These results support the contention that ergometrine and ADTN stimulate dopamine receptors in the striatum, the dimethylether of ADTN being ineffective. We have presented evidence for the involvement of cyclic AMP in the behavioural response to these drugs.

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Inhibition of dopamine-sensitive adenylate cyclase in rat basal ganglia and other hormone sensitive adenylate cyclase systems by neuroleptic drugs.

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Homogenates of brain areas containing dopaminergic synapses respond to low concentrations of dopamine by increased cyclic AMP production. The action of dopamine is inhibited by neuroleptic drugs, and mimicked by dopamine agonists (Kebabian, Petzold & Greengard, 1972; Miller, Horn, Iversen & Pinder, 1974). Neuroleptics also inhibit other adenylate cyclase systems (Wolff & Jones, 1970). In the present study we have investigated the specificity of inhibition of these various systems and their possible significance to the mode of action of neuroleptics.

Dopamine-sensitive adenylate cyclase in rat striatum was assayed as previously described (Miller *et al.*, 1974). Stimulation produced by dopamine (10^{-4}M) in striatal homogenates was inhibited by (+)-butaclamol with an IC50 of 1.8×10^{-7} M. (−)-butaclamol, however, was ineffective. Butaclamol is a neuroleptic of novel structure and is the first to exhibit optical isomerism. Its interaction with the dopamine receptor appears to differ from any other neuroleptic so far described. It is known that the neuroleptic activity of butaclamol resides solely in the (+) isomer. This again illustrates the close correspondence between neuroleptic activity and dopamine receptor blockade.

The inhibition caused by (+)-butaclamol was competitive with dopamine except at high drug concentrations (10^{-5}M). Inhibition of the effect of dopamine by other neuroleptics at concentrations approximately equal to their IC50 values proved also to be competitive. This included drugs of the phenothiazine, butyrophenone and dibenzodiazepine classes such as thioridazine, haloperidol, clozapine and loxapine. Phenothiazines and butyrophenones also both acted within one minute of their addition to the assay system.

Neuroleptic drugs were also tested on other hormone sensitive adenylate cyclase systems such as the β-adrenoceptor linked system in isolated adipocytes and the glucagon-sensitive system in liver plasma membranes. Inhibition of adenylate cyclase in these systems occurs at higher drug concentrations than in the dopamine-sensitive systems. Thus, for example, the IC50 for chlorpromazine in the β-adrenoceptor system was approximately 2×10^{-4}M against a maximally stimulating concentration of 10^{-4}M noradrenaline, whereas the corresponding IC50 for the dopamine-sensitive cyclase was 1×10^{-6}M. Inhibition of the glucagon-sensitive system occurred at chlorpromazine concentrations (10^{-4}M) which did not inhibit basal enzyme activity in liver plasma membranes although at higher drug concentrations basal activity was

inhibited. Inhibition of the glucagon-sensitive system by chlorpromazine (5×10^{-4}M) was non-competitive. In this system α and β-flupenthixol were both equally effective at 10^{-4}M, and haloperidol was ineffective at 10^{-4}M. This does not parallel the neuroleptic properties of these compounds. The effects of neuroleptics on the glucagon or noradrenaline-sensitive adenylate cyclases occurred at concentrations at which other membrane bound enzymes are also affected and probably reflect the general membrane stabilizing effects of neuroleptics rather than direct drug/receptor interactions of the type that occur with the dopamine-sensitive system.

Richard Miller is an M.R.C. Scholar.

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Some effects of age upon irreversible inhibition of cardiac MAO

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It has been suggested, from evidence derived from the use of irreversible inhibitors such as clorgyline that the monoamine oxidase (MAO) in many tissues exists in two forms, called 'species A' and 'species B' (Johnston, 1968). At present, it is not clear whether these two species of enzyme represent differences in localization, e.g., intra- and extraneuronal MAO (Goridis & Neff, 1971), or differential binding of membrane lipid to the same enzyme protein (Tipton, Houslay & Garrett, 1973). In the rat heart, tyramine is metabolized solely by species A while benzylamine is metabolized by both A and B (Lyles & Callingham, 1974). With this latter substrate, the pattern of inhibition produced by clorgyline depends upon the age of the animal.

Hearts were removed from male Wistar rats and male CFLP mice and homogenized in 0.001 M potassium phosphate buffer at pH 7.4. Assay of MAO activity involved the use of ^{3}H-tyramine and ^{14}C-benzylamine as substrates. Inhibitor concentrations varied from 5×10^{-11} to 5×10^{-4} M.

In rats of mean body weight 151 g, benzylamine oxidation produced a double sigmoid inhibition curve with clorgyline. The plateau region of this curve, which occurred at inhibitor concentrations of 5×10^{-8} to 5×10^{-7} M, suggested that species A represented about 35% of the total enzyme activity. In younger rats of body weight 63 g, the plateau shifted to indicate a smaller (23%) proportion of species A. In rats weighing 36 g, the inhibition curve was essentially a single sigmoid typical of species B. However, in older rats weighing 317 g and 414 g, the plateau shifted in the opposite direction suggesting an increased proportion of species A (50% and 70%, respectively). Confirmation of this was obtained using the MAO inhibitor deprenyl. This compound while producing similar inhibition curves to clorgyline, is more active on species B. Benzylamine oxidation again resulted in a double sigmoid inhibition curve, and the plateau region also shifted to indicate an increase in the proportion and amount of species A with increase in the age of the rat. On this evidence, it is suggested that the increase in the specific activity of rat heart MAO seen in the growing rat is mediated largely by an increase in the amount of species A.

In the adult mouse heart, the inhibition curves using clorgyline were different from those in the rat. Using tyramine as substrate, a double sigmoid curve was found, with species A representing about 20% of the total activity. However, benzylamine produced a single sigmoid curve characteristic of species B.

It would appear that the use of the irreversible MAO inhibitors, clorgyline and deprenyl, with a variety of substrates can be used to investigate the possibility of selective changes in the nature of the enzyme that may accompany changes in its specific activity.

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