Drugs. Physostigmine salicylate (Ph.Nord.), atropine sulphate (Ph.Nord.), methylatropine bromide (Ph.Nord.) and amantadine hydrochloride (a gift from Orion Oy, Finland) were dissolved in 0.9 % NaCl solution. Biperiden (a gift from Knoll AG, FRG) was dissolved in 3 % lactic acid and adjusted to pH 5.5 with 2 N NaOH. 55.5 mg of bromocriptine methanesulphonate (a gift from Sandoz Ltd, Switzerland) was dissolved in few drops of 70 % ethanol and 3 % lactic acid and diluted with 5 % glucose solution. 10.0 mg of pimozide (a gift from Orion Oy, Finland) was dissolved in one drop of concentrated acetic acid, then diluted with water. Doses are given as the base and the drugs were administered in a volume of 1 ml/kg (except pimozide, which was given in a volume of 2 ml/kg).

Results

Physostigmine-induced tremor started within 3 – 5 min after injection, reached its highest intensity within 6 – 7 min and returned gradually to control values during the next 20 min (Fig. 3A or B). The intensity of the tremor increased with increasing doses of physostigmine (Figs. 1 and 2). Physostigmine in a dose of 1.0 mg/kg caused lethal convulsions instead of tremor as indicated in Figs. 1 and 2 by a vertical dotted line.

Preliminary experiments using wide dose ranges of atropine, methylatropine, biperiden, amantadine, bromocriptine and pimozide showed that these substances lacked tremorogenic activity except amantadine in doses larger than 70 mg/kg. On the basis of these experiments suitable doses and pretreatment times were selected for the studies of drug interaction. In order to obtain a tremor response it was necessary to increase the dose of physostigmine to 1.3 – 3.0 mg/kg in combination with atropine (Fig. 1A), biperiden (Fig. 1C), amantadine (Fig. 2A) and bromocriptine (Fig. 2B), because these drugs totally inhibited the tremorogenic effect of small physostigmine doses.

Atropine reduced the physostigmine-induced tremor in a dose-related manner (Fig. 1A) and protected the rats against the lethal dose of physostigmine. Rats treated wih atropine 1.2 mg/kg tolerated physostigmine in doses as high as 3.0 mg/kg. A small dose of methylatropine (0.3 mg/kg) significantly potentiated tremor activity (Fig. 1B). However, the potentiating effect of methylatropine diminished when its dose was increased, and in the dose of 1.2 mg/kg it slightly inhibited physostigmine-induced tremor. Biperiden dose-dependently reduced physostigmine-induced tremor (Fig. 1C). However, the dose of biperiden 1.0 mg/kg protected only 5 out of 10 rats against lethal convulsions caused by 1.7 mg/kg of physostigmine. Thus, the efficacy of biperiden in antagonizing the effect of physostigmine was less than that of atropine.

Amantadine significantly reduced tremor activity (Fig. 2A). However, amantadine in a dose of 3 mg/kg did not reduce the tremor more than in a dose of 1 mg/kg. Amantadine parallelly displaced the log dose-response curve of physostigmine to the right. Bromocriptine also antagonized significantly the tremor induced by physostigmine but this antagonism was not dose-related (Fig. 2B). Bromocriptine doses 0.1 – 10 mg/kg were all apparently equally

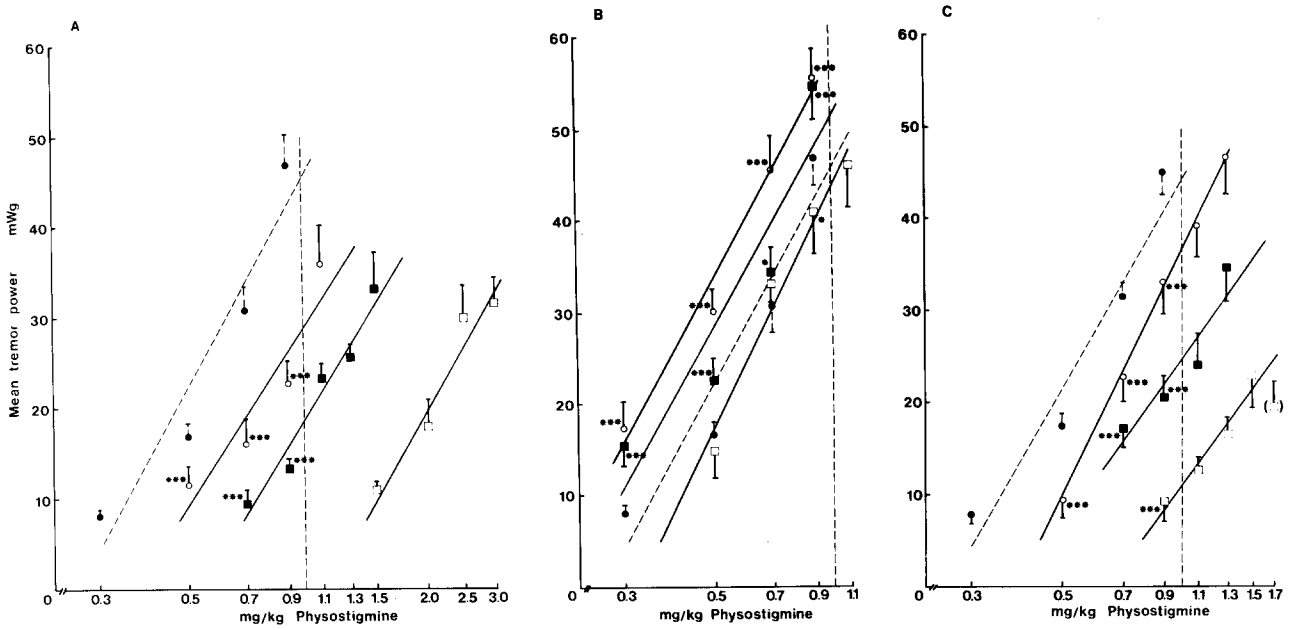

Fig. 1. The effects of atropine (**A**), methylatropine (**B**) and biperiden (**C**) on physostigmine-induced tremor. The mean tremor power in rats treated with physostigmine (0.3 – 0.9 mg/kg) alone is presented by broken lines and closed circles. The effect of drug pretreatment is shown by the curves with solid lines; (**A**) (○) atropine 0.3 mg/kg, (■) atropine 0.6 mg/kg and (□) atropine 1.2 mg/kg; (**B**) (○) methylatropine 0.3 mg/kg, (■) methylatropine 0.6 mg/kg and (□) methylatropine 1.2 mg/kg; (**C**) (○) biperiden 0.01 mg/kg, (■) biperiden 0.1 mg/kg and (□) biperiden 1.0 mg/kg. The parenthesis in (**C**) indicates that biperiden did not protect the animals against convulsions. The vertical broken lines indicate the lethal dose (1 mg/kg) of physostigmine, when administered alone. The vertical bars show the SEM from 9 to 13 rats. The asterisks indicate significant differences ($*P < 0.05$, $**P < 0.01$, $***P < 0.001$) from physostigmine control values

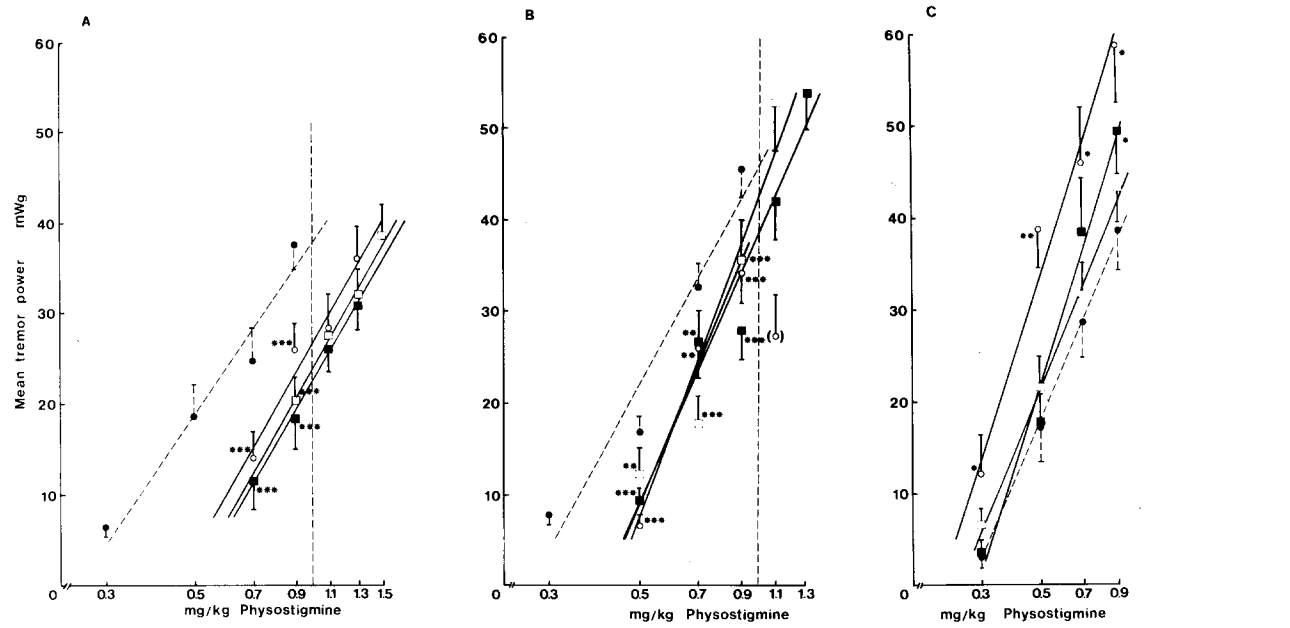

Fig. 2. The effects of amantadine (**A**), bromocriptine (**B**) and pimozide (**C**) on physostigmine-induced tremor. The mean tremor power in rats treated with physostigmine (0.3 – 0.9 mg/kg) alone is presented by broken lines and closed circles. The effect of drug pretreatment is shown by the curves with solid lines; (**A**) (○) amantadine 0.3 mg/kg, (■) amantadine 1.0 mg/kg and (□) amantadine 3.0 mg/kg; (**B**) (○) bromocriptine 0.1 mg/kg, (■) bromocriptine 1.0 mg/kg and (□) bromocriptine 10.0 mg/kg; (**C**) (○) pimozide 0.2 mg/kg, (■) pimozide 0.4 mg/kg and (□) pimozide 0.8 mg/kg. The parenthesis in (**B**) indicates that bromocriptine did not protect the animals against convulsions. For further explanation see legend to Fig. 1

potent in this respect. Pimozide significantly potentiated physostigmine-induced tremor in the dose of 0.2 mg/kg but not in larger doses (Fig. 2C).

The calculated regression parameters for the log dose-response curves in Figs. 1 and 2 are given in Table 1. The significant correlation coefficients (r) show that there exist a linear log dose-response relationship between the mean tremor power and the dose of physostigmine in these curves. The slopes (b) of the straight lines indicate that the log dose-response curve of physostigmine is parallelly displaced by

Table 1. The calculated regression parameters for the log dose-response curves of physostigmine without and after pretreatment

Pretreatment (dose mg/kg)	Regression parameters[a]			
	r	b	a	*n*
Atropine				
0	0.960*	78.7	45.7	20
0.3	0.932	69.4	29.0	10
0.6	0.977*	71.0	18.8	9
1.2	0.976*	72.7	−2.4	10
Methylatropine				
0	0.960*	78.7	45.7	20
0.3	0.990**	80.2	57.5	9
0.6	0.918	76.7	51.0	13
1.2	0.981*	89.2	43.9	12
Biperiden				
0	0.975*	72.7	43.3	12
0.01	0.999***	86.6	36.3	12
0.1	0.912	60.0	24.7	12
1.0	0.979*	60.1	10.8	10
Amantadine				
0	0.978*	68.5	37.3	20
0.3	0.976*	75.5	26.7	12
1.0	0.998**	73.2	22.5	11
3.0	0.992**	75.8	23.8	12
Bromocriptine				
0	0.969*	78.5	45.8	10
0.1	0.991**	108.1	40.3	10
1.0	0.973*	102.7	38.7	9
10.0	0.953*	117.9	43.0	10
Pimozide				
0	0.999***	102.6	33.8	11
0.2	0.984*	131.0	53.9	12
0.4	0.989*	137.0	42.8	10
0.8	0.998**	105.3	37.6	12

[a] The parameters of the regression equations were calculated using the method of least squares. The regression lines are in the form $y = b \log x + a$, where y is the mean tremor power, x the dose of physostigmine, b the slope of the line and a the y-intercept. The significance of the correlation coefficients (r) is given by * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$. The regression lines are calculated from 4–5 mean values, each based on n number of rats

atropine, methylatropine and amantadine, but not by biperiden, bromocriptine and pimozide.

Figure 3A and B show the time course of the tremor and hypothermia induced by physostigmine without and after pretreatment with anticholinergic and dopaminergic drugs. Both tremor and hypothermia appeared at about 3–5 min after physostigmine injection. Atropine (0.6 mg/kg) and biperiden (0.1 mg/kg) but not methylatropine (0.3 mg/kg) significantly reduced the tremor at all intervals of the observation period. The tremor-reducing effect of amantadine (0.3 mg/kg) was clear at all time points studied. Although bromocriptine (0.1 mg/kg) reduced (Fig. 2B) and pimozide (0.2 mg/kg) enhanced (Fig. 2C) the tremor when calculated as the total tremor power during 20 min, their effects were not as clearly seen at the time points given in Fig. 3B. Neither the anticholinergic nor the dopaminergic drugs altered the hypothermic effect of physostigmine (Fig. 3A and B).

Discussion

Based on the parallel shift of the log dose-response curve to the right it is suggested that atropine exerts a competitive antagonism on the physostigmine-induced tremor. This agrees with the finding that atropine prevented both physostigmine- and oxotremorine-induced tremor in the rat (eg. Holmstedt and Lundgren 1966; Sethy and Van Woert 1973a). Our results obtained with small doses of methylatropine, which poorly penetrates into the brain, confirmed our previous finding that methylatropine significantly potentiates physostigmine-induced tremor (Gothóni et al. 1981). Methylatropine alone did not cause any tremor. This paradoxical event is difficult to account for. As tremor can be modified by influences of peripheral origin (eg. Rondot and Bathien 1978), one explanation could be that methylatropine changes the afferent input to the central nervous system. The antitremor effect of biperiden, a drug acting mainly on central muscarinic receptors (Haas and Jantos 1969), resembled that of atropine. But biperiden did not as effectively as atropine antagonize the lethal convulsions induced by large doses of physostigmine. At the lowest dose (0.01 mg/kg) studied biperiden caused a parallel shift of the log dose-response line to the right, but with higher doses the lines got less steep suggesting a non-competitive antagonism.

Amantadine has been shown to enhance synthesis and release of dopamine in the rat brain (Scatton et al. 1970; Farnebo et al. 1971). Moreover, Heimans et al. (1972) have reported that amantadine inhibits the neuronal re-uptake of dopamine. Thus, amantadine should counteract the physostigmine-induced cholinergic hyperactivity by activating the dopaminergic system. However, Cox and Tha (1975) have reported that amantadine itself produces tremor in doses above 70 mg/kg, which is in accordance with our own findings. In the present experiments amantadine significantly reduced tremor but only to a certain degree. Amantadine in a dose of 3 mg/kg did not reduce the tremor more than in a dose of 1 mg/kg.

Bromocriptine activates dopamine receptors in the basal ganglia (Fuxe et al. 1974; Ungerstedt 1978). In this study, bromocriptine significantly antagonized physostigmine-induced tremor, but increasing its dose did not increase this antagonistic effect. In contrast to amantadine bromocriptine did not cause a parallel shift of the dose-response curve to the right as shown by the increased slopes nor did it protect the rats from lethal convulsions. Thus it is possible that bromocriptine does not act on all dopamine receptors modifying tremor.

Pinder et al. (1976) have reported that pimozide is a specific blocker of central dopamine receptors. In our experiments pimozide significantly potentiated the tremor in the dose of 0.2 mg/kg but not in larger doses. This is in accordance with the findings of Al-Shabibi and Dogget (1979) that pimozide slightly potentiated tremor induced by oxotremorine.

Because the physostigmine-induced hypothermia lasted significantly longer than the tremor, it seems unlikely that the tremor was due to a shivering effect.

Our results suggest that stimulation of the dopaminergic system can reduce the tremor induced by the hyperactivity of the striatal cholinergic system but only to a certain extent. Such a conclusion was made by Ahtee and Kääriäinen (1974) concerning the antagonism of these two systems on the dopamine metabolism in the striatum. Furthermore, Jurna et

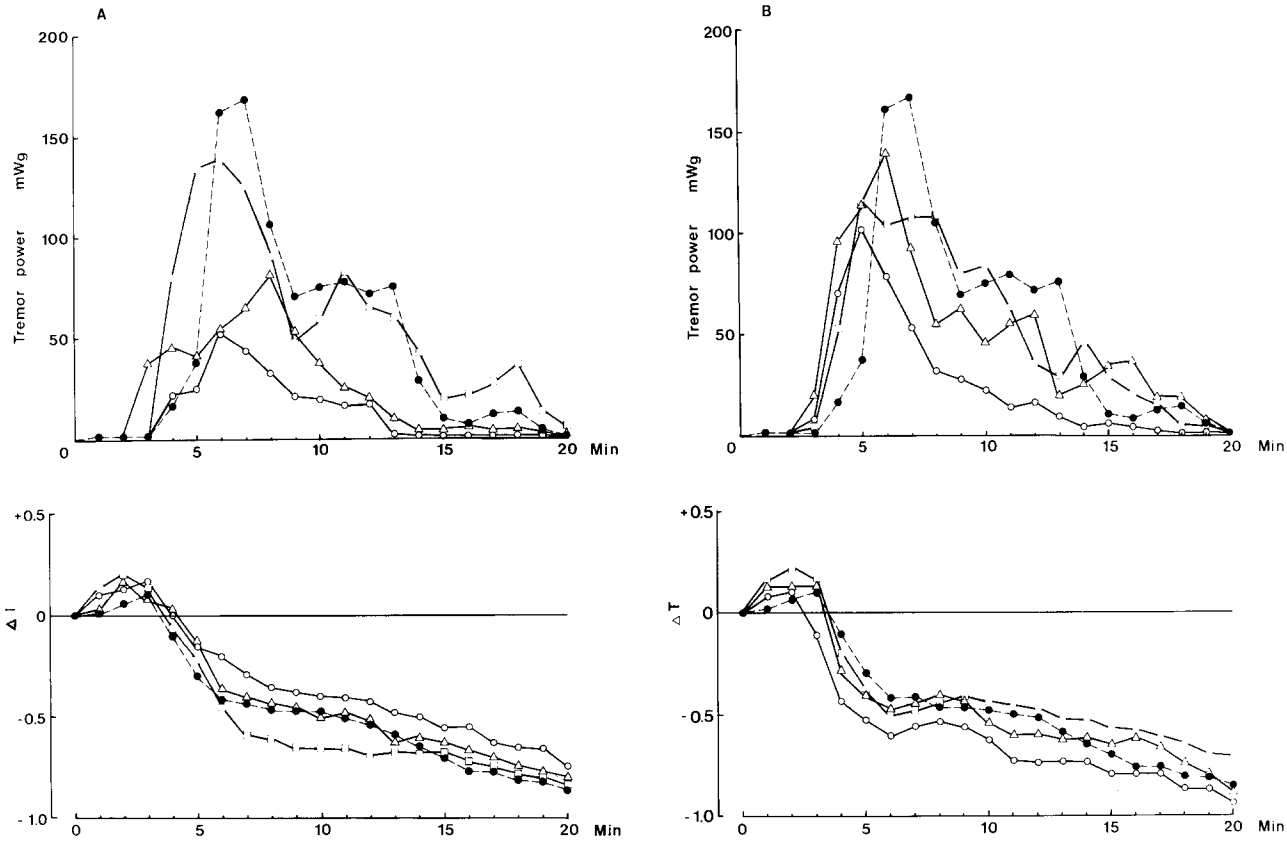

Fig. 3. Time course of the effects of atropine, methylatropine and biperiden (**A**), and of amantadine, bromocriptine and pimozide (**B**) on the physostigmine-induced tremor (*upper panels*) and body temperature (*lower panels*). The effect of 0.9 mg/kg physostigmine without pretreatment is presented by broken lines and closed circles. The effect of drug pretreatment is shown by the curves with solid lines. The (○) in (**A**) represent atropine 0.6 mg/kg, the (□) methylatropine 0.3 mg/kg and the (△) biperiden 0.1 mg/kg. The (○) in (**B**) represent amantadine 0.3 mg/kg, the (□) bromocriptine 0.1 mg/kg and the (△) pimozide 0.2 mg/kg. ΔT in the lower panels gives the difference (°C) in the body temperature of the rats between zero time and at various times after the injection of physostigmine. Each point is a mean from 5–6 rats

al. (1969) attributed the physostigmine-induced changes in the activities of alpha and gamma motoneurones, through which the central nervous system controls muscle action, to an imbalance between the dopaminergic and the cholinergic systems in the brain.

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