Lack of supersensitivity to L-5-hydroxytryptophan following chronic methysergide treatment

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Supersensitivity to 5-hydroxytryptamine (5-HT) precursors and agonists after destruction of central 5-HT nerve terminals, but not after chronic 5-HT depletion by synthesis inhibition, has been reported by Trulson, Eubanks & Jacobs (1976a) and Trulson, Ross & Jacobs (1976b) using rats. This is unlike findings with the central dopamine system, in which both chronic depletion of dopamine following inhibition of its synthesis and nerve terminal destruction, produce supersensitivity (Dominic & Moore, 1969; Thornburg & Moore, 1973). Chronic receptor blockade also produced supersensitivity in the central dopamine system (Klawans & Rubovits, 1972; Tarsy & Baldessarini, 1974). In an attempt to parallel these latter two studies, we have tested the effects of chronic 5-HT receptor blockade on the behavioural response to L-5-hydroxytryptophan (L-5-HTP) as an additional probe into the nature of the mechanisms mediating supersensitivity in these two systems.

A behavioural syndrome that specifically reflects the activity in central 5-HT mediated synapses (Jacobs, 1976) was used to test for supersensitivity to L-5-HTP. Behavioural observations were made with the rats placed individually in round plastic buckets (20 cm high × 35 cm in diameter) with metal screen lids and sawdust covering the floor. After L-5-HTP administration, the rats were examined for the presence of the following signs: resting tremor (especially of the head and forelimbs); rigidity or hypertonicity (evaluated by grasping the rat around the body and by flexing the hindlimbs); hindlimb abduction (a splaying out of the hindlimbs); Straub tail; lateral head weaving (slow side-to-side movements of the head); and reciprocal forepaw treading (rhythmic dorsoventral movements of the forelimbs). If at least four of these six signs were present, the syndrome was rated "present".

Male Sprague-Dawley rats (250–300 g) received daily subcutaneous injections of methysergide maleate (1 or 10 mg kg^{-1} day^{-1}) or saline for 14 consecutive days. Subgroups were then administered L-5-HTP (125, 150, 175 or 200 mg kg^{-1}), at 24 or 72 h after the last injection of methysergide. The presence or absence of the behavioural syndrome was then scored during the hour following L-5-HTP injection. ED50 values were estimated by probit analysis (Bliss, 1952). The statistical significance between control and experimental groups was evaluated using Student's *t*-tests. That the 10 mg kg^{-1} dose of methysergide was effective in blocking the behavioural effects studied in the present work was reported by Jacobs (1974).

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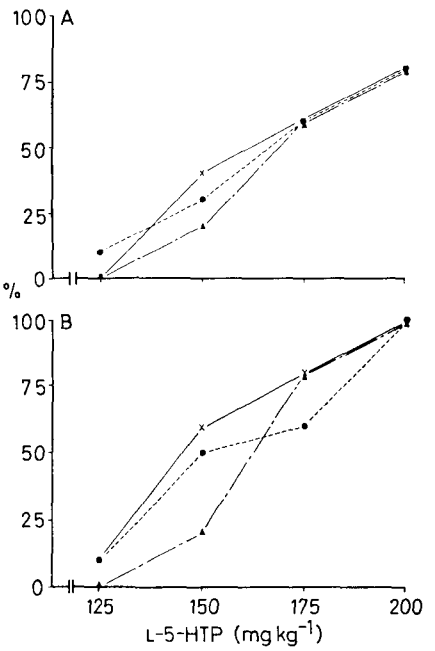

FIG. 1. Effects of various doses of L-5HTP in producing the syndrome A–24 h, B–72 h after termination of chronic methysergide (×—×, 1 mg kg^{-1} day^{-1}; ▲- -▲, 10 mg kg^{-1} day^{-1}) or saline (●- - -●) administration. Each point represents the % of the animals displaying the syndrome at each dose of L-5HTP. n = 10 for the saline group and n = 5 in all other cases.

Chronic administration of methysergide produced no evidence for supersensitivity to L-5-HTP. After the final injection of methysergide, the ratios of ED50 values for control *vs* methysergide-treated rats were at 24 h—1·02 and 0·98, and at 72 h 1·05 and 0·98, for the 1 and 10 mg kg^{-1} doses of methysergide respectively (Fig. 1A, B). The overall response to L-5-HTP in rats chronically administered methysergide was not significantly different from control rats in any of the four groups ($P>0·2$).

We previously reported that destruction of central 5-HT nerve terminals with intraventricular 5,7-dihydroxytryptamine (5,7-DHT) produced a dramatic supersensitivity to 5-HT precursors and agonists (Trulson & others, 1976a, b). Chronic inhibition of 5-HT synthesis with *p*-chlorophenylalanine, however, produced no evidence for supersensitivity. We therefore hypothesized that the supersensitivity that develops after 5,7-DHT administration may be dependent upon destruction of the presynaptic nerve terminal

with little or nor concomitant change in the sensitivity of the postsynaptic receptor. The present finding that chronic blockade of 5-HT receptors also produces no supersensitivity supports this hypothesis, but is inconsistent with the finding in guinea-pigs that chronic administration of methysergide, using one of the pretreatment regimens used in the present study (1 mg kg^{-1} day^{-1} for 14 days), produced a significant supersensitivity to L-5-HTP, using myoclonus as a behavioural index (Klawans, D'Amico & Patel, 1975).

Although 5-HTP is known to release central catecholamines, this is not a factor in preventing the development of supersensitivity in the present study since 5-HTP also releases central catecholamines in rats pretreated with 5,7-dihydroxytryptamine, but a very dramatic supersensitivity to 5-HTP is observed in these animals (Trulson & others, 1976a). Furthermore, 5-methoxy-*NN*-dimethyltryptamine, (5-MDMT), a direct-acting 5-HT agonist which does not release catecholamines, does not elicit a supersensitive response in rats chronically treated with the tryptophan hydroxylase inhibitor, *p*-chlorophenylalanine (Trulson & others, 1976a) nor does it elicit a supersensitive response in rats chronically treated with methysergide.

In contrast to the lack of supersensitivity after chronic 5-HT receptor blockade or synthesis inhibition, a pronounced supersensitivity occurs following receptor blockade or synthesis inhibition in the dopamine system. Chronic pretreatment with dopamine receptor blockers such as haloperidol, potentiates amphetamine- or apomorphine-induced stereotyped behaviour (Klawans & Rubovits, 1972; Tarsy & Baldessarini, 1974). Also catecholamine depletion produced by synthesis inhibition potentiates the behavioural response to catecholamine precursors and agonists (Dominic & Moore, 1969; Thornburg & Moore, 1973). Together, these data suggest that the enhanced responsiveness to dopamine precursors and agonists after the various treatments is a manifestation of supersensitive postsynaptic dopamine receptors. Direct neurochemical evidence of an increase in adenyl cyclase activity associated with dopamine receptors following nerve terminal destruction or chronic dopamine receptor blockade also supports this hypothesis (Palmer, 1972; Huang, Ho & Daly, 1973).

Several other lines of evidence suggest that there are fundamental differences between central dopaminergic and 5-HT systems. For example, there is convincing evidence that the activity of dopaminergic neurons of the substantia nigra are regulated by a neuronal feedback (Aghajanian & Bunney, 1974), while this does not appear to be so for 5-HT-containing raphé neurons (Mosko & Jacobs, 1977). In addition, catecholamine synthesis is regulated by end-product inhibition (Neff & Costa, 1966; Spector, Gordon & others, 1967), while 5-HT synthesis appears to lack such a regulatory process (Jequier, Robinson & others, 1969; Lin, Neff & others, 1969). Furthermore, the local availability of dopamine in nerve endings is regulated by a presynaptic receptor-mediated process (Kehr, 1974; Carlsson, 1975), while, again, this apparently is not a feature of 5-HT neurons (Andén, Fuxe & Hokfelt, 1966; Bedard, Carlsson & Lindquist, 1972). The present finding coupled with our previous report that supersensitivity does not develop in the 5-HT system after either chronic receptor blockade or 5-HT depletion may be indicative of another basic difference between catecholamine and 5-HT neurons in the mammalian central nervous system.

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Lack of effect of chronic haloperidol administration on the prolactin-lowering actions of piribedil

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If impulse traffic along a neuronal tract is interrupted either surgically or pharmacologically for a prolonged time, post-synaptic supersensitivity can develop to the neurotransmitter involved (Trendelenburg, 1966; Fleming, McPhillips & Westfall, 1973). This phenomenon has been demonstrated in central dopaminergic systems after a variety of treatments. Ungerstedt (1971) reported that 6-hydroxydopamine-induced destruction of nigrostriatal dopamine neurons in rats causes supersensitivity to the behavioural effects of apomorphine, a drug believed to act directly on dopamine receptors (Andén, Rubenson & others, 1967). Withdrawal of a chronic diet of α-methyltyrosine, a tyrosine hydroxylase inhibitor, in mice leads to an enhanced response to the locomotor stimulant effects of (+)-amphetamine, which is thought to exert these effects by increasing dopamine concentrations at central receptors (Dominic & Moore, 1969) and of apomorphine, a direct acting dopamine agonist (Gudelsky, Thornburg & Moore, 1975). Finally, prolonged treatment of rats with a variety of neuroleptics induces supersensitivity to the behavioural actions of apomorphine (Gianutsos, Drawbaugh & others, 1974; Tarsy & Baldessarini, 1974). Electrophysiological evidence for the development of supersensitivity of dopamine receptors in the caudate nucleus after chronic impulse interruption has also been presented (Siggins, Hoffer & Ungerstedt, 1974; Yarbrough, 1975).

Since prolactin release from the anterior pituitary is under tonic inhibitory control by tuberoinfundibular dopamine neurons (MacLeod, 1976), the measurement of serum prolactin concentrations is an easily accessible, easily quantifiable estimate of tuberoinfundibular dopamine activity. Agents which increase central dopamine activity lower circulating prolactin concentrations, while agents which reduce this activity increase serum prolactin (Neill, 1974; MacLeod, 1976; Mueller, Simpkins & others, 1976).

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We set out to determine whether supersensitivity to the prolactin-lowering effects of piribedil (Mueller & others, 1976), a direct-acting dopamine agonist (Corrodi, Farnebo & others, 1972), occurs after chronic dopamine receptor blockade by haloperidol. If supersensitivity does develop, the dose-response curve for piribedil-induced lowering of prolactin in neuroleptic-treated animals will show a shift to the left as compared with animals receiving vehicle chronically. This would be presumptive evidence of similarity between dopamine receptors involved in the control of prolactin secretion and other central dopamine receptors.

A problem encountered when measuring decreases in prolactin concentrations in male rats is that basal hormone concentrations are already so low that dose-related decreases cannot accurately be determined using the prolactin radioimmunoassay. This problem was avoided by pretreating all animals with α-methyltyrosine 30 min before injecting piribedil. This tyrosine hydroxylase inhibitor effectively increases circulating prolactin concentrations by decreasing endogenous dopamine without blocking postsynaptic dopamine receptors and therefore would not be expected to interfere with the prolactin-lowering actions of piribedil, as these actions are exerted through direct stimulation of the postsynaptic dopamine receptors.

Male Sprague-Dawley rats (Spartan Research Animals, Haslett, MI) 150–175 g, received haloperidol (McNeil Laboratories; 2·5 mg kg^{-1}, s.c.) every 12 h for 7 consecutive days, then haloperidol (5·0 mg kg^{-1}, s.c.) every 12 h for an additional 7 days. This schedule of haloperidol administration is more intensive than those used previously to demonstrate an enhanced response to apomorphine (Tarsy & Baldessarini, 1974; Yarbrough, 1975), a drug having dopaminergic agonistic properties which are similar to those of piribedil (Corrodi & others, 1972; Thornburg & Moore, 1974, 1975). A second group of rats received vehicle (0·3 % tartaric acid) twice daily for 14 consecutive days. 72 h after the last injection, rats received DL-α-methyltyrosine