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Citalopram Antagonizes the Stimulation by Lysergic Acid Diethylamide of Presynaptic Inhibitory Serotonin Autoreceptors in the Rat Hypothalamus

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ABSTRACT

Slices of rat hypothalamus prelabeled with [^{3}H]-5-hydroxytryptamine ([^{3}H]-5-HT) were superfused and the release of the labeled transmitter was elicited either by electrical stimulation or by fenfluramine. Whereas the electrically stimulated release of [^{3}H]-5-HT was completely abolished by removing calcium from the superfusion medium, the fenfluramine-induced release of [^{3}H]-5-HT was calcium-independent. Methiothepin increased, in a concentration-dependent manner, the [^{3}H]-5-HT release induced by electrical stimulation but had no effect on that elicited by fenfluramine. The ^{3}H-transmitter release elicited by electrical stimulation was inhibited by lysergic acid diethylamide (LSD) in a concentration-dependent manner, but the release induced by fenfluramine was not modified by LSD. The reduction by LSD of [^{3}H]-5-HT overflow elicited by electrical stimulation was antagonized by methiothepin, but unaffected by phentolamine or by sulpiride. Low concentrations (10–1000 nM) of citalopram, a 5-HT uptake inhibitor, antagonized the inhibition by LSD of electrically evoked release of [^{3}H]-5-HT. These concentrations of citalopram did not modify by themselves the overflow of [^{3}H]-5-HT elicited by electrical stimulation. It is concluded that the modulation of [^{3}H]-5-HT release by presynaptic serotonin autoreceptors is not operational when the neurotransmitter is released through a calcium-independent mechanism. The potent presynaptic inhibition by LSD of serotonergic neurotransmission may contribute to the central actions of this drug. The interaction between citalopram and LSD at the level of [^{3}H]-5-HT release does not seem to involve a competitive interaction at the same receptor site. The possibility that neuronal uptake of 5-HT and the presynaptic 5-HT autoreceptor may be linked in a functional manner cannot be excluded.

The existence of presynaptic inhibitory autoreceptors modulating the release of several neurotransmitters has been demonstrated both in the peripheral and in the central nervous system. The noradrenergic system is one of the best studied (for reviews see Langer, 1974, 1977, 1980; Starke, 1977). In the serotonergic system, the presence of presynaptic inhibitory autoreceptors has been shown in hypothalamic synaptosomes (Cerrito and Raiteri, 1979) and in cerebral cortex slices (Göthert and Weinheimer, 1979; Baumann and Waldmeier, 1981). In these two systems, the neurotransmitter appears to regulate its own release *via* a negative feedback mechanism, mediated through the activation of presynaptic inhibitory autoreceptors.

The aim of the present experiments was to compare in rat hypothalamic slices the effects of 5-HT receptor agonists and antagonists on the calcium-dependent release of 5-HT induced by electrical stimulation and that elicited by fenfluramine, which displaces 5-HT from vesicular stores through a calcium-independent mechanism. It is reported here that LSD inhibits the release of [^{3}H]-5-HT elicited by electrical stimulation and that this inhibition is competitively antagonized by methiothepin, a 5-HT receptor antagonist. Furthermore, low concentrations of citalopram, a 5-HT uptake inhibitor, antagonized the inhibitory effect of LSD on electrically evoked overflow of [^{3}H]-5-HT.

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Methods

Male Sprague-Dawley rats (Charles River strain) weighing 180 to 200 g were killed by decapitation and the brains were quickly removed. Slices from hypothalamus of 0.4 mm thickness were incubated for 30 min at 37°C in Krebs' solution containing 0.1 μM [^{3}H]-5-HT creatinine sulphate (Amersham, Paris, France; specific activity 12 Ci/mmol) and bubbled with a mixture of 95% O_2-5% CO_2. The composition of the Krebs' solution was the following (millimolar concentrations): NaCl, 118.0; KCl, 4.7; $CaCl_2$, 1.3; $MgCl_2$, 1.2; NaH_2PO_4, 1.0; $NaHCO_3$, 25.0; glucose, 11.1; Na_2 EDTA, 0.004; and ascorbic acid, 0.11. At the end of the incubation period, one slice was set up in a special chamber and superfused with Krebs' solution, which was continuously oxygenated. The temperature of the superfusate was 37°C and the rate of superfusion was 0.5 ml/min for experiments with electrical stimulation and 1 ml/min for those with fenfluramine. After incubation with [^{3}H]-5-HT, the slices were superfused for 44 min or for 60 min before eliciting release of the labeled transmitter with fenfluramine or electrical stimulation, respectively. Starting 8 min before the first period of stimulation, samples of the superfusate Krebs' solution were collected at 4-min

ABBREVIATIONS: 5-HT, 5-hydroxytryptamine; LSD, lysergic acid diethylamide.

intervals. When electrical stimulation was used each slice was stimulated for 2 min at 60 min (S_1) and 104 min (S_2) after the onset of superfusion, using an electrical field generated in the chamber between two platinum electrodes (3 Hz, rectangular pulses of 20 mA current strength and 2 msec duration). Exposure to fenfluramine (10 μM) was carried out for 2 min at 44 min (S_1) and 88 min (S_2) after the onset of superfusion. At the end of the experiment, the slices were solubilized with 0.5 ml of Toluene 100 (Packard Instrument Company, Inc., Downers Grove, IL). The radioactivity in the slices and superfusate samples was determined by liquid scintillation spectrometry. The first stimulation period (S_1) was always control and the drugs, which were added to the perfusion medium 20 min before S_2, remained present throughout the rest of the experiment. In some experiments, methiothepin, phentolamine, sulpiride or citalopram were added 20 min before the first period of electrical stimulation (S_1) and remained present until the end of the experiment.

The amount of tritium released per 4-min sample was expressed as a fraction of the total tissue tritium content at the onset of the respective collection period. The overflow of tritium induced by electrical stimulation or by fenfluramine was calculated as the total increase of radioactivity above the resting outflow obtained in the sample immediately preceding the onset of stimulation.

In order to quantify drug-induced changes of stimulated tritium overflow, the ratio S_2/S_1 was calculated indicating the ratio of fractional release between the second and the first period of stimulation.

Statistical calculations were performed according to conventional procedures by the use of nonparametric Mann-Whitney test (Siegel, 1956). The following drugs were used: *d*-LSD bitartrate, phentolamine methanesulfonate, fenfluramine, methiothepin, S-sulpiride and citalopram (Lu 10.171).

Results

Differences between the release of [^{3}H]-5-HT evoked by fenfluramine or electrical stimulation from rat hypothalamic slices. As stated under "Methods," the rate of superfusion of the hypothalamic slices was 0.5 ml/min for experiments with electrical stimulation and 1 ml/min for those with fenfluramine because the results were more reproducible under these experimental conditions. This difference of rate of superfusion was shown not to influence the comparison of results between electrical stimulation and fenfluramine. (Separate experiments with electrical stimulation applied at a superfusion rate of 1 ml/min gave results identical with those obtained at 0.5 ml/min; data not shown.)

In the controls, the absolute values of fractional release of [^{3}H]-5-HT elicited by the first period of electrical stimulation or of exposure to fenfluramine (10 μM) (S_1) were, in percentage of the total tissue radioactivity, 1.86 ± 0.07 (n = 39) and 1.97 ± 0.21 (n = 24), respectively.

The fraction of the total tissue radioactivity released in each of two consecutive periods (S_1 and S_2) of either electrical stimulation or exposure to 10 μM fenfluramine did not differ significantly (table 1).

When calcium ions were removed from the Krebs' solution before the second period of stimulation, the release of [^{3}H]-5-HT induced by electrical stimulation was entirely abolished. On the other hand, the release of [^{3}H]-5-HT elicited by fenfluramine was not modified by the absence of calcium in the medium (table 1).

Exposure to methiothepin (0.01–1 μM) 20 min before the second period of electrical stimulation increased in a concentration-dependent manner the stimulation-evoked release of [^{3}H]-5-HT (fig. 1A). These concentrations of methiothepin did not modify the release of [^{3}H]-5-HT induced by 10 μM fenfluramine (fig. 1B). During exposure to 0.01 to 1 μM methiothepin, there were no changes in the spontaneous outflow of radioactivity (data not shown).

Exposure to LSD (0.1–1 μM) markedly decreased the electrically evoked release of [^{3}H]-5-HT (fig. 2A). These concentrations of LSD did not modify the release of [^{3}H]-5-HT induced by 10 μM fenfluramine (fig. 2B). During exposure to LSD in concentrations up to 1 μM, there were no changes in the spontaneous outflow of radioactivity (data not shown).

Inhibition by LSD of the [^{3}H]-5-HT release elicited by electrical stimulation in slices of the rat hypothalamus and interaction of some drugs with LSD. The complete concentration-response curve of LSD on the release of [^{3}H]-5-HT elicited by electrical stimulation is shown in figure 3. Exposure to LSD (0.001–1 μM) before S_2 decreased the stimulation-evoked release of [^{3}H]-5-HT in a concentration-dependent manner. As already mentioned, these concentrations of LSD did not affect the spontaneous outflow of radioactivity.

As shown in figure 3, in the presence of 0.1 μM methiothepin, there was a shift to the right in the concentration-response curve to LSD (0.001–1 μM) on electrically evoked release of [^{3}H]-5-HT. When the concentration of methiothepin was increased to 1 μM the inhibitory effects of LSD (0.001–0.1 μM) were completely antagonized and only the highest concentration of LSD (1 μM) gave a significant inhibition (35%) of the release of [^{3}H]-5-HT evoked by electrical stimulation (data not shown).

Exposure to the *alpha* adrenoceptor blocking agent, phentolamine, at 0.1 μM, did not antagonize the inhibitory effects of LSD on the electrically evoked release of [^{3}H]-5-HT (fig. 4). The concentration of phentolamine used in these experiments (0.1 μM) did not modify, by itself, the overflow of [^{3}H]-5-HT elicited by electrical stimulation, although it antagonized the inhibition of serotonergic neurotransmission elicited by *alpha*-

TABLE 1

Calcium dependency of [^{3}H]-5-HT release elicited by electrical stimulation but not by fenfluramine from slices of the rat hypothalamus

S_1 is the absolute value of fractional release ($\times 10^{-2}$) during the first period of electrical stimulation or exposure to fenfluramine. S_2 is the absolute value of fractional release during the second period of electrical stimulation or exposure to fenfluramine. S_2/S_1 is the ratio of fractional release between the second and the first period of stimulation. Calcium (1.3 mM) was present during S_1 in all experimental groups. When indicated, calcium was removed from the superfusion medium 20 min before S_2. Shown are mean values ± S.E.M. n = number of experiments per group.

Experimental Group	n	Ca^{++}	Fractional Release × 10^{-2}		
			S_1	S_2	S_2/S_1
		mM			
Electrical stimulation	4	1.3 (S_1-S_2)	2.45 ± 0.23	2.45 ± 0.19	1.02 ± 0.08
	3	0 (S_2)	1.91 ± 0.27	0.04 ± 0.04*	0.02 ± 0.02*
Fenfluramine	7	1.3 (S_1-S_2)	2.55 ± 0.28	2.89 ± 0.48	1.16 ± 0.18
	6	0 (S_2)	2.03 ± 0.61	2.80 ± 1.01	1.28 ± 0.11

* $P < .05$ when compared to the corresponding value obtained in the presence of calcium.

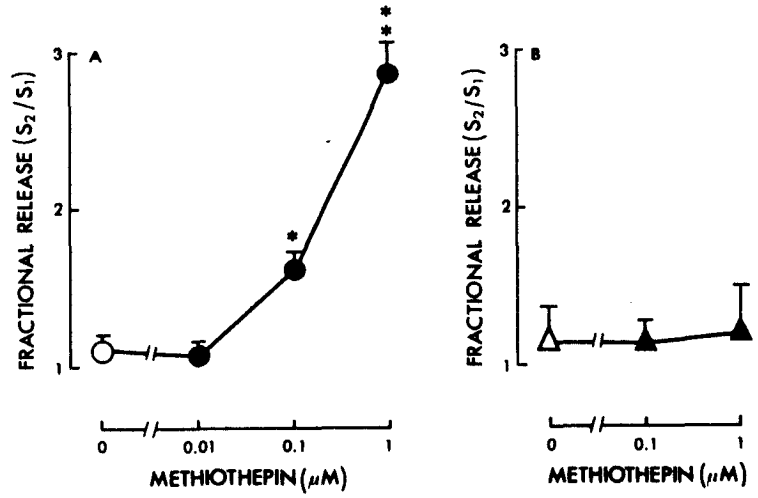

Fig. 1. Effect of methiothepin on [^{3}H]-5-HT release elicited by electrical stimulation or fenfluramine from slices of the rat hypothalamus. A, ordinate: fraction of the total tissue radioactivity released by a 2-min period of electrical stimulation (3 Hz, 20 mA, 2 msec) expressed as the ratio (S_2/S_1) obtained between the second period of stimulation carried out in the presence of the drug (S_2) and the first period (S_1). ○, control; ●, methiothepin. B, ordinate: fraction of the total tissue radioactivity released by a 2-min period of exposure to fenfluramine (10 μM) expressed as the ratio (S_2/S_1) obtained between the second period of exposure to fenfluramine carried out in the presence of the drug (S_2) and the first period (S_1). △, control; ▲, methiothepin. Abscissae: concentrations of methiothepin (micromolar). Methiothepin in the concentrations indicated was added to the medium 20 min before S_2. Each point represents mean values ±S.E.M. of at least four experiments per group. *P < .05; **P < .005 when compared to the corresponding control value. The absolute values of fractional release of radioactivity elicited by electrical stimulation or fenfluramine are shown in table 1.

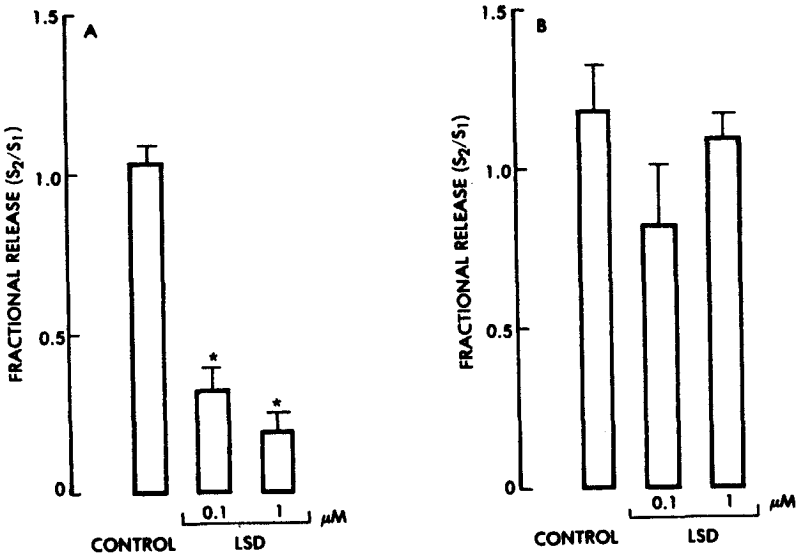

Fig. 2. Effect of LSD on [^{3}H]-5-HT release elicited by electrical stimulation or fenfluramine from slices of the rat hypothalamus. A, ordinate: fraction of the total tissue radioactivity released by a 2-min period of electrical stimulation (3 Hz, 20 mA, 2 msec) expressed as the ratio (S_2/S_1) obtained between the second period of stimulation carried out in the presence of the drug (S_2) and the first period (S_1). B, ordinate: fraction of the total tissue radioactivity released by a 2-min period of exposure to fenfluramine (10 μM) expressed as the ratio (S_2/S_1) obtained between the second period of exposure to fenfluramine carried out in the presence of the drug (S_2) and the first period (S_1). LSD in the concentrations indicated was added to the medium 20 min before S_2. Shown are mean values ± S.E.M. of at least six experiments per group. *P < .001 when compared to the corresponding control value. The absolute values of fractional release of radioactivity elicited by electrical stimulation or fenfluramine are shown in table 1.

2 adrenoceptor agonists such as clonidine (Moret and Langer, 1982).

Exposure to the dopamine receptor blocking agent sulpiride (1, 10 and 100 μM) before S_2 had no effect on the release of [^{3}H]5-HT elicited by electrical stimulation (data not shown). At 1 μM, sulpiride failed to decrease the inhibitory effect of LSD on the electrically evoked overflow of [^{3}H]-5-HT (fig. 4). In fact, our results show that the inhibitory effect of 0.01 μM LSD was slightly but significantly increased in the presence of sulpiride (fig. 4).

Antagonism by citalopram of the inhibition by LSD of the release of [^{3}H]-5-HT elicited by electrical stimulation. Citalopram, a selective inhibitor of neuronal uptake of 5-HT, at 0.001 to 10 μM, did not modify, by itself, the electrically evoked overflow of [^{3}H]-5-HT (table 2). At concentrations of citalopram of 0.1 μM and higher, there was a small but significant increase in the spontaneous outflow of radioactivity (table 2). At 0.01 μM, citalopram reduced significantly the inhibitory action of LSD on transmitter release (fig. 5A). When 0.1 or 1 μM citalopram was used, the inhibition by LSD of [^{3}H]-5-HT release elicited by electrical stimulation was completely antagonized (fig. 5, B and C, respectively).

Discussion

One of the criteria necessary to postulate the existence of presynaptic autoreceptors is the modulation by receptor agonists and antagonists of the calcium-dependent release of a neurotransmitter (Langer, 1980). Chase *et al.* (1969), using a very high frequency of electrical stimulation (100 Hz), found that the [^{3}H]-5-HT release from brain slices was not calcium-dependent. In contrast to the results of Chase *et al.* (1969) and in agreement with the results obtained in rat brain synaptosomes (Mulder *et al.*, 1975; Lane and Aprison, 1977) and in rat cerebral cortex slices (Farnebo, 1971; Göthert, 1980), under our experimental conditions the electrically induced tritium overflow from slices of the rat hypothalamus prelabeled with [^{3}H]-5-HT was completely abolished by exposure to calcium-free Krebs' solution.

The sympathomimetic amine, tyramine, displaces catecholamines from vesicular storage sites through a calcium-independent mechanism (Lindmar and Muscholl, 1965). Fenfluramine, which causes a depletion of 5-HT from brain stores (Neckers *et al.*, 1976) and whose effects on tryptaminergic terminals can be compared with those of tyramine on catecholaminergic nerve

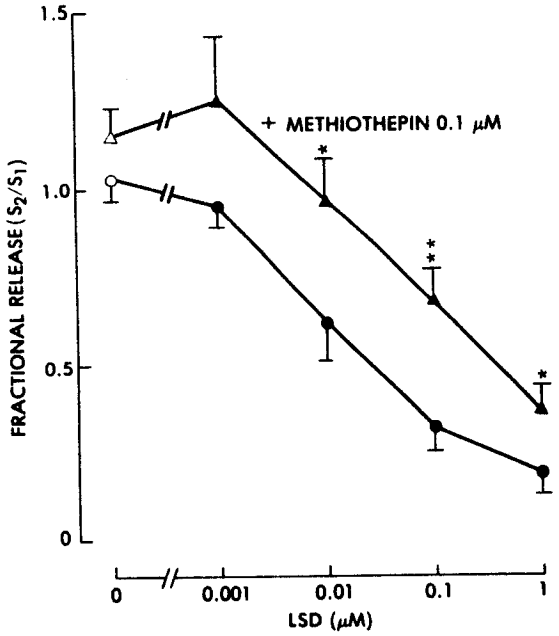

Fig. 3. Methiothepin antagonizes the inhibition by LSD of the release of [^{3}H]-5-HT elicited by electrical stimulation from slices of the rat hypothalamus. Ordinate: fraction of the total tissue radioactivity released by a 2-min period of electrical stimulation (3 Hz, 20 mA, 2 msec) expressed as the ratio (S_2/S_1) obtained between the second period of stimulation carried out in the presence of the drug (S_2) and the first period (S_1). ○, control; ●, LSD; △, control in the presence of 0.1 μM methiothepin during S_1 and S_2; ▲, LSD added before S_2 in the presence of 0.1 μM methiothepin. Abscissa: concentrations of LSD (micromolar). LSD in the concentrations indicated was added to the medium 20 min before S_2. Methiothepin (0.1 μM) was added to the medium 20 min before S_1 and maintained for the rest of the experiment. Each point represents mean values ± S.E.M. of at least six experiments per group. *$P < .05$; **$P < .01$ when compared to the corresponding value obtained in the absence of methiothepin. LSD, when present alone, significantly decreased [^{3}H]-5-HT release at 0.01 μM ($P < .01$), 0.1 μM ($P < .001$) and 1 μM ($P < .001$), when compared to the control value in the absence of drugs.

endings, was used in order to release [^{3}H]-5-HT from slices of the rat hypothalamus. In contrast to the results obtained with electrical stimulation, the release elicited by fenfluramine was not reduced by the absence of calcium ions in the external medium. A similar result with amphetamine was obtained by Homan and Ziance (1981) in chopped rat cerebral cortex. These authors found that the release of [^{3}H]-5-HT induced by *d*-amphetamine was not dependent upon calcium, whereas the potassium-induced release was calcium-dependent.

Since the release of [^{3}H]-5-HT by fenfluramine involves displacement of the stored neurotransmitter and does not require the presence of calcium ions, the present results resemble those obtained for noradrenergic and for dopaminergic transmission: presynaptic modulation of transmitter release is observed for electrical stimulation but not for the calcium-independent release of the neurotransmitter evoked by tyramine (Starke and Montel, 1974; Arbilla and Langer, 1979; Kamal *et al.*, 1981).

The fact that LSD and methiothepin modified the calcium-dependent release of [^{3}H]-5-HT elicited by electrical stimulation but failed to affect the release induced by fenfluramine, which is calcium-independent, supports the proposal of Göthert (1980) for the role of calcium ions in the presynaptic regulation of serotonin release. It is likely that the inhibition by serotonin receptor agonists of 5-HT release is mediated by a decrease in the availability of calcium ions for the stimulus-release coupling, probably by decreasing the affinity of the voltage-sensitive permeability channel of the cell membrane for calcium ions.

Cerrito and Raiteri (1979) have shown that presynaptic serotonergic autoreceptors can be stimulated by exogenous 5-HT to mediate a decrease in the potassium-induced [^{3}H]-5-HT release from rat hypothalamic synaptosomes, and that methiothepin counteracts the inhibitory effect of 5-HT. These results are compatible with a presynaptic location of the serotonin inhibitory autoreceptors. Similar results with exogenous 5-HT inhibiting the electrically evoked release of [^{3}H]-5-HT were obtained by Göthert and Weinheimer (1979), by Baumann and

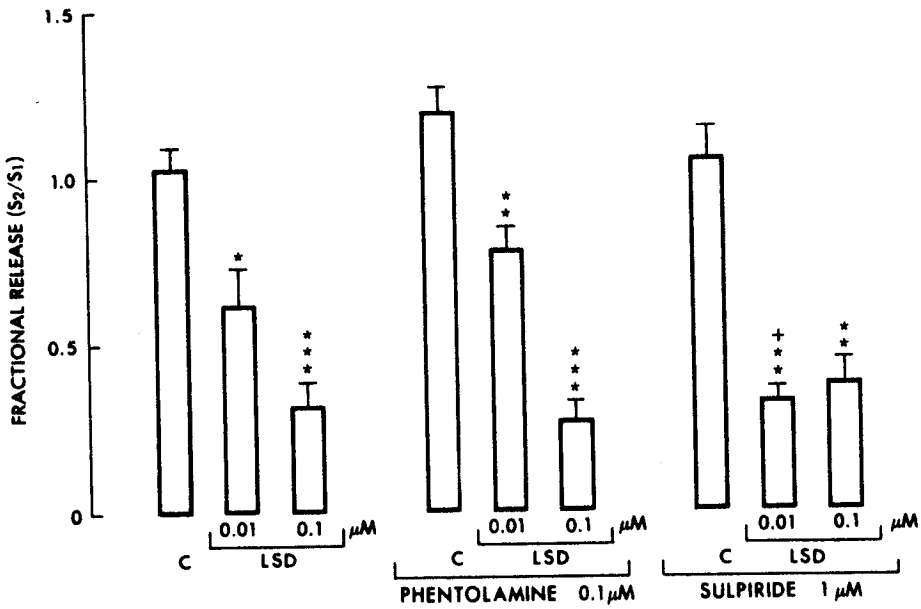

Fig. 4. Failure of phentolamine or sulpiride to antagonize the inhibition by LSD of [^{3}H]-5-HT release elicited by electrical stimulation from slices of the rat hypothalamus. Ordinate: fraction of the total tissue radioactivity released by a 2-min period of electrical stimulation (3 Hz, 20 mA, 2 msec) expressed as the ratio (S_2/S_1) obtained between the second period of stimulation carried out in the presence of the drug (S_2) and the first period (S_1). LSD in the concentrations indicated was added to the medium 20 min before S_2. Phentolamine (0.1 μM) or sulpiride (1 μM) was added to the medium 20 min before S_1 and maintained throughout the rest of the experiment. Shown are mean values ± S.E.M. of at least six experiments per group. *$P < .01$; **$P < .005$; ***$P < .001$ when compared to the corresponding control value (C). †$P < .05$ when compared to the corresponding value obtained with LSD (0.01 μM) in the absence of sulpiride.

TABLE 2

Effect of citalopram on the release of [^{3}H]-5-HT elicited by electrical stimulation from slices of the rat hypothalamus

Citalopram was added to the medium 20 min before the second period of stimulation. Shown are mean values ± S.E.M. *n* = number of experiments per group.

Experimental Group	*n*	Fractional Release × 10^{-2}[a]		Fractional Release, S_2/S_1[b]	Fractional Release Sp_2/Sp_1[c]
		S_1	S_2		
Control	8	1.77 ± 0.25	1.72 ± 0.17	1.05 ± 0.10	0.78 ± 0.01
Citalopram					
0.001 μM	4	1.93 ± 0.22	1.67 ± 0.21	0.86 ± 0.04	0.81 ± 0.02
0.01 μM	5	1.76 ± 0.20	1.86 ± 0.22	1.05 ± 0.08	0.81 ± 0.02
0.1 μM	8	1.73 ± 0.22	1.90 ± 0.29	1.11 ± 0.13	0.88 ± 0.02**
1 μM	6	1.67 ± 0.31	1.67 ± 0.30	1.00 ± 0.09	0.86 ± 0.02*
10 μM	10	1.64 ± 0.09	1.45 ± 0.14	0.91 ± 0.10	1.06 ± 0.02***

[a] Fraction of the total tissue radioactivity released by a 2-min period of electrical stimulation (3 Hz, 20 mA, 2 msec) 60 min (S_1) and 104 min (S_2) after the end of the incubation with [^{3}H]-5-HT.

[b] Ratio of fractional release obtained between the second period of stimulation (S_2) and the first control period (S_1).

[c] Ratio between the spontaneous outflow of radioactivity obtained during the 4 min preceding the second stimulation (Sp_2) and the corresponding fraction of radioactivity released before the first period (Sp_1).

* $P < .05$; ** $P < .005$; *** $P < .001$ when compared to the corresponding control value.

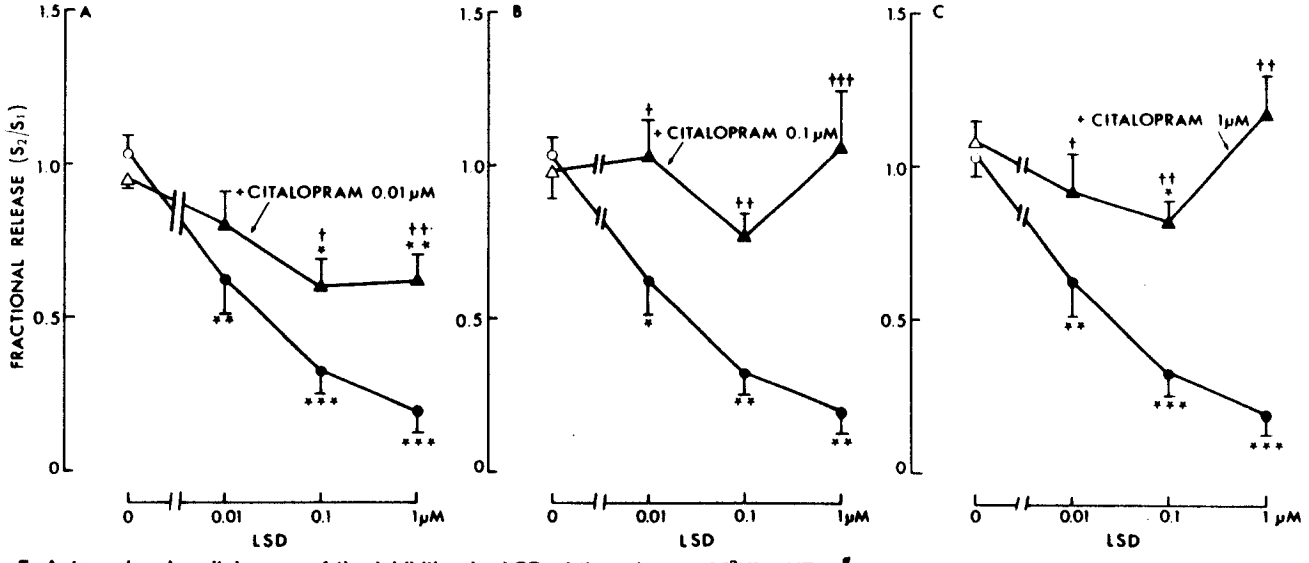

Fig. 5. Antagonism by citalopram of the inhibition by LSD of the release of [^{3}H]-5-HT elicited by electrical stimulation from slices of the rat hypothalamus. Ordinate: fraction of the total tissue radioactivity released by a 2-min period of electrical stimulation (3 Hz, 20 mA, 2 msec) expressed as the ratio (S_2/S_1) obtained between the second period of stimulation carried out in the presence of the drug (S_2) and the first period (S_1). ○, control; ●, LSD; △, control in the presence of citalopram during S_1 and S_2; ▲, LSD added before S_2 in the presence of citalopram. Abscissa: concentrations of LSD (micromolar). LSD in the concentrations indicated was added to the medium 20 min before S_2. Citalopram in the concentrations indicated was added to the medium 20 min before S_1 and maintained for the rest of the experiment. Each point represents mean values ± S.E.M. of at least four experiments per group. *when compared to the corresponding control value. +when compared to the corresponding value obtained with LSD in the absence of citalopram. A, effects of 0.01 μM citalopram. *$P < .05$; **$P < .01$; ***$P < .001$; †$P < .05$; ††$P < .005$. B, effects of 0.1 μM citalopram. *$P < .01$; **$P < .001$; †$P < .05$; ††$P < .01$; †††$P < .005$. C, effects of 1 μM citalopram. *$P < .05$; **$P < .01$; ***$P < .001$; †$P < .05$; ††$P < .005$.

Waldmeier (1981) in rat brain cortex slices and by S. Z. Langer and C. Moret in rat hypothalamic slices (unpublished observations). It should be pointed out, however, that in order to observe an inhibition of [^{3}H]-5-HT release by exogenous serotonin, it is necessary to inhibit the neuronal uptake of 5-HT. When LSD is used as a 5-HT receptor agonist, there is no need to inhibit neuronal uptake of the neurotransmitter.

Katz and Kopin (1969) and Farnebo and Hamberger (1971) have shown that LSD reduces the stimulation-induced overflow of [^{3}H]-5-HT, but these authors used rather high concentrations of LSD (1 μM and higher), and the frequency of stimulation used to elicit [^{3}H]-5-HT release was also fairly high (100 and 10 Hz, respectively). Hamon *et al.* (1974) have shown that the potassium-evoked release of [^{3}H]-5-HT was inhibited by LSD in hippocampal and striatal slices. In brain stem slices, the inhibitory effect of LSD and the enhancing effect of methiothepin on potassium-induced release of [^{3}H]-5-HT have been shown by Bourgoin *et al.* (1977). These authors suggested that LSD was reducing serotonergic neurotransmission through the stimulation of presynaptic inhibitory receptors. In rat hypothalamic slices we found similar results but under our experimental conditions the potency of LSD to reduce the electrically evoked overflow of [^{3}H]-5-HT is considerably higher than previously reported.

It should be noted that LSD can stimulate *alpha* adrenoceptors (Gillespie and McGrath, 1975), as well as dopamine receptors (Von Hungen *et al.*, 1974 Da Prada *et al.*, 1975). On the other hand, it is well known that methiothepin blocks competitively dopamine receptors (Zivkovic *et al.*, 1975).

The presence of inhibitory *alpha* adrenoceptors on the serotonin nerve terminals has been demonstrated using norepinephrine as an *alpha* adrenoceptor agonist by Göthert and

Huth (1980) in the rat cortex and by Frankhuyzen and Mulder (1980) in the rat hippocampus. Similar results have also been obtained in the rat hypothalamus using clonidine as the *alpha*-2 adrenoceptor agonist (Moret and Langer, 1982). Phentolamine at 0.1 μM, a concentration which did not modify, by itself, the electrically evoked release of [3H]-5-HT, competitively antagonized the inhibitory effect of clonidine on the electrically evoked release of [3H]-5-HT (Moret and Langer, 1982). This concentration (0.1 μM) of phentolamine did not, however, modify the inhibitory effect of LSD on [3H]-5-HT release elicited by electrical stimulation, indicating that LSD did not inhibit serotonergic neurotransmission by acting on *alpha* adrenoceptors present on the serotonin nerve terminals of the rat hypothalamus. Phentolamine does not antagonize the inhibitory effect of exogenous 5-HT on stimulation-evoked release of [3H]-5-HT (Göthert and Huth, 1980), a result confirmed by our experiments in rat hypothalamic slices.

Sulpiride is well known to antagonize the inhibitory effect of apomorphine on the electrically evoked release of [3H]dopamine from the rabbit caudate nucleus (Langer *et al.*, 1980a; Arbilla and Langer, 1981). At 1 μM sulpiride, a concentration which effectively blocks dopamine receptors, the antagonist did not modify the inhibition by LSD of electrically induced overflow of [3H]-5-HT. In addition, at 0.01 μM, LSD inhibited [3H]-5-HT overflow more in the presence of sulpiride than in its absence. At present we do not have an explanation for this unexpected result. These findings show that LSD does not act on dopamine receptors to reduce the electrically evoked release of [3H]-5-HT.

The present study shows that LSD possesses a potent inhibitory effect on the electrically evoked, calcium-dependent [3H]-5-HT release in slices of the rat hypothalamus. This action appears to be mediated through serotonergic inhibitory autoreceptors, inasmuch as methiothepin but not phentolamine or sulpiride prevented the inhibition by LSD of 5-HT release.

The mechanism of action of LSD is considered very complex because this drug is known to be a 5-HT receptor antagonist in peripheral studies and a partial agonist in central nervous system studies. Our experiments showed that it behaves as a full 5-HT receptor agonist at the level of the presynaptic autoreceptor inasmuch as it produced nearly full inhibition of electrically evoked [3H]-5-HT release. Within the range of concentrations of LSD studied (up to 1 μM), there were no indications of partial agonist effects at the level of [3H]-5-HT release.

Peripheral pharmacological studies have determined the existence of two types of 5-HT receptors (Apperley *et al.*, 1980). In addition, binding studies have described two 5-HT receptor sites, 5-HT_1 and 5-HT_2, in the central nervous system, and LSD appears to bind to both receptor subtypes (Peroutka and Snyder, 1979). At the moment it is unknown whether the presynaptic 5-HT autoreceptors resemble the 5-HT_1 or 5-HT_2 receptors. However, it is unlikely that the presynaptic inhibitory 5-HT autoreceptors are of the 5-HT_2 subtype because neither spiroperidol nor mianserin have an effect on these release-modulating receptors (C. Moret and S. Z. Langer, unpublished observations). Additional studies are required to characterize further the 5-HT receptor subtype involved in the presynaptic modulation of serotonin release.

Citalopram, a selective inhibitor of serotonin uptake, did not modify the electrically evoked release of [3H]-5-HT in a wide range of concentrations. The antagonism of the inhibitory effect of LSD observed with low concentrations of citalopram appears to be noncompetitive. These results should be considered in the context of similar results by Pelayo *et al.* (1980) in central noradrenergic neurons. These authors found that noradrenaline uptake inhibitors, cocaine or desipramine, reduce the effects of clonidine on stimulation-evoked [3H]-noradrenaline release from rat occipital cortex slices but fail to modify the inhibition induced by the catecholamine α-methylnoradrenaline. Similar results were observed by Moret and Langer (1982) in rat hypothalamic slices, where cocaine and citalopram antagonize the inhibitory effect of clonidine on electrically evoked release of [3H]-5-HT.

From the results of the present study, two mechanisms of action can be invoked to explain the antagonism by citalopram of the inhibitory action of LSD on serotonergic neurotransmission. The first possibility involves a specific interaction between citalopram and the 5-HT autoreceptor site. However, in contrast to methiothepin, citalopram did not, by itself, increase the overflow of [3H]-5-HT elicited by electrical stimulation. In addition, whereas methiothepin antagonized the effects of LSD in a competitive manner, citalopram did so in a noncompetitive manner. This would imply that citalopram acts at a site different from the 5-HT autoreceptor, which could be the uptake recognition site for 5-HT. A second alternative may be related to the amount of 5-HT present in the synaptic cleft, which may be increased in the presence of citalopram and therefore compete with LSD for the 5-HT autoreceptor. We have shown that the IC_{50} for the inhibition of [3H]-5-HT uptake in the rat hypothalamus for citalopram is 0.040 μM (Langer *et al.*, 1980b). The complete antagonism of the effect of LSD by 0.1 and 1 μM citalopram could be explained by the fact that 5-HT uptake was completely inhibited. At 0.01 μM citalopram, however, the uptake of 5-HT was not completely inhibited, but the effects of LSD were still significantly antagonized.

One cannot exclude the possibility that there is an interaction between the amount of 5-HT increased by citalopram in the synaptic gap and the presynaptic 5-HT autoreceptor. However, it is tempting to speculate that the site of neuronal 5-HT uptake and the presynaptic 5-HT autoreceptor site may be linked in a functional manner. The latter possibility may be of pharmacological and therapeutic importance because several inhibitors of neuronal uptake of 5-HT are used as antidepressants.

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