Short communications

Modification of the tremorigenic activity of physostigmine

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Drugs which reduce central catecholamine activity increased the tremorigenic action of physostigmine (0·25 mg/kg) in the rat. The potentiation of physostigmine tremor induced by these neuroleptic drugs was quantitated by measuring the ratio of post- to prephysostigmine motor activity; this ratio may be a useful index of catecholamine/cholinergic balance. Of the compounds tested, chlorpromazine HCl (15 mg/kg) and reserpine (10 mg/kg) had the greatest potentiating effect on the tremor-producing action of physostigmine. L-DOPA (500 mg/kg) reduced the tremor induced by physostigmine in rats pre-treated with reserpine, but not in rats pre-treated with chlorpromazine.

Normal extrapyramidal function may depend upon a sensitive balance between inhibitory dopaminergic neurones and excitatory cholinergic fibres in the basal ganglia (McGeer, Boulding, Gibson & Foulkes, 1961; Barbeau, 1962). Large doses of the acetylcholinesterase inhibitor, physostigmine, produce tremors in animals, presumably by increasing acetylcholine levels in the basal ganglia (Frances & Jacob, 1971). In patients with Parkinson's disease, physostigmine aggravates tremor, rigidity, and hypokinesia at a dose which has no neurological effects in normal subjects (Duvoisin, 1967; Weintraub & Van Woert, 1971; Van Woert & Weintraub, 1971). Anticholinergic drugs and L-dihydroxyphenylalanine (L-DOPA) clinically improve this disease and reduce or prevent the adverse neurological effects of physostigmine (Duvoisin, 1967; Weintraub & Van Woert, 1971; Van Woert & Weintraub, 1971). If the hypersensitivity to physostigmine in Parkinson's disease is due to decreased brain dopamine (Ehringer & Hornykiewicz, 1960), drugs which alter dopamine metabolism might also affect the neurological response to physostigmine. Recent studies in our laboratory demonstrated that serial intracisternal injections of 6-hydroxydopamine in dogs produced hypokinesia and frequently rigidity and tremor which were aggravated by intravenous physostigmine and reversed by L-DOPA and benztropine (Van Woert, Ambani & Bowers (1972) in press). We suggested that the physostigmine hypersensitivity induced by 6-hydroxydopamine was due to the degeneration of catecholamine containing neurones produced by this amine.

This communication describes the results of further experiments in rats designed to correlate the tremorigenic activity of physostigmine with drug-induced changes in catecholamine metabolism. We were particularly interested in the effect of drugs which produce Parkinsonism such as reserpine, phenothiazines, and haloperidol on physostigmine tremor.

Methods. — 152 Sprague-Dawley rats weighing 180 to 220 g were used in the experiments. Tremor and motor activity were measured on a 501 activity platform (Lafayette Instruments, Lafayette, Indiana) set at a sensitivity adjustment of 10 which minimized counts due to exploratory movements, but still recorded tremor. Two rats were placed on the activity platform at one time for each measurement. After a 3 min period to permit the rats to familiarize themselves with the new environment, activity was recorded for 15 minutes. In the same set of rats measurements were obtained before and after intraperitoneal injections of physostigmine salicylate (0·25 mg/kg), before and at various time intervals after administration of the drug being evaluated. The following drugs were tested for their effect on physostigmine-induced tremor: reserpine (10 mg/kg), chlorpromazine HCl (15 mg/kg), haloperidol (10 mg/kg), trifluoperazine HCl (1 mg/kg), thioridazine (0·5 mg/kg), α-methyl-*p*-tyrosine (200 mg/kg), *p*-chlorophenylalanine (316 mg/kg), gamma-butyrolactone (250 mg/kg), ethopropazine (30 mg/kg) and diazepam (3 mg/kg). Each drug was administered to a total of 10 rats. Dosage was primarily based on the production of similar degrees of sedation with each drug, in order to produce a similar minimal pre-physostigmine spontaneous locomotor activity. All drugs were injected intraperitoneally. α-Methyl-*p*-tyrosine and *p*-chlorophenylalanine were dissolved in distilled water by adjusting the pH to 11·0 with 0·5 N NaOH and then lowering the pH to 9·4 with 1·0 N HCl just prior to injection. The other

drugs were dissolved in 0·9% NaCl w/v (normal saline). L-DOPA (500 mg/kg) was injected as a suspension in normal saline.

The ratio of the post-physostigmine activity to pre-physostigmine activity was calculated in each set of rats and was called the activity index. The activity index is a measure of the tremorigenic effect of physostigmine in these animals. Tremor intensity also was evaluated subjectively on a 0–4+ scale by the authors and the results obtained by the two methods were similar.

Results.—No tremors developed after injection of 0·1 mg/kg physostigmine and mild to moderate tremors of the extremities were evident in 70% of the animals after 0·5 mg/kg. At a dose of 0·25 mg/kg, physostigmine induced minimal tremor from 10 to 15 min after injection in about 50% of the rats; this dose was used in all subsequent experiments. Physostigmine, as well as the other drugs all exerted a sedative effect so that the animals usually remained stationary in the middle of the platform. The ratio of post-physostigmine activity to pre-physostigmine activity (activity index) was less than 1 in the untreated control rats (Fig. 1) because of the greater sedative than tremorigenic effect of this dose (0·25 mg/kg). One hour after the intraperitoneal injection of chlorpromazine, physostigmine produced moderate to severe tremor in all 4 extremities of the treated rats. This was reflected by a 26-fold increase in the activity index compared to control animals (Fig. 1). The tremor started 5 min after the physostigmine injection and lasted about 30 minutes. The degree of tremor produced by physostigmine also was enhanced markedly 1 h after reserpine as evidenced by a 23-fold increase in the activity index. L–DOPA (500 mg/kg), administered 1 h prior to physostigmine, reduced the tremorigenic effect of physostigmine in rats pre-treated with reserpine but had no effect on tremors produced by physostigmine in rats pretreated with chlorpromazine. Benztropine (4 mg/kg), administered 0·5 h prior to physostigmine, decreased the tremorigenic activity of physostigmine induced by both reserpine and chlorpromazine. Other drugs which alter catecholamine metabolism, ethopropazine, trifluoperazine, thioridazine, α-methyl-*p*-tyrosine, haloperidol and γ-bu-

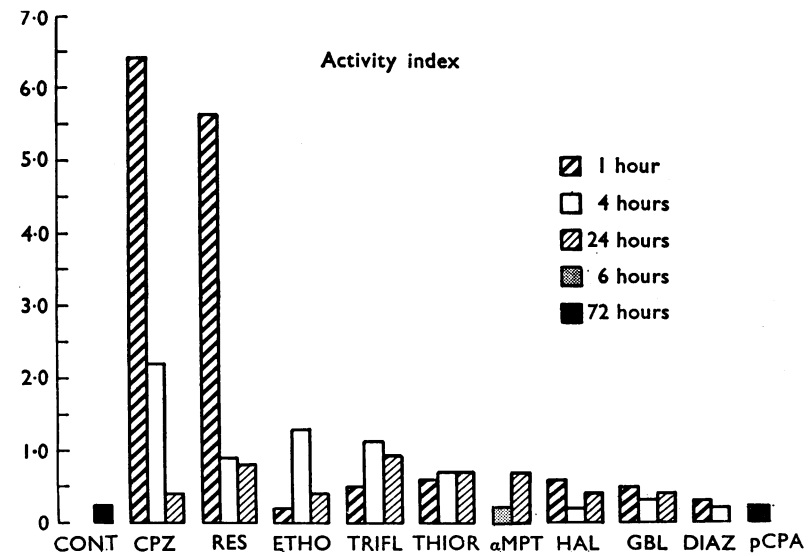

FIG. 1. Effect of various drugs on physostigmine-induced tremor. The activity index (ratio of post-physostigmine counts to pre-physostigmine counts) indicates the tremorigenic action of physostigmine (0·25 mg/kg) as affected by different compounds. The hours indicated are the time intervals between the neuroleptic drug administration and the physostigmine injection. Each value is the mean of 5 groups of rats. The abbreviations are: CONT, control; CPZ, chlorpromazine; RES, reserpine; ETHO, ethopropazine; TRIFL, trifluoperazine; THIOR, thioridazine; α-MPT, α-methyl-*p*-tyrosine; HAL, haloperidol; GBL, γ-butyrolactone; DIAZ, diazepam; *p*CPA, *p*-chlorophenylalanine.

tyrolactone produced a lesser degree of enhancement of physostigmine-induced tremor. Diazepam and *p*-chlorophenylalanine had no effect on the tremorigenic activity of physostigmine.

Discussion.—Our results indicate that various drugs which reduce catecholaminergic activity increased the tremorigenic action of 0·25 mg/kg of physostigmine, a dose which alone produced minimal tremor in 50% of the animals. Of the drugs tested, reserpine and chlorpromazine had the greatest potentiating effect on the tremor-producing action of physostigmine. L-DOPA reduced the intensity of the tremor produced by 0·25 mg/kg of physostigmine in the rat pre-treated with reserpine. This to explain the potentiation of physostigtigmine in the reserpine pre-treated rat may be due to the depletion of catecholamines and that L-DOPA reduces this hypersensitivity by restoring catecholamine levels. This mechanism is similar to that postulated to explain the potentialion of physostigmine tremor in dogs by 6-hydroxydopamine and its reversal by L-DOPA (Van Woert *et al.*, 1972). In humans, L-DOPA is therapeutically effective in reserpine-induced and idiopathic Parkinsonism (Bruno & Bruno, 1966; Cotzias, Van Woert & Schiffer, 1967) both of which are associated with decreased brain catecholamine levels.

In rats treated with chlorpromazine, L-DOPA did not reduce the tremors caused by physostigmine. This finding is compatible with the hypothesis that chlorpromazine blocks dopaminergic post-synaptic receptor sites (Van Rossum, 1968) and it is also consistent with the observation that L-DOPA does not improve extrapyramidal symptoms associated with chlorpromazine administration in man (Yaryura-Tobias, Wolpert, Dana & Merlis, 1970).

Diazepam and *p*-chlorophenylalanine which have little, if any, significant effect on catecholamine metabolism did not alter the tremorigenic action of physostigmine. α-Methyl-*p*-tyrosine and γ-butyrolactone were evaluated because of their effects on dopamine metabolism. α-Methyl-*p*-tyrosine lowers brain catecholamines by inhibiting their synthesis (Engelman, Horwitz, Jequier & Sjoerdsma, 1968); γ-butyrolactone increases brain dopamine, presumably by decreasing dopamine release from neuronal storage sites (Roth & Suhr, 1970). The incidence of extrapyramidal side effects of both α-methyl-*p*-tyrosine (Engelman, Horwitz, Jequier & Sjoerdsma, 1968) and γ-butyrolactone (Solway & Sadove, 1965) are low in humans and these drugs had only minimal potentiating effects on physostigmine tremor. In comparison with chlorpromazine, thioridazine showed less potentiation of the tremorigenic action of physostigmine and has a smaller effect on striatal dopamine metabolism (Laverty & Sharman, 1965). These observations are compatible with the lower incidence of extrapyramidal dysfunction observed with thioridazine (Ayd, 1961). However, there is no correlation between the incidence of extrapyramidal side effects reported for ethopropazine, trifluoperazine and haloperidol and their ability to potentiate physostigmine tremor. Ethopropazine is a phenothiazine with significant anticholinergic activity and it is used in the treatment of Parkinson's disease. Trifluoperazine and haloperidol produce a higher incidence of extrapyramidal reactions than chlorpromazine or reserpine (Ayd, 1961). Multiple factors such as dosage, duration of therapy and species sensitivity to these drugs may be important in determining their relative potentiating effect on physostigmine tremor and might account for the lack of correlation between animal experiments and clinical observations. In addition to their catecholaminergic inhibitory action, reserpine and chlorpromazine can also facilitate central cholinergic activity (Malhotra & Pundlik, 1959; Maickel, 1968; Dasgupta & Mukherjie, 1956; Arterberry, Bonifaci, Nah & Quiney, 1963). The relative importance of these two mechanisms is obscure, but it seems reasonable to suggest that striatal dopaminergic-cholinergic balance is involved in at least some instances in which the tremorigenic action of physostigmine is enhanced by a neuroleptic drug.

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