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ANTI-TREMOR ACTION OF C_{10}DICHOL, A PERIPHERAL ACETYLCHOLINE SYNTHESIS INHIBITOR

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1 Anti-tremor action of decamethylene bis-(hydroxyethly)-dimethylammonium bromide (C_{10}Dichol), a peripheral acetylcholine synthesis inhibitor, was investigated.

2 C_{10}Dichol inhibited tremor induced by oxotremorine, nicotine and physostigmine and afforded partial protection from physostigmine-induced mortality in mice.

3 In non-paralysing doses, C_{10}Dichol antagonized the neuromuscular effects of oxotremorine, nicotine and physostigmine.

4 Prior administration of C_{10}Dichol failed to prevent tremor and neuromuscular paralysis induced by harmine and arecoline.

5 In the absence of any antimuscarinic property of C_{10}Dichol, its neuromuscular effects appeared to be causually related to its anti-tremor action.

6 This study reveals a possibility for the development of peripherally acting anti-Parkinson drugs.

Introduction

Skeletal neuromuscular effects of decamethylene bis-(hydroxyethyl)-dimethylammonium bromide (C_{10}Dichol) and its inhibitory influence on acetylcholine (ACh) synthesis are well known (Barlow & Zoller, 1962; Bowman & Hemsworth, 1965; Hemsworth, 1971). The structure of C_{10}Dichol prevents its entering the central nervous system (Figure 1).

There is increasing evidence in the literature for skeletal neuromuscular involvement in the genesis as well as the prevention of Parkinson-like features induced by chemical agents that are recommended for screening procedures in the evaluation of antiParkinson drugs (Cahen & Lynes, 1951; Everett, Blockus & Shepperd, 1956; Ganguly, 1976; Das & Ganguly, 1977; Das, Ganguly & Vedasiromoni, 1978; Ganguly, Nath, Ross & Vedasiromoni, 1978; Ganguly & Das, 1979). Of these agents, the muscarinic agonist oxotremorine (Oxo-T), is unique in producing the typical features of Parkinson's disease (Friedman & Everett, 1964; Jenden, 1968). This effect of Oxo-T is mediated at least in part through a presynaptic cholinergic

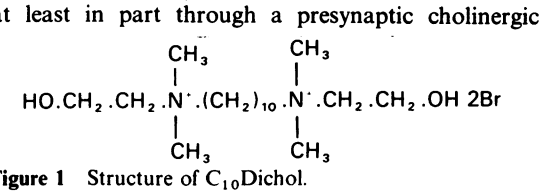

Figure 1 Structure of C_{10}Dichol.

dominance at the myoneural junction resulting from activation of presynaptic muscarinic receptors on the motor nerve endings (Das *et al.*, 1978; Ganguly & Das, 1979). With the use of Oxo-T as a screening model, neuromuscular involvement has been demonstrated in the antitremor action of propranolol (Ganguly, 1976) and of TK-174 (1,1-bis-(4-amino-phenyl)-propyl-(3)-amine), a substance that does not permeate the blood brain barrier (Leszkovszky & Tardos, 1971).

Since the cholinergic dominance at extra- and intra-fusal neuromuscular sites has been found to be involved in the production of experimental Parkinsonism, it was considered worthwhile to look for anti-Parkinson action of C_{10}Dichol because of its inhibitory effects on ACh-synthesis and neuromuscular transmission. C_{10}Dichol was preferred to hemicholinium-3 as an ACh-synthesis blocking agent in the present investigation because the latter has limited therapeutic possibilities due to its high systemic toxicity (Thies & Brooks, 1961). Although toxicity studies with C_{10}Dichol could not be undertaken in the present investigation because an insufficient amount was available, the observation that cats tolerate an intravenous dose of the drug as high as 15 mg/kg (Bowman & Hemsworth, 1965) indicates the low toxicity of C_{10}Dichol.

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Methods

Tremor in mice

Tremor scores were assessed visually in groups of mice (5/group) according to the method of Spencer (1965). Score values were determined at 5, 10, 20, 40 and 80 min intervals following intraperitoneal injection of the tremorogenic agents. C_{10}Dichol (0.5 to 4 mg/kg i.p.) was injected 2 h before the administration of the tremorogenic agents. The control groups of mice received saline pretreatment and were tested with each of the experimental groups.

Rat isolated phrenic nerve-diaphragm preparation

The preparations were set up according to the original method of Bülbring (1946). Muscle contractions were recorded isometrically with a force displacement transducer (Encardio-Rite model ET3; tension range 10 mg to 10 kg) on a multichannel recorder (Encardio-Rite) at 0.25 mm/s employing a differential preamplifier (Encardio-Rite model 532; f.r. d.c. 100 Hz). The phrenic nerve was stimulated at 0.2 Hz with supermaximal pulses of 0.2 ms duration.

Isolated rectus abdominis muscle of frog

This preparation was set up in the conventional manner using a 10 ml bath containing aerated frog-Ringer solution. Muscle contractions were recorded with a straw lever having an 8 to 10 fold magnification.

Physostigmine-induced lethality

Protection against physostigmine-induced lethality was assessed by the method of Nose & Kojima (1970). For this purpose the LD_{99} of physostigmine as determined by Nose & Kojima (1970) was employed.

Rat blood pressure and electrocardiogram

Experiments were carried out in heparinised (100 u/rat i.v.) albino rats (150 to 230 g) anaesthetized with urethane (1.4 g/kg s.c.). Blood pressure was recorded conventionally from the left carotid artery together with the electrocardiogram from lead II. Drugs were administered through the cannulated right femoral vein.

Drugs

The following drugs were used: acetylcholine chloride (E. Merck), arecoline hydrochloride (BDH), harmine hydrochloride (Sigma), nicotine sulphate (Sigma), oxotremorine sesquifumarate (Aldrich), physostigmine sulphate (E. Merck), (+)-tubocurarine chloride (Sigma) and C_{10}Dichol. Doses and concentrations of the drugs refer to appropriate salts.

Results

Tremor

The simple subjective scoring method of Spencer (1965) was used, as results obtained with it closely paralleled those obtained with an objective tremor recording device (Ganguly, 1976). Prior administration (2 h) of C_{10}Dichol in the dose-range of 0.5 to 4 mg/kg (i.p.) inhibited the tremor induced by Oxo-T (0.25 mg/kg i.p.), nicotine (0.43 mg/kg i.p.) and physostigmine (0.75 mg/kg i.p.). The maximal intraperitoneal anti-tremor dose of 4 mg/kg of C_{10}Dichol used in the present study may not affect neuromuscular transmission as the neuromuscular blocking dose of the agent is in the range of 5 to 10 mg/kg with intravenous administration in intact animals at low frequency indirect stimulation (0.1 Hz; Bowman & Hemsworth, 1965). The approximate ED_{50} of C_{10}Dichol in affording protection against tremor was 2 mg/kg (i.p.), with all the three tremorogens (Figure 2).

Tremor induced by harmine (10 mg/kg i.p.) and arecoline (10 mg/kg i.p.) remained unaffected in mice pretreated with C_{10}Dichol (0.5 to 4 mg/kg i.p.).

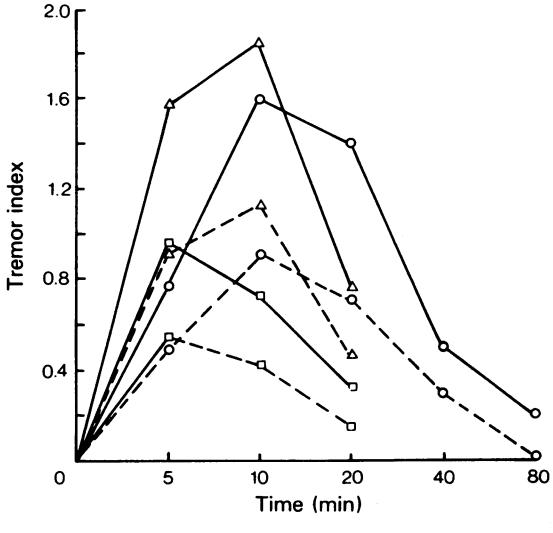

Figure 2 Inhibition (about 50%) by C_{10}Dichol (2 mg/kg i.p. 2 h beforehand) of tremor in mice induced by oxotremorine (0.25 mg/kg i.p.; ○), nicotine (0.43 mg/kg i.p.; △) and physostigmine (0.75 mg/kg i.p. □). Continuous lines indicate control and broken lines after C_{10}Dichol pretreatment. Values represent mean of 6 experiments (s.e. < 0.005 at all points).

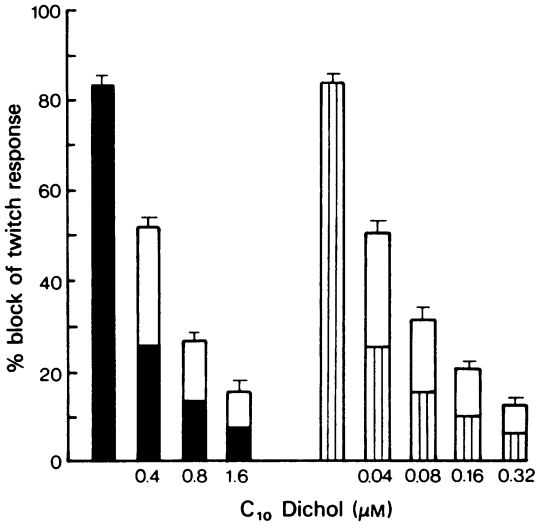

Figure 3. Histogram illustrating the dose-dependent antagonism by C_{10}Dichol of the neuromuscular block induced by nicotine and oxotremorine on indirect twitch responses of rat diaphragm. Values represent mean of 6 experiments; vertical bars show s.e. Solid columns 0.06 mM nicotine; striped columns: 0.01 mM oxotremorine; open columns: plus C_{10}Dichol.

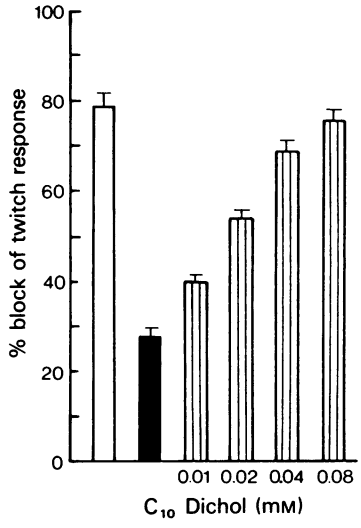

Figure 4. Histogram showing the inhibition of (+)-tubocurarine (Tc) blockade on indirectly elicited twitch responses of rat diaphragm by preincubation with physostigmine and dose-dependent antagonism of this effect of physostigmine in presence of C_{10}Dichol. Open column: 3.75 μM Tc; Solid column: Tc plus 4 μM physostigmine; striped columns: Tc plus physostigmine plus various concentrations of C_{10}Dichol. Values represent mean of 6 experiments (vertical lines show s.e. mean).

Neuromuscular transmission

After preincubation (5 min) of the preparation with C_{10}Dichol, the submaximal paralytic effects of Oxo-T (10 μM) and nicotine (0.06 mM) on indirect twitch responses of the rat phrenic nerve-diaphragm preparations were prevented (Figure 3). This effect of C_{10}Dichol was concentration-dependent. The neuromuscular blocking effects of harmine (0.2 mM) and arecoline (5 mM) in the same preparation were unaltered in the presence of C_{10}Dichol.

In another set of experiments, interaction of C_{10}Dichol with physostigmine was studied by examining the effect of C_{10}Dichol on the ability of physostigmine to antagonize the neuromuscular blocking effect of (+)-tubocurarine (Tc). Prior incubation (5 min) with physostigmine (4 μM) completely reversed the paralytic effect of Tc on indirect twitch responses of the diaphragm and this antagonistic effect of physostigmine was reversed dose-dependently by C_{10}Dichol (0.01 to 0.08 mM) when added to the bathing fluid 5 min before physostigmine (Figure 4). Indirect twitch responses of the rat diaphragm were unaffected by incubation with C_{10}Dichol alone in the dose-range that reversed the effect of physostigmine on Tc block.

In the frog isolated rectus abdominis muscle, previous administration of C_{10}Dichol (0.01 to 0.08 mM) potentiated the submaximal response to ACh (5 μM) and antagonized the blockade of this response by Tc (1.5 μM).

Physostigmine lethality and muscarinic receptors

The ability of C_{10}Dichol to protect mice from physostigimine-induced mortality was evaluated by use of the intraperitoneal LD_{99} i.e., 2 mg/kg of physostigmine (Nose & Kojima, 1970). Intraperitoneal administration of C_{10}Dichol (0.5 to 2 mg/kg) 2 h before physostigmine afforded maximal protection of about 50% (18/35 mice). Increase in the dose of C_{10}Dichol above 2 mg/kg failed to afford any further protection.

C_{10}Dichol had no antimuscarinic action for the vasodepressor and bradycardiac effects of Oxo-T and of ACh (injected intravenously) in rats pretreated (1 h) with C_{10}Dichol (2 mg/kg i.v; Figure 5).

Discussion

The present study reveals the possibility of developing peripherally acting drugs against Parkinson's disease.

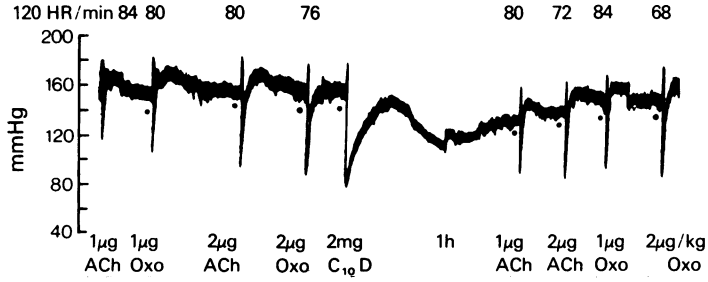

Figure 5 Rat blood pressure and heart rate (HR): showing failure of C_{10}Dichol (C_{10}D) to inhibit the bradycardiac and vasodepressor effects of oxotremorine (Oxo) and acetylcholine (ACh).

This conclusion is based on the observations that C_{10}Dichol, which impairs the synthesis of ACh and does not pass the blood brain barrier (see Introduction), is able to inhibit tremor induced by Oxo-T, physostigmine and nicotine and affords partial protection against physostigmine-induced lethality in mice (see Results). These tests are widely recognised as useful screening procedures for evaluation of anti-Parkinson drugs (Friedman & Everett, 1964; Jenden, 1968; Nase & Kojima, 1970)

Peripheral skeletomotor involvement in the 'Oxo-T model' of Parkinsonism and a presynaptic muscarinic-dopaminergic link in the motor nerve endings have been demonstrated in recent years (for references, see Introduction). Like Oxo-T, the presynaptic effects of nicotine and physostigmine on motor nerve terminals mediated through excess ACh are well documented (Barstad, 1962; Randić & Straughan, 1964; Chiou & Long, 1969; Chiou, 1973). It is thus possible to conclude that C_{10}Dichol antagonized the neuromuscular and tremorogenic effects of Oxo-T, nicotine and physostigmine by reducing the availability of ACh at the myoneural junction. In contrast, neither the neuromuscular effects of arecoline and harmine which are mediated postsynaptically (Chaudhuri & Ganguly, 1974) nor their tremorogenic effects were prevented by C_{10}Dichol. Indeed, it is important to mention in this connection that in a human trial, the incapacitating spasticity, rigidity and tremor of Parkinson's disease could be relieved with intramuscular repository curare even in patients whose symptoms were unrelieved by standard anti-Parkinson therapy (Berger, 1959), an observation that has remained obscure so far.

Protection by the peripherally acting drug C_{10}Dichol against physostigmine-induced tremor and mortality demonstrates the involvement of myoneural junction in these effects of physostigmine. Thus our results fail to confirm the suggestion that physostigmine-induced lethality is entirely central in origin (Nose & Kojima, 1970). It was suggested earlier that nicotine tremor has both central and peripheral components (Everett *et al.* 1956) and that neuromuscular blocking agents prevent the same (Cahen & Lynes, 1951).

The effects of C_{10}Dichol on the frog isolated rectus abdominis muscle and on the rat blood pressure indicate that neither the postsynaptic nicotinic receptors of the skeletal muscle nor the peripheral muscarinic receptors are involved in the anti-tremor action of the drug. The mild anticurare effect of C_{10}Dichol on the isolated rectus muscle was presumably due to its weak anticholinesterase property (Barlow, 1955). Absence of any impairing influence of the maximum dose of C_{10}Dichol used in the present study (0.08 mM) on indirect twitch responses of rat diaphragm, coupled with the observation that the twitch tension of cat anterior tibialis muscle to indirect stimulation (0.1 Hz) was only marginally affected by an intravenous dose of 5 mg/kg of C_{10}Dichol (Bowman & Hemsworth, 1965) suggest that the drug may not affect the normal neuromuscular function while producing its anti-tremor action.

The present experiments further confirm the hypothesis that cholinergic dominance at the extra- and intra-fusal neuromuscular junction is involved in Oxo-T induced experimental Parkinsonism (Fackler, Ross, Haase & Cleveland, 1977; Cleveland, Ross, Ganguly, Kuschmierz & Haase, 1978; Das *et al.*, 1978; Ganguly *et al.*, 1978; Ganguly & Das, 1979).

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