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Studies on the Specificity of Neurochemical and Behavioral Effects of LSD-25

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SPARBER, S B , L P DWOSKIN AND M S KLEVEN *Studies on the specificity of neurochemical and behavioral effects of LSD-25* PHARMACOL BIOCHEM BEHAV **24**(2) 341–345, 1986 —Brain perfusion experiments of conscious rats engaged in operant behavior and administered fluoxetine or LSD, with or without prior injection of 5-HTP, indicate there is probably more than one functional pool of 5-HT in the CNS Furthermore the fact that prior loading with the precursor is necessary before unmasking an effect of LSD suggests the LSD-sensitive pool is newly synthesized and represents only a small fraction of total CNS serotonin Separating the effects of LSD's behavioral action into pausing (disruption) and depressed responding rate, with or without pausing, enabled us to demonstrate blockade of the disruption by methysergide without blockade of the decreased responding rate Mianserin blocks both effects of LSD's action We suggest that behavioral effects of low doses of LSD are due to sympathetic arousal and may offer a model for agitated depression and/or anxiety and that drugs of the mianserin-type may prove useful for treating some forms of anxiety, as well as depression

LSD-25 5-HTP Neurochemical effects Behavioral effects

THE complex manner in which serotonergic neurotransmission may be altered by acute or chronic drug treatment is being studied at the biochemical level by many laboratories The importance of reuptake recognition and carrier sites [28], the involvement or coupling of serotonergic transmission to β-adrenergic receptors and β-stimulated cyclic 3′,5′-AMP formation [33] and the relevance of other up- and down-regulatory processes for presumptive endogenous modulatory ligands [5, 15, 33] are three avenues of approach for trying to determine the importance of this transmitter in the etiology of some affective disorders and in trying to understand the disparity between the immediate biochemical actions of most antidepressant drugs and the requirement that chronic treatment is invariably necessary before a therapeutic response emerges The additional recent demonstrations of at least two serotonin receptors [17, 20, 25, 26] that may mediate functional changes of a different type (e g , control release of transmitter from serotonin neurons, inhibit, excite or facilitate postsynaptic effector neuronal activity), have further complicated the lives of psychopharmacologists and neuropharmacologists interested in hallucinogenic drugs such as lysergic acid diethylamide (LSD) and mescaline These agents have been promoted over the years as both serotonergic antagonists and agonists Our own interests date back to the late 1960's and early 1970's and were predated and instigated in part by the earlier work of Freedman and coworkers [1–4, 11, 12] In fact, when we initially started to use these drugs, they had attained "tool" status and our first studies were designed to devise a procedure for quantifying the typical hallucinogen-induced behavioral pausing by animals maintained on fixed ratio schedules of reinforcement [2,31] so that behaviorally "equivalent" doses could be used in dose-response studies of the behavioral and neurochemical effects of these and other psychotropic drugs We hoped that some of the mechanisms of action of the major psychotropic drugs could be studied *in vivo* by combined use of concurrent behavior analysis and brain perfusion methods The experimental designs and methods employed proved almost too sensitive because, instead of validating the similar and selective actions of LSD and mescaline upon "release" of serotonin and its metabolite (5-HIAA) into push-pull perfusate, these two drugs seemed, at behaviorally equivalent doses, to act differently from each other Not only did these hallucinogens differentially affect the appearance of radiolabeled 5-HT (and metabolites) in perfusate [34], subsequent studies indicated that their effects upon the pattern of behavior maintained by a fixed interval schedule of reinforcement were somewhat different [35] Furthermore, the potential utility of these drugs as tools for studying the acute and chronic effects of other psychotropic drugs was borne out by the demonstration that chronic amphetamine treatment modified 5-HT neurotransmission at a dose which had no apparent effect acutely After chronic treatment and tolerance to its behavioral suppressing action, amphetamine "released" radiolabeled 5-HT in addition to its expected "releasing" effect upon radiolabeled norepinephrine Mescaline produced a significantly greater effect upon [^{14}C]5-HT after chronic amphetamine relative to the hallucinogen's initial effect upon this transmitter [32] This early demonstration of a modulatory interaction between the two transmitters predated by several years the morphological and biochemical

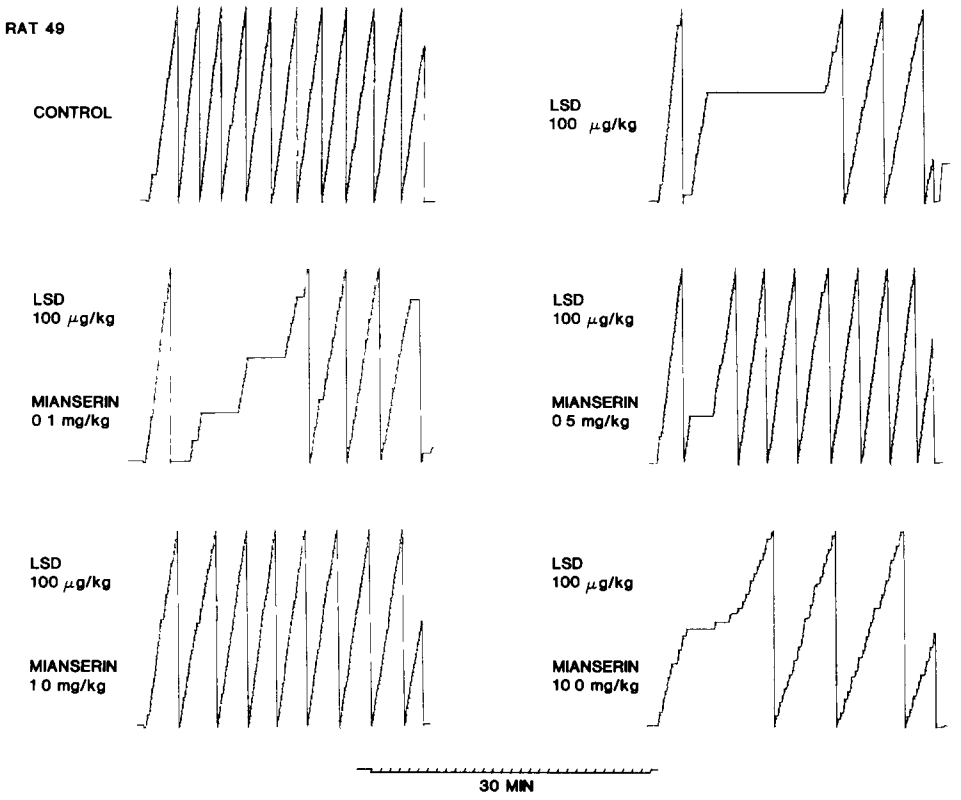

FIG 1 Representative cumulative records for rat 49 illustrating the effect of LSD (100 μg/kg) upon fixed ratio 15 responding for food and antagonism of the LSD-induced disruption (pausing) and decreased responding Mianserin (0 1–10 mg/kg, IP) was administered 30 min prior to the operant session and LSD was administered immediately prior to the session While a combination of 10 mg mianserin/kg plus 100 μg LSD/kg produced a behavioral effect reminiscent of a lower dose of LSD (e g , 50 μg/kg) alone, 2 5 or 5 0 mg mianserin/kg completely blocked the disruption and decreased responding rate

data which also predict such a functional interaction [24,27]

While we were encouraged by the relative replication and extension of much of the work comparing and contrasting the phenethylamine hallucinogens with the indoleamine hallucinogens by Rech and coworkers [29], who also concluded there were fundamental differences in the mechanism whereby these two classes of hallucinogens acted to alter fixed ratio (pausing) behavior, they nevertheless reinforced the prevalent idea that alterations in 5-HT neurotransmission was somehow mediating the effects of these drugs We were surprised when, with the availability of HPLC coupled to electrochemical detection, we were unable to confirm our original observation that LSD diminished the amount of 5-HT (and metabolite) in push-pull perfusate One might argue that pulse-labeling CNS stores with [^{14}C]5-HT was potentially too nonspecific and could have been taken up by nonserotonin neurons This possibility is unlikely because the prime alternative candidates for uptake are the catecholamine neurons and behaviorally equieffective acute doses of amphetamine did not "release" [^{14}C]5-HT [32,34] Furthermore, Freedman's group demonstrated small, but significant increases in steady state levels of 5-HT and decreases in 5-HIAA [30], suggesting diminished utilization Gallager and Aghajanian [14] reported that radiolabeled 5-HT, synthesized from radiolabeled tryptophan (specific for serotonergic neurons) was reduced in lateral ventricular perfusate (same site as our perfusion) after LSD treatment After determining that fluoxetine, a selective 5-HT reuptake inhibitor [37], could reduce levels of endogenous 5-HIAA in push-pull perfusate [18], we were convinced that an alteration in serotonergic neurotransmission, brought about by other 5-HT-selective drugs, should have been reflected by some sort of change in 5-HIAA levels in perfusate Our inability to induce a change in 5-HIAA in perfusate by such a potent psychoactive compound as LSD, which has a different psychopharmacological profile than fluoxetine, was somewhat encouraging, but it did not resolve the above mentioned source of our consternation The apparent resolution of this problem, which also helps explain several of the aforementioned observations (e g , a rather small maximum change in steady-state levels of 5-HT/5-HIAA after LSD), was prompted by the fact that Gallager and Aghajanian [14] studied newly synthesized, radioactive serotonin (by definition) and that earlier studies by Glowinski *et al* [16] suggested that intraventricularly injected radiolabeled biogenic amines were taken up and stored in a readily releasable pool This indicated that the amount of 5-HIAA contributed by the LSD-sensitive pool may have been too small, relative to the total pool or source of 5-HIAA in ventricular perfusate, to discriminate even large changes brought about

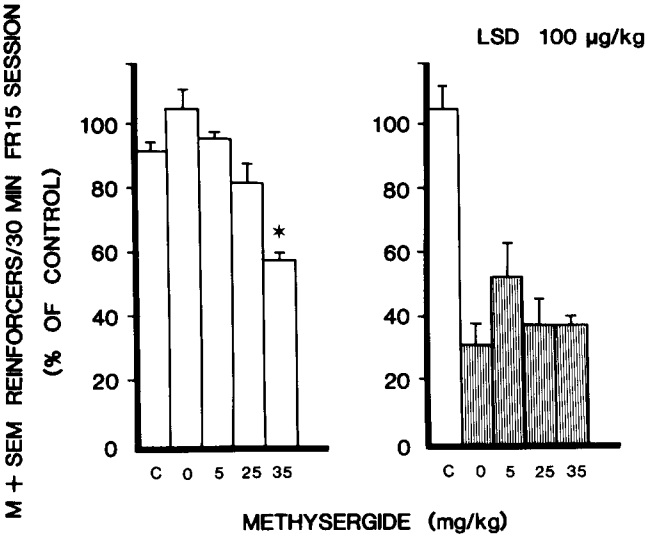

FIG 2A Lack of antagonism of the behavioral suppressant (responding rate) effect of LSD by methysergide Data are expressed as the percent of the number of reinforcers earned by rats engaged in fixed ratio 15 responding on the control days prior to drug administration days Clear bars illustrate the effect of methysergide (5 0–35 0 mg/kg, IP) 30 min prior to the 30 min sessions Striped bars illustrate the effect of LSD (100 µg/kg) administered immediately prior to the session C designates the experimental condition in which only vehicles were administered *$p<0$ 001 by Duncan's test

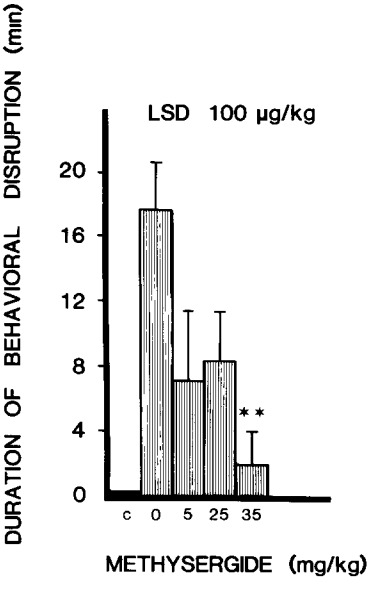

FIG 2B Methysergide antagonized the disruption in responding (pausing, see Fig 1) induced by LSD (100 µg/kg) The measure of behavioral disruption is the number of min taken to earn 5 reinforcers after a period of 2 min during which no reinforcers were earned C designates the experimental condition in which only vehicles were administered, and the thickened abscissa indicates there was no disruption of responding Lower doses of methysergide (0 1–5 0 mg/kg) showed partial antagonism of the disruption without significantly antagonizing the decreased responding rate **$p<0$ 01 compared to LSD alone, by Duncan's test

by injection of LSD If it was a newly synthesized pool (i e , perhaps functionally more active), then small changes in total CNS transmitter may have attended dramatic functional (behavioral) changes Furthermore, such a newly synthesized pool could be easily released by precursor loading Indeed, shortly after IP injection of rats with a small, behaviorally inactive, dose of 5-HTP (i.e , 5 mg/kg), the content of 5-HIAA in brain perfusate was significantly elevated As predicted, LSD was then capable of reducing the content of 5-HIAA, confirming the previous work and demonstrating there are at least two, pharmacologically distinct, functional pools of this transmitter in the CNS [18,22] It also helps interpret the rather meager changes reported by Freedman's group [30] and suggests that the LSD-sensitive pool may comprise only a small percentage of the total CNS content of 5-HT, at any time, under normal conditions

We should now like to address the nature of the selectivity or unitary mechanism whereby LSD produces its behavioral effects in animals Early binding, neurophysiological studies raised some doubt about a unitary mechanism (receptor-mediated) action of LSD While it may be argued that LSD mimics serotonin, with high affinity for presynaptic (auto)-receptors and perhaps lower affinity for postsynaptic receptors, it is thought that most neuronal inhibitory actions of 5-HT (and LSD) are not blockable by even the newer, so-called second generation antagonists The demonstration that 5-HT also has excitatory activity in the CNS, which is blockable with some but not all 5-HT antagonists, as well as with LSD [17,21], raises some interesting questions relating to the site (pre- vs postsynaptic) and type (agonist vs antagonist) actions responsible for LSD's behavioral effects Cogent arguments for a postsynaptic site of LSD's action have been put forth, since destruction or loss of function of serotonergic neurons do not block LSD's action but may in fact enhance it [2,4] The original suggestion that LSD is a serotonin antagonist in peripheral organs [13] and that it may be a partial agonist (partial antagonist) or a true antagonist in the CNS, speaks to the potential, numerous ways in which LSD may affect behavior Our recent experiments and a review of recent literature lead us to believe that LSD acts upon at least two receptors and/or recognition sites to affect function The most parsimonious interpretation of the presynpatic or autoreceptor effects, which cannot be discounted as a site of action, and the postsynaptic receptor effects argues for an agonist action of LSD on the former, and an antagonist (i e , 5-HT-blocking) action on most of the latter There may also be postsynaptic receptors which recognize LSD as a 5-HT agonist but respond with excitation [21], rather than inhibition, as do the serotonin autoreceptors The relative distribution and type of receptors (including nonserotonergic) may determine the behavioral profile or actions of LSD at a given dose and the selectivity or specificity of drugs which may antagonize such behavioral actions Depending upon the sensitivity of the biobehavioral assay, one might not be able to detect multiple sites of action of LSD, as they are expressed behaviorally For example, while tests for discriminative stimulus properties of LSD may be sensitive to relatively low doses of the

hallucinogen and be amenable to studies with selective 5-HT_2 antagonists, the fact that behavioral pausing (an effect which has almost come to be a hallmark of action of this class of drugs) is not studied, suggests a limited number of LSD-sensitive sites may contribute to its discriminative stimulus properties Indeed, the perceptual distortions and hallucinations caused by these drugs in humans would tend to preclude their ability or willingness to respond "normally" in cognitive and/or psychomotor tests Therefore, such tests may have a limited utility for studying the pharmacology of LSD but may prove fruitful as tests for LSD-antagonists toward some, but not all, of its actions Support for this interpretation is presented by Colpaert and Janssen [8], who argue for a 5-HT_2 site mediating much of LSD's discriminative stimulus properties (in a paradigm which requires emission of an operant for food reinforcement to demonstrate stimulus properties), even though LSD also binds to 5-HT_1 sites

If a biobehavioral assay is insensitive in a different way, because the behavioral endpoint requires a very high dose of drug (e g , the so-called 5-HT syndrome), it too may not allow one to study LSD for multiple sites of action because at lower doses the elicited behavior disappears If some behavioral effects of LSD can be clearly demonstrated at 15–150 μg/kg [35] in the rat, which requires much larger doses to elicit the 5-HT syndrome, it is likely that the latter effect is more easily blockable by less specific drugs, even drugs which render the subject incapable of displaying the behavior In any event, if we assume that LSD acts presynaptically as an agonist on autoreceptors and postsynaptically as an antagonist, the sum of its actions would be a reduction of serotonergic activity in that pathway One should not necessarily expect to see reduced activity of LSD after presynaptic lesioning, especially if up-regulation of postsynaptic 5-HT (mianserin) recognition sites occurs [15] and these sites are LSD-sensitive

While comparing and contrasting the ability of mianserin and methysergide to block the suppressant action of LSD upon fixed ratio behavior [9,10], a serendipitous observation allowed us to study these agents upon two separate components of LSD's action which may reflect different sites or receptor effects At low doses (e g , 50 μg/kg, IP) LSD does not easily produce the typical long pausing alluded to above but tends to suppress fixed ratio behavior Although we have defined pausing as a disruption in responding whereby at least two minutes have elapsed without the rat receiving a reinforcer during responding maintained by fixed ratio schedules [31], it is possible that subliminal (miniature) increases in postreinforcement pausing, qualitatively identical to an obvious disruption (pausing) in behavior, is responsible for the apparent decrease in "running rate " We think this is unlikely due to the nature of the differential antagonist action of a broad range of doses of mianserin and methysergide towards the two actions of LSD Even though high doses of mianserin may mimic LSD in operant [10], or substitute for LSD in stimulus discrimination studies [7], low, behaviorally inactive doses (e g , 0 1–2 5 mg/kg) can completely block both the disruption (pausing) and decreased responding rate after injection of LSD (Fig 1) Pretreatment of rats with methysergide blocked only the disruption in a range of doses from 0 1–35 mg/kg Nevertheless, the number of reinforcers earned (an indirect measure of running rate in the absence of pausing) never exceeded about 50% of control (Fig 2), a value essentially identical to that observed after treating rats with only 50 μg LSD/kg, a dose which did not cause much disruption (as defined by us, vide supra)

If we assume that lower doses of LSD affect mainly sites with higher affinity than those sites responsible for behavioral effects observed at only higher doses (qualitatively different behaviors, not just behaviors of greater intensity), we can conclude that pausing and other behaviors induced by LSD at its low affinity site would be blocked by methysergide-type drugs and that methysergide is more selective than mianserin towards one or more (5-HT) receptors responsible for the pausing However, not knowing which site or receptor(s) are responsible for the entire constellation of LSD's behavioral action, we may conclude that mianserin is a more specific antagonist of LSD

Obviously it is not possible to attribute the capacity of LSD to induce pausing in animals performing an operant as analogous to the hallucinogenic experience in humans, but methysergide may also be hallucinogenic, to some small degree, in humans Therefore, one is tempted to suspect the analogy would hold if it were somehow testable What, then, can we make of the rate-suppressing action of LSD at lower doses and its blockade by mianserin? It has long been known that subhallucinogenic doses of LSD induce an amphetamine-like experience (i e , sympathomimetic action) and similarities between low doses of LSD and moderate doses of amphetamine upon fixed interval responding in rats have been reported [35] One might therefore predict that drugs of the mianserin-type would also be effective in treating symptoms associated with sympathetic arousal state, such as anxiety and opiate withdrawal We have recently found mianserin to be effective in blocking both acute and chronic opiate withdrawal [23] and the behavioral suppression induced by isobutylmethylxanthine [19], a drug used to induce a quasimorphine withdrawal syndrome [6] Since the so-called selective 5-HT_2 or LSD-antagonists also block the discriminative stimulus properties of moderate doses of LSD, and are likewise effective antidepressants, the use of these behavioral approaches may offer some insight into the etiology of some forms of affective disorders which are manifest, in part, by agitation and anxiety They may also offer some heuristic value for predicting efficacy of drugs for treating such disorders

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