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ANTIMUSCARINIC DRUGS—EFFECT ON BRAIN ACETYLCHOLINE AND TREMORS IN RATS*

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Abstract—The effect of cholinergic and antimuscarinic drugs on rat brain acetylcholine (ACh) concentration and on tremors has been studied. The cholinergic drugs (physotigmine and oxotremorine) increased brain ACh concentration and produced tremors. Antimuscarinic drugs (atropine, benztropine and trihexyphenidyl) decreased brain ACh concentration, blocked oxotremorine-induced tremors and prevented the oxotremorine-induced increase in brain ACh. Physostigmine-induced tremors and elevation in brain ACh concentration were not altered by pretreatment with benztropine and trihexyphenidyl. However, atropine reduced physostigmine-induced tremors and prevented the increase in brain ACh concentration. The results suggest that oxotremorine- and physostigmine-induced tremors are causally related to an increase in brain ACh concentration.

OXOTREMORINE increases the concentration of brain acetylcholine (ACh) and produces tremors in animals.[1,2] Holmstedt and Lundgren[1] and Slater and Rogers[3] postulated that there is a causal relationship between the oxotremorine-induced rise in brain ACh and the tremors, since both effects occurred simultaneously and both were blocked by prior treatment with atropine, hemicholinium-3 and triethylcholine. Further support for this relationship was provided by Bartolini *et al.*[4] who observed that the largest increase in ACh, produced by oxotremorine, occurred in the region of the basal ganglia, an area of the brain frequently implicated in tremor production. Direct injection of tremorine into the basal ganglia produces tremors in rats.[5]

Physostigmine, an inhibitor of acetylcholinesterase, also increases brain ACh content[6] and produces tremors in animals[7] as well as in patients with Parkinson's disease.[8] We previously determined that L-Dopa, an anti-Parkinsonian drug, did not prevent the oxotremorine- and physostigmine-induced increase in brain ACh concentration and tremors in rats.[9]

Numerous antimuscarinic drugs have been demonstrated to block tremorine and oxotremorine-induced tremors.[2,10,11] If drug-induced tremors are due to elevated brain ACh levels, then an antimuscarinic agent which blocks tremor induction might prevent the increase in brain ACh concentration produced by oxotremorine or physostigmine. In the present study, we have tested this hypothesis by determining the effects of three antimuscarinic compounds, atropine, benztropine (Cogentin) and trihexyphenidyl (Artane), on brain ACh concentration and on tremors in rats injected with either saline, physostigmine or oxotremorine.

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METHODS

Male Sprague–Dawley rats weighing 180–250 g were used in this study. Animals were kept under constant diurnal lighting and temperature conditions for at least 1 week prior to their use. All animals were killed approximately the same time (7:00 to 9:00 a.m.) of the day.

Physostigmine salicylate, oxotremorine sesquifumarate, atropine sulfate and benztropine mesylate were dissolved in 0·9% sodium chloride solution. Trihexyphenidyl hydrochloride was suspended in 1% sodium carboxymethyl cellulose (CMC). The doses of the drugs were calculated on the basis of the salts of the compounds. All drugs were administered intraperitoneally; control rats received equal volumes of CMC and saline.

Rats were decapitated by guillotine 20 min after the injection of physostigmine or oxotremorine, 30 min after atropine and 60 min after benztropine or trihexyphenidyl. The time interval between the administration of physostigmine, oxotremorine and atropine and decapitation of rats corresponded to the peak effect of these compounds on brain acetylcholine concentration.[9,12] The maximum reduction in brain ACh content after the treatment with benztropine and trihexyphenidyl occurred between 60 and 120 min as shown in Fig. 1. Therefore, a 60-min time interval was selected for the subsequent experiments with these two drugs. The same time intervals were used

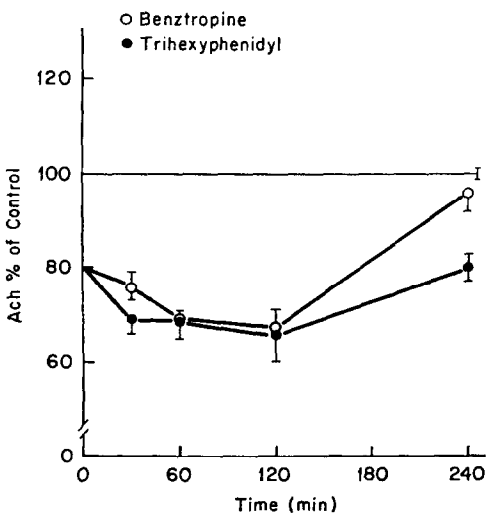

FIG. 1. Time course of changes in whole brain ACh concentration produced by benztropine (50 mg/kg) and trihexyphenidyl (40 mg/kg). Results are expressed as the per cent of control values (18·2 ± 0·4 nmoles/g). Each point is the mean ± S. E. of 4–6 determinations.

in the drug interaction studies. Brains were removed within 15 sec after decapitation and immediately homogenized in 0·4 N perchloric acid (4°) containing propionylcholine iodide as an internal standard. ACh was extracted and assayed by the method of Jenden *et al.*[13] and Hanin *et al.*[14] Analysis was performed on a Packard (model 7631) dual column gas chromatograph equipped with dual flame ionization detectors and a Honeywell recorder. Coiled columns (6 ft, 6 mm o.d.) were packed with Pennwalt 223 amine packing (Applied Science). The temperature of the flame detectors and

injection port was 210° and 250°, respectively, and the column temperature was 165°. The carrier gas was nitrogen at a flow rate of 65 ml/min. Hydrogen flow was maintained at 30 ml/min and oxygen flow at 300 ml/min. Levels of ACh are expressed as nmoles/g of wet brain.

Tremors were graded visually by both the authors during the entire 20-min period, between the injection of physostigmine or oxotremorine and decapitation. The effect of pretreatment with each dose of antimuscarinic drug on physostigmine- and oxotremorine-induced tremors was recorded as no change, decreased or completely blocked (no tremors).

RESULTS

Oxotremorine (3·6 mg/kg) and physostigmine (1 mg/kg) significantly ($P < 0·001$) increased whole brain ACh concentration in rats (Figs. 2–7). Atropine in the dose of 10, 20 and 40 mg/kg significantly decreased brain ACh concentration (Figs. 2 and 3).

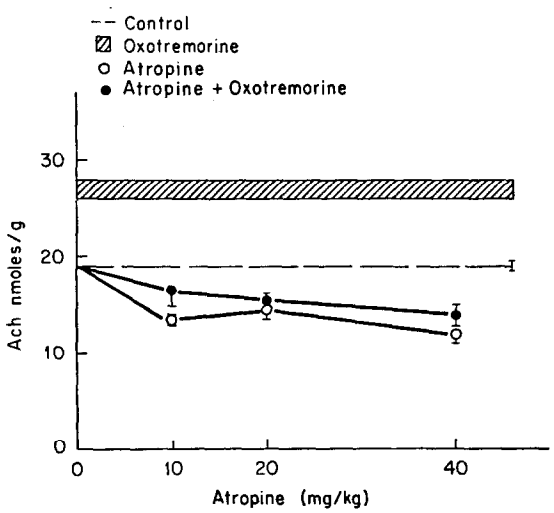

FIG. 2. Effect of various doses of atropine on whole brain ACh concentration in control and oxotremorine-treated (3·6 mg/kg) rats. Each point is the mean ± S. E. of 4–7 determinations.

Pretreatment with atropine significantly ($P < 0·001$) reduced the ACh elevation produced by oxotremorine and physostigmine (Figs. 2 and 3). Both benztropine (10, 25 and 50 mg/kg) and trihexyphenidyl (10, 20 and 40 mg/kg) significantly reduced brain ACh concentration (Figs. 4–7). Pretreatment with both of these drugs significantly ($P < 0·01$) inhibited oxotremorine-induced increase in brain ACh concentration (Figs. 4 and 5). However, physostigmine-induced elevation in brain ACh was not altered by pretreatment with both benztropine and trihexyphenidyl (Figs. 6 and 7).

Tremors produced by oxotremorine were decreased or completely blocked by pretreatment with the three antimuscarinic drugs at all dose levels (Table 1). Physostigmine-induced tremors were significantly decreased by pretreatment with atropine (10, 20 and 40 mg/kg) but were not affected by pretreatment with benztropine (10, 25 and 50 mg/kg) and trihexyphenidyl (Table 1).

TABLE 1. EFFECT OF ANTIMUSCARINIC DRUGS ON OXOTREMORINE- AND PHYSOSTIGMINE-INDUCED TREMORS*

Antimuscarinic drug	Effect on oxotremorine-induced tremors	Effect on physostigmine-induced tremors
Atropine (10, 20, 40 mg/kg)	Completely blocked	Decreased
Benztropine (10, 25, 50 mg/kg)	Completely blocked	No change
Trihexyphenidyl (10, 20, 40 mg/kg)	Decreased	No change

* The conditions of the experiments including injection schedules and dosage of drugs are the same as in Figs. 2–7. Rats injected with only the antimuscarinic drugs or saline-injected control animals did not develop tremors.

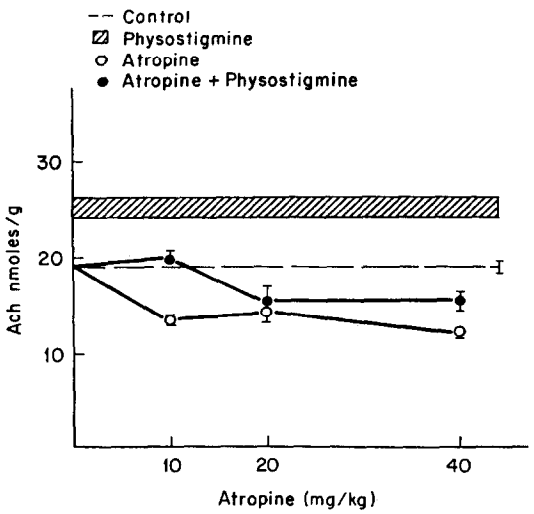

FIG. 3. Effect of various doses of atropine on whole brain ACh concentration in control and physostigmine-treated (1 mg/kg) rats. Each point is the mean ± S. E. of 4–7 determinations.

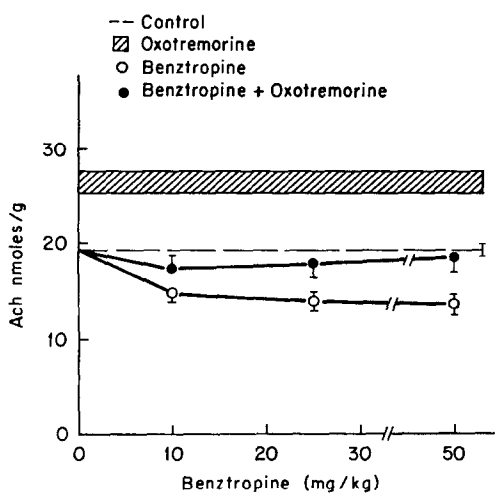

FIG. 4. Effect of various doses of benztropine on whole brain ACh concentration in control and oxotremorine-treated (3·6 mg/kg) rats. Each point is the mean ± S. E. of 4–7 determinations.

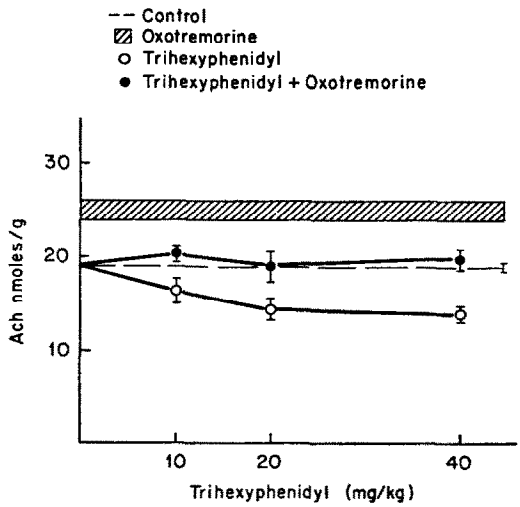

FIG. 5. Effect of various doses of trihexyphenidyl on whole brain ACh concentration in control and oxotremorine-treated (3·6 mg/kg) rats. Each point is the mean ± S. E. of 4–9 determinations.

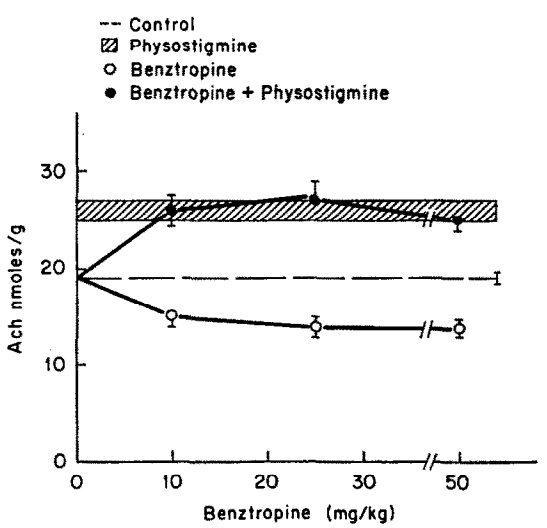

FIG. 6. Effect of various doses of benztropine on whole brain ACh concentration in control and physostigmine-treated (1 mg/kg) rats. Each point is the mean ± S. E. of 4–7 determinations.

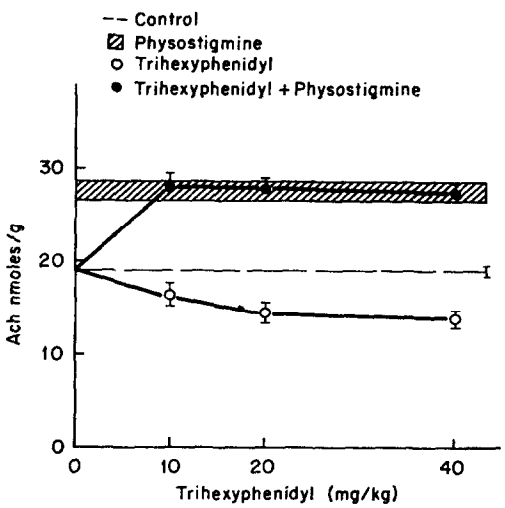

FIG. 7. Effect of various doses of trihexyphenidyl on whole brain ACh concentration in control and physostigmine-treated (1 mg/kg) rats. Each point is the mean ± S. E. of 4–9 determinations.

DISCUSSION

Our data demonstrate a correlation between the rise in brain ACh concentration and the tremors induced by either physostigmine or oxotremorine. All three antimuscarinic drugs, atropine, benztropine and trihexyphenidyl, decreased or completely blocked oxotremorine-induced tremors as well as prevented an increase in brain ACh. Physostigmine-induced tremors and the increase in brain ACh were diminished by pretreatment with atropine. However, benztropine and trihexyphenidyl had no effect on either tremors or brain ACh content in rats treated with physostigmine. These findings are consistent with the hypothesis of Holmstedt and Lundgren[1] that there is a causal relationship between the increase in brain ACh levels and tremors. However, Cox and Potkonjak[12,15] have cited some drugs which were exceptions to this hypothesis. Reserpine, diethyldithiocarbamate and α-methyl-metatyrosine inhibited oxotremorine tremors but did not reduce brain ACh content.[15]

Although benztropine and trihexyphenidyl did not reduce physostigmine tremors in the rat, these two antimuscarinic drugs block the aggravation of tremors by physostigmine in patients with Parkinson's disease.[8] The reason for this apparent inconsistency is not obvious, but it could be due to the biochemical and pathological alterations which are found in this disease or perhaps due to a species variation in response to these drugs.

Several mechanisms have been suggested to explain the anti-tremor action of antimuscarinic drugs. We have confirmed and extended the observation that antimuscarinic drugs decrease brain ACh concentration and this action parallels their anti-tremor effect. Atropine decreases cerebral cortex ACh by enhancing the neuronal release of this neurotransmitter.[16,17] Our results suggest that benztropine and trihexyphenidyl may be acting at different ACh storage sites than atropine, since they have different effects on physostigmine-induced increase in brain ACh. Antimuscarinic compounds also block post-synaptic muscarinic receptor sites; this is the most

commonly accepted mechanism of anticholinergic action in the peripheral and central nervous system. If the anti-tremorigenic action of benztropine and trihexyphenidyl is due to central muscarinic receptor blockage, then these two drugs would have been expected to prevent the tremors induced by physostigmine as well as oxotremorine. Recently Coyle and Snyder[18] postulated that the anti-Parkinsonian and anti-tremor effects of some antimuscarinic drugs may be due to their inhibition of neuronal dopamine uptake, thus potentiating dopamine action on the receptor sites. It is of interest that benztropine and trihexyphenidyl blocked the uptake of dopamine by striatal neurons whereas atropine has slight or no effect on dopamine uptake.[18,19] Thus, although all antimuscarinic drugs have anticholinergic action, their individual effects on ACh and dopamine metabolism may be quite different and the mechanism of their antitremorigenic action remains unexplained.

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