Inhibition of tyrosine hydroxylase but not dopamine-β-hydroxylase facilitates the action of behaviourally ineffective doses of neuroleptics

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The behavioral effects of a wide variety of neuroleptic agents (including drugs belonging to the butyrophenone, phenothiazine and diphenylbutylpiperidine groups) are markedly enhanced by the tyrosine hydroxylase inhibitor, α-methyl-*p*-tyrosine (AMT) (Ahlenius & Engel, 1971, 1973; Carlsson, Persson & others, 1972; Carlsson, Roos & others, 1973). Neuroleptics are known to induce an increase in catecholamine turnover (Carlsson & Lindqvist, 1963) presumably as compensation for their receptor blocking activity. This suggests that the potentiating effects of AMT on the action of neuroleptics may be due to its inhibition of neuroleptic-increase in catecholamine turnover, thereby reducing the amount of neurotransmitter available to compete with the neuroleptic for receptor sites. Although neuroleptics are thought to act primarily by blocking brain dopamine receptors (Matthysse, 1973), AMT inhibits the synthesis of both noradrenaline and dopamine (Spector, Sjoerdsma & Udenfriend, 1965). Furthermore, *in vitro* studies of dopamine-sensitive adenylate cyclase in the caudate nucleus indicate that noradrenaline is capable of stimulating dopamine receptors (Kebabian, Clement-Cormier & others, 1975). These data suggest that the use of a tyrosine hydroxylase inhibitor such as AMT is not sufficiently selective to permit a determination of whether potentiation of neuroleptic activity is due entirely to prevention of dopamine synthesis, as has been suggested (Ahlenius & Engel, 1973) or whether the additional inhibition of noradrenaline synthesis may also play a role.

The present experiment sought to investigate this question more thoroughly by combining behaviorally ineffective doses of neuroleptics, with inhibitors of either tyrosine hydroxylase or dopamine-β-hydroxylase (DBH). Since inhibition of DBH selectively blocks noradrenaline synthesis, this technique should allow us to determine whether noradrenaline plays a role in the neuroleptic-potentiating action of tyrosine hydroxylase inhibition. To the extent that DBH inhibition facilitates neuroleptic action, noradrenaline must also be considered to be involved when AMT is combined with neuroleptics.

The behavioral end point used was tail-pinch-induced eating. This is a phenomenon, recently discovered (Antelman & Szechtman, 1975), in which food satiated rats within seconds of application of a mild pinch to the tail begin to sniff and then take up and eat food pellets in a manner identical in every respect to eating precipitated by hunger. The phenomenon appears to depend on the integrity of the nigrostriatal dopamine system (Antelman & Szechtman, 1975).

Eighty-eight male Sprague-Dawley rats, 250–350 g were housed in pairs and maintained on a natural day-night cycle with food and water freely available. All testing was done during the daytime. Behavioral tests were conducted in shallow metal bowls between 13·5 and 17·5 inches in diameter, each containing 6–10 pellets of Purina rat chow. A 10 inch surgical haemostat insulated at the tips with foam rubber was used for tail pinch. All animals were adapted to the bowls for 10 min before the onset of testing. Following adaptation, the haemostat was applied approximately 1 inch from the tip of an animal's tail and locked into the first notch. Animals received five, 20 s tail-pinch trials under a particular condition, each separated by 5–8 min. Each animal was tested under both pre- and post-drug (or vehicle) condi-

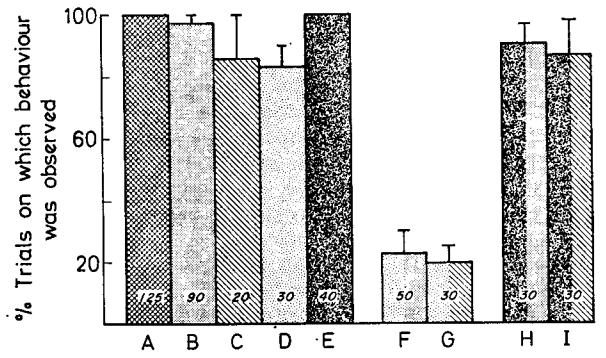

FIG. 1. The effects on tail-pinch behaviour of combining inhibitors of either tyrosine hydroxylase (AMT) or dopamine-β-hydroxylase (FLA-63) with behaviorally ineffective doses of the dopamine receptor blockers, haloperidol and pimozide. Numbers on each bar refer to total number of trials with a given treatment. Number of animals is equal to number of trials (5). A—combined vehicles. B—haloperidol (0·1 mg kg^{-1}). C—pimozide (0·5 mg kg^{-1}). D—AMT (2 × 100 mg kg^{-1}). E—FLA-63 (25 mg kg^{-1}). F—AMT + haloperidol. G—AMT + pimozide. H—FLA-63 + haloperidol. I—FLA-63 + pimozide.

tions, for a combined total of 10 trials. The criterion for inclusion of any animal in a drug experiment required that the animal show eating within 20 s on each of the five pre-drug trials. All animals tested met this criterion.

The neuroleptics employed were haloperidol (Haldol, McNeil Laboratories) a butyrophenone and pimozide a diphenylbutylpiperidine. The tyrosine hydroxylase inhibitor used was AMT (the (±)-methylester hydrochloride) and the DBH inhibitor was FLA-63[bis(4-methyl-1-homopiperazinylthiocarbonyl)disulphide] (Corrodi, Fuxe & others, 1970). All drugs were administered intraperitoneally (1 ml kg^{-1}). Haloperidol was diluted for injection with saline. Pimozide was dissolved in tartaric acid with a resulting pH of close to 2·1. FLA-63 was dissolved in N HCl and titrated to pH 5·5–6·0 with N NaOH. AMT was used after dissolution in water.

Following the determination that the doses of AMT (2 × 100 mg kg^{-1}, given 6 and 2 h before testing) and FLA-63 (25 mg kg^{-1}, 7·5 h before testing) used in these studies were ineffective in altering tail pinch behaviour, these agents were combined with doses of haloperidol (0·1 mg kg^{-1}, 1 h before testing), and pimozide (0·5 mg kg^{-1}, 4 h before testing) which also failed to block this behaviour (Fig. 1).

Fig. 1 illustrates that previously ineffective doses of both haloperidol and pimozide had a dramatic and highly significant blocking action on tail-pinch behaviour when they were combined with AMT ($P < 0{\cdot}001$, *t*-test comparison with vehicle-treated controls following a one-way analysis of variance), but no blocking effect whatever when given in combination with FLA-63. Indeed, FLA-63 actually resulted in a significant prolongation of tail-pinch-induced eating following cessation of tail-pinch, whether given singly or in combination with neuroleptics ($P < 0{\cdot}0005$, U-test).

Our results clearly suggest that the potentiating effects of AMT on the action of neuroleptics may, in fact, be due to inhibition of dopamine synthesis. Although these data would appear to provide strong support for the similar conclusion of Ahlenius & Engel (1973), caution must nevertheless be exercised in generalizing the results until similar experiments have been carried out on other behaviours.

This study was supported by USPHS grants MH 24114 (S.M.A.) and MH 1951 (A.E.F.). Dr. Paul Janssen, Janssen Pharmaceutica, kindly supplied us with pimozide.

June 6, 1975

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The involvement of serotoninergic and noradrenergic systems in the compulsive gnawing in mice induced by imipramine and apomorphine

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Antidepressant agents such as imipramine and amitriptyline alter the effect of apomorphine from running and licking to intense gnawing behaviour in mice, which has been termed as "potentiation" (Pedersen, 1967). This action was considered due to enhancement of dopaminergic and inhibition of cholinergic systems in the central nervous system (Pedersen, 1967, 1968). Imipramine has been shown to block both 5-hydroxytryptamine (5-HT) and noradrenaline uptake at the neuronal levels (Carlsson, Corrodi & others, 1969). Friedman, Shopsin & others (1974) have shown that serotoninergic rather than adrenergic neuronal systems are involved in the antidepressant effects of imipramine. These results suggest that both catecholamines and 5-HT may play an important role in the compulsive gnawing syndrome produced by apomorphine in combination with imipramine.

The present studies were, therefore, designed to re-evaluate the importance of serotoninergic and adrenergic systems in the imipramine-apomorphine induced gnawing behaviour, by using various agents known to modify synthesis and storage of 5-HT and noradrenaline.

The gnawing activity was measured in mice of either sex, 19–21 g, in groups of 6, in a cage with corrugated paper covering the floor (Ther & Schramm, 1962). After administration of the test substance intraperitoneally, apomorphine (10 mg kg^{-1}) was injected subcutaneously at varying intervals. Imipramine (60 mg kg^{-1}) was injected 15 min before apomorphine. The bites in the paper caused by gnawing were counted for 10% of the total surface and the mean and s.e. of at least 6 groups of animals per test compound were calculated.