The actions of neuroleptic drugs and putative serotonin receptor antagonists on LSD and quipazine-induced reductions of brain 5-HIAA concentrations

J. H. JACOBY*, J. J. POULAKOS, *Department of Pharmacology, College of Medicine and Dentistry of New Jersey, New Jersey Medical School, 100 Bergen Street, Newark, New Jersey 07103, U.S.A.*

LSD and quipazine reduce brain 5-hydroxyindoleacetic acid (5-HIAA) concentrations (Rosecrans, Lovell & Freedman, 1967; Freedman & Boggan, 1974; Grabowska, Antkiewicz & Michaluk, 1974a; Fuller, Snoddy & others, 1976; Hamon, Bourgoin & others, 1976; Jacoby, Howd & others, 1976) presumably as a result of inhibitory feed-back mechanisms initiated by stimulation of serotonin (5-HT) receptors [although contributing to the quipazine effect is a blockade of 5-HT reuptake and a slight inhibition of monoamine oxidase (Medon, Leeling & Phillips, 1973; Green, Youdim & Grahame-Smith, 1976; Fuller & others, 1976; Hamon & others, 1976; Jacoby & others, 1976)]. Conversely, blockade of 5-HT receptors with methiothepin leads to an elevation of brain 5-HIAA concentrations (Monachon, Burkard & others, 1972; Fuller & Perry, 1974; Jacoby, Shabshelowitz & others, 1975), a biochemical event compatible with a compensatory increase of 5-HT synthesis subsequent to receptor blockade. However, other putative 5-HT receptor antagonists such as cyproheptadine and methysergide do not initiate such a compensatory response (D'Amico, Patel & Klawans, 1976; Jacoby & Bryce, 1976), although methysergide at doses far higher than those commonly used does accelerate the accumulation of 5-HT after monoamine oxidase inhibition (Sofia & Vassar, 1975). Thus, we have suggested (Jacoby & Bryce, 1976 recently Jacoby, Poulakos & Bryce, 1977) that if indeed cyproheptadine and methysergide are central 5-HT antagonists, then they act upon receptors which differ from those initiating a feed-back loop. To further test this hypothesis we have attempted to prevent the LSD- or quipazine-induced decrease of brain 5-HIAA concentrations by prior treatment with putative 5-HT receptor antagonists. Furthermore, since LSD (Von Hungen, Roberts & Hill, 1974) and quipazine (Grabowska, Antkiewicz & Michaluk, 1974b) exert at least some of their actions by stimulating dopamine receptors, we have also attempted to study the effects of prior treatment with dopamine antagonists on the reduction of brain 5-HIAA concentrations seen subsequent to administration of these latter two drugs.

Male Sprague-Dawley rats (Marland Breeding Farms, Hewitt, N.J.) fasted overnight were used to study the effects of the agonists on brain 5-HIAA concentrations. For the study of drug interaction with LSD, the antagonists were given 15 min before the hallucinogen, and animals were killed 60 min after the second injection. Brains were quickly removed, frozen on dry ice and subsequently assayed for 5-HT and 5-HIAA (Curzon & Green, 1970). Since quipazine can interfere with the fluorimetric assay of 5-HT (Green & others, 1976), but not 5-HIAA (although the increase of brain 5-HT obtained by the use of a non-interfering assay yielded results which were compatible with other published results, Fuller & others, 1976; Hamon & others, 1976; Jacoby & others, 1976) we modified our experimental design to study the effects of putative receptor antagonists on the quipazine-induced impairment of brain 5-HIAA accumulation after probenecid administration. Thus, animals were administered either quipazine or antagonists, or a combination of both drugs 10 min before probenecid (200 mg kg^{-1}, i.p.) and killed 60 min after the second injection.

LSD, as previously reported (Rosecrans & others, 1967; Freedman & Boggan, 1974) reduced brain

Table 1. *Effect of receptor antagonist pretreatment on LSD-induced reduction of brain 5-hydroxyindoles.* Animals (n = 6) were pretreated with saline or one of the following receptor antagonists (i.p.): methysergide (3 mg kg^{-1}), cyproheptadine (5 mg kg^{-1}), metergoline (5 mg kg^{-1}), cinanserin (25 mg kg^{-1}), propranolol (40 mg kg^{-1}), chlorpromazine (10 mg kg^{-1}), clozapine (10 mg kg^{-1}) or haloperidol (10 mg kg^{-1}) 15 min before LSD (1 mg kg^{-1}, i.p.) and killed 60 min after the latter injection.

Treatment	Brain 5-HT ng g^{-1} (mean ± s.e.m.)	Brain 5-HIAA ng g^{-1} (mean ± s.e.m.)
Saline—saline	522 ± 32	522 ± 29
Saline—LSD	583 ± 25	371 ± 8[a]
Methysergide—LSD	581 ± 25	386 ± 11
Cyproheptadine—LSD	559 ± 16	347 ± 10
Metergoline—LSD	579 ± 48	358 ± 28
Cinanserin—LSD	548 ± 17	364 ± 19
Propranolol—LSD	595 ± 12	368 ± 11
Chlorpromazine—LSD	604 ± 12	418 ± 10[b]
Clozapine—LSD	596 ± 8	419 ± 13[b]
Haloperidol—LSD	522 ± 17	459 ± 18[c]

[a] $P < 0.001$ compared with saline-treated animals.
[b] $P < 0.05$ compared with saline–LSD treated animals.
[c] $P < 0.001$ compared with saline–LSD treated animals.

* Correspondence.

5-HIAA concentrations (Table 1). We did not observe a concomitant increase of brain 5-HT. The commonly utilized 5-HT receptor antagonists, methysergide, cyproheptadine, methergoline and cinanserin, did not prevent the reduction of 5-HIAA. Recently, propranolol has also been reported to act as an effective 5-HT receptor antagonist at high doses (Green & Graham-Smith, 1976) and to bind to 5-HT receptors *in vitro* (Middlemiss, Blakeborough & Leather, 1977), yet this drug was also unable to prevent the 5-HIAA-lowering effect of LSD. (Propranolol is also ineffective in blocking LSD-stimulated adenylate cyclase: Von Hungen & others, 1974). However, pretreatment with neuroleptic compounds that exert a primary action by blocking dopamine receptors, i.e., haloperidol, chlorpromazine and clozapine, did interfere with the LSD-induced reduction of brain 5-HIAA concentrations. Haloperidol appeared to be most effective in exerting this blockade.

Similar results were achieved when the effects of these drugs on quipazine-induced alterations of brain 5-HIAA accumulation following probenecid were studied (Table 2). Quipazine, as expected, reduced the accumulation of 5-HIAA after probenecid. The putative 5-HT receptor antagonists cyproheptadine, methysergide, methergoline and cinanserin, had no effect on this response. Haloperidol, as observed when administered before LSD, interfered with the actions of quipazine on 5-HIAA accumulation. However, chlorpromazine and clozapine were ineffective in blocking this action of quipazine.

Table 2. *Effect of receptor antagonist pretreatment on quipazine-induced alterations of brain 5-HIAA accumulation following probenecid.* Animals were pretreated with quipazine (10 mg kg^{-1}, i.p.) and saline or one of the following receptor antagonists (i.p.): methysergide (3 mg kg^{-1}), cyproheptadine (5 mg kg^{-1}), cinanserin (25 mg kg^{-1}), chlorpromazine (10 mg kg^{-1}), clozapine (10 mg kg^{-1}), haloperidol (10 mg kg^{-1}) followed 10 min later by an injection of probenecid (200 mg kg^{-1}, i.p.) and killed 60 min after the latter injection. Student's *t*-test was used to determine differences between probenecid treated animals receiving saline or drug pretreatment.

Treatment	Brain 5-HIAA ng g^{-1} (mean ± s.e.m.)	% of control value
Saline	686 ± 45	—
Saline + quipazine	557 ± 26[b]	81
Methysergide + quipazine	554 ± 27[b]	81
Cyproheptadine + quipazine	581 ± 26[c]	85
Cinanserin + quipazine	524 ± 32[b]	76
Saline	696 ± 36	—
Chlorpromazine + quipazine	475 ± 20[a]	68
Clozapine + quipazine	564 ± 27[b]	81
Haloperidol + quipazine	658 ± 33	95

[a] $P < 0.001$ compared with animals receiving saline before probenecid.
[b] $P < 0.01$ compared with animals receiving saline before probenecid.
[c] $P < 0.05$ compared with animals receiving saline before probenecid.

The ability of dopamine receptor blockade to interfere with the reduction of brain 5-HIAA accumulation following either LSD or quipazine administration contrasts with the failure of commonly used putative 5-HT receptor antagonists to exert a similar action (Tables 1 and 2). Indeed, other instances of an impairment of the response to LSD by dopamine receptor antagonists have been noted. For example, several neuroleptics have been reported to block the *in vitro* production of cAMP (Von Hungen & others, 1974) and the increase in motor activity (Grabowska, 1974; Kelly & Iversen, 1975; Pieri, Pieri & Haefely, 1974) after LSD. Cyproheptadine and methysergide failed to prevent the LSD stimulation of motor activity (Grabowska, 1974). These findings suggest that the reduction of brain 5-hydroxyindoles after LSD and quipazine may not result from an activation of an inhibitory feed-back loop initiated by 5-HT receptor stimulation, but such a reduction may result from an interaction with dopamine receptors that then act in some undefined manner to impair transmitter release from 5-HT-containing neurons. Such an hypothesis is in apparent contradiction of observations showing that stimulation of dopamine receptors with apomorphine results in an elevation of brain 5-hydroxyindoles (Grabowska, 1975) an effect which is also blocked by dopamine receptor antagonists. While our results do not present evidence to suggest that the putative 5-HT antagonists used are ineffective as central 5-HT blocking agents (these agents do not block the depressant effects of 5-HT in several brain areas; Haigler & Aghajanian, 1974) they do bring into question as recently suggested (Mosko & Jacobs, 1977), the existence of a 5-HT-feed-back loop. This doubt arises because of the present demonstration that dopaminergic but not 5-HT antagonists block the action of LSD and quipazine on brain 5-HIAA, as well as observations by others showing that manipulations leading to decreased synaptic catecholamine availability increase brain 5-HIAA concentrations (Johnson, Kim & Boukma, 1972; Blondaux, Juge & others, 1973; Kostowski, Samanin & others, 1974; Stein, Jouvet & Pujol, 1974). Furthermore, stimulation of noradrenergic receptors with clonidine impairs the methiothepin-induced elevation of 5-HIAA concentrations (Lloyd & Bartholini, 1974) (an effect commonly attributed to an action upon 5-HT receptors).

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2, 4-Dinitrophenol inhibition of transport of 5-hydroxyindoleacetic acid from the cerebrospinal fluid and spinal cord

BRANIMIR ŽIVKOVIĆ, MARIN BULAT*, *Institute 'Rudjer Boškovic', Bijenička c. 54, 41001 Zagreb, Yugoslavia*

5-Hydroxyindoleacetic acid (5-HIAA) in the lumbar cerebrospinal fluid (csf) of patients is often analysed to obtain insight into metabolism of 5-hydroxytryptamine in the central nervous system. Recent experiments indicate that this acid in the lumbar csf is derived from the adjacent spinal cord (Bulat, Lacković & others, 1974; Jakupčević, Lacković & others, 1977) rather than from the brain (Bulat, 1977) or blood (Bulat & Živković, 1973). Probenecid is a competitive inhibitor of 5-HIAA transport from the spinal cord (Bulat, 1974), lumbar csf and cisternal csf (Živković & Bulat, 1971; Wolfson, Katzman & Escriva, 1974). To find if this transport of 5-HIAA requires metabolic energy derived from ATP we treated cats with 2,4-dinitrophenol which inhibits the formation of ATP by uncoupling oxidative phosphorylation (Davson, 1967).

Adult cats (2·5–3·5 kg) of either sex were lightly anaethetised with thiopentone sodium anaesthesia (50 mg kg^{-1}, i.p.). Laminectomy was performed at the lumbar (L5-L7 vertebrae) and thoracic (T11 vertebra)

* Correspondence.