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5-HT_{2A} receptor antagonists inhibit potassium-stimulated γ-aminobutyric acid release in rat frontal cortex

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Abstract

Several drugs selective for the serotonin 5-HT_{2A} receptor were tested for their effects on spontaneous and K^+-evoked [^{3}H]γ-aminobutyric acid (GABA) release from slices of rat frontal cortex. Under K^+ stimulation, the antagonists ketanserin, spiperone, *R*-(+)-α-(2,3-dimethoxyphenyl)-1-[2-(4-fluorophenethyl)]-4-piperidinemethanol (MDL 100,907) and ritanserin inhibited GABA release by 12–31%. Rats were treated with the serotonin-depleting agent *para*-chlorophenylalanine and with the serotonergic neurotoxin *para*-chloroamphetamine. In *para*-chlorophenylalanine-treated animals, stimulated GABA release in the presence of ketanserin remained depressed. In animals treated with both *para*-chlorophenylalanine and *para*-chloroamphetamine, ketanserin or the hallucinogenic agonist (2,5-dimethoxy-4-iodophenyl)-2-aminoethane (2C-I) each appeared to decrease stimulated GABA release but this was not significant. However, when ketanserin and 2C-I were both present in the superfusion buffer an additive inhibitory effect was observed, and GABA release was decreased 30%. These results suggest that serotonin facilitates GABA release in cortex via 5-HT_{2A} receptors and that the functional response of this system is resistant to serotonin depletion.

Keywords: 5-HT (5-hydroxytryptamine, serotonin); 5-HT_{2A} receptor; γ-Aminobutyric acid; Hallucinogen; Ketanserin

1. Introduction

The neurotransmitter serotonin has been linked to a variety of biological processes including sleep, appetite, sexual activity, depression, and cognitive function. There are multiple serotonin receptor subtypes, and studies suggest the involvement of one or another subtype in specific mental processes. The serotonin 5-HT_{2A} receptor, for example, has been implicated as the site of action for hallucinogenic drugs (McKenna et al., 1989; Pierce and Peroutka, 1989; Titeler et al., 1988), and may therefore play a role in sensory perception, emotion, and cognition.

Autoradiographic studies with 5-HT_{2A}-selective radioligands have revealed high densities of 5-HT_{2A} binding sites in layer IV of the mammalian cerebral cortex (Appel et al., 1990; Blue et al., 1988; Lidow et al., 1989). This region of the cerebral cortex is the primary terminal projection layer for specific sensory afferents relayed by the thalamus and contains large numbers of intrinsic γ-aminobutyric acid-releasing (GABAergic) neurons (Hendry et al., 1986; Jones, 1988; Kisvardy et al., 1986). These neurons play a major role in sensory information processing and would seem to be likely targets for hallucinogenic drugs. It is not known, however, whether 5-HT_{2A} receptors are involved in modulating these neurons. The present studies were initiated to address this question.

It was hypothesized that drugs acting at 5-HT_{2A} receptors on GABAergic interneurons in the cerebral cortex would alter the amount of GABA these cells release in vitro. Therefore, 5-HT_{2A}-selective drugs, including the hallucinogen 2-(2,5-dimethoxy-4-iodophenyl)-aminoethane (2C-I) (Shulgin and Shulgin, 1991) and the nonhallucinogenic drugs ketanserin, *R*-(+)-α-(2,3-dimethoxyphenyl)-1-[2-(4-fluorophenethyl)]-4-piperidinemethanol (MDL 100,907) (Sorensen et al., 1992), ritanserin, and spiperone were tested for their effects on spontaneous and stimulated GABA release from slices of rat frontal cortex. It was further hypothesized that serotonin depletion would reduce or abolish antagonist drug effects, so some rats were

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treated with the serotonin-depleting agent *para*-chlorophenylalanine alone and in combination with the serotonergic neurotoxin *para*-chloroamphetamine prior to testing ketanserin and 2C-I.

2. Materials and methods

2.1. Drugs

Para-chloroamphetamine was synthesized by modifications of standard methods and 2C-I was synthesized as described (Cozzi, 1994); all analytical data were in agreement with the expected final structures. MDL 100,907 was a gift from Marion Merrell Dow. All other compounds were obtained from commercial sources.

2.2. Animals and brain slice preparation

Male Sprague Dawley rats (175–200 g) were obtained from Harlan Laboratories, Indianapolis, IN, USA. The animals were group-housed under a 12 h light/dark schedule and received food (Lab Blox, Purina) and water ad libitum.

Following decapitation, the rat brain was rapidly removed and dissected over ice. A coronal cut through both hemispheres was made caudal to the neostriatum, and the hemispheres were separated by a midline sagittal cut. The frontal cortex was then trimmed of striatal, thalamic, and piriform cortex tissue. During the dissection, the tissue was regularly moistened with ice-cold, aerated (5% CO_2 in O_2) modified Krebs-Ringer bicarbonate (KR) buffer containing (mM): NaCl (124.3), KCl (2.95), $MgSO_4$ (1.30), KH_2PO_4 (1.25), $NaHCO_3$ (26.0), $CaCl_2$ (2.41), *d*-glucose (10.4). A McIlwain tissue chopper was used to prepare 10–15 350 μm coronal cortical slices from each hemisphere, beginning at the level of the caudal border of the neostriatum and proceeding rostrally to the prefrontal area. The cortical slices were then transferred to a holding bottle containing 100 ml of aerated 37°C KR buffer for a 1 h preincubation. The time from decapitation to incubation in warm buffer was approximately 5 min.

2.3. Superfusion: GABA release

The GABA neurotransmitter pool in the brain slices was labeled with exogenous [^{3}H]GABA prior to the release experiments. Eighteen intact slices were selected from the preincubation bottle on the basis of visual appearance and were transferred to a radiolabeling incubation buffer containing 30 ml of aerated 37°C KR buffer and [^{3}H]GABA (1 μCi/ml, final concentration 25 nM, along with 1 μM unlabeled GABA as a carrier) for a 30 min incubation period. Carboxymethoxylamine (10 μM), a transaminase inhibitor, was present in the incubation buffer and all subsequent control and drug buffers to block the metabolism of the labeled neurotransmitter (Levy et al., 1973; Szerb, 1983). After the 30 min labeling incubation, one intact slice was transferred to each of 12 superfusion chambers of a superfusion apparatus (Brandel model SF12). The superfusion apparatus allows the slices to be continuously superfused with buffer and permits the collection of superfusate from each chamber. A solid-state temperature controller maintains the superfusion buffer at 37°C, and gas delivery probes allow the buffer to be continuously aerated. After transfer to the chambers, slices were superfused for a 30 min washout period with KR buffer (0.5 ml/min) to achieve a basal level of spontaneous neurotransmitter release.

For spontaneous release control runs, 10 serial 2-min fractions were collected after the 30-min washout period. To test the effect of 2C-I on spontaneous GABA release, five spontaneous release fractions were collected (after washout), and then various concentrations of 2C-I were introduced into the buffer flow line and five additional release fractions were collected. When antagonists were tested alone or in the presence of 2C-I, the antagonists were introduced after four spontaneous release fractions were collected, so they were present prior to the introduction of 2C-I. Once drugs were introduced, they remained present for the remainder of the experiment. The amount of tritium released (assessed by liquid scintillation spectroscopy, Packard model 4430) in the presence of drugs was compared to the amount released during control runs and is expressed as percentage released. Percentage released for any fraction was calculated by dividing the amount of tritium released during that fraction by the total tissue slice tritium present at the start of that fraction collection period and multiplying by 100. The tissue slice tritium present at the start of a collection period is the sum of the tritium released during that collection period, all subsequent collection periods, and the slice tritium content at the end of the experiment.

Drug effects were also determined under K^+-evoked release conditions. For K^+-stimulated release control runs, 20 2-min fractions were collected after the 30-min washout period. During fractions 3 and 13 (S1 and S2), 40 mM K^+ KR buffer (composition identical to regular KR except KCl was increased to 40 mM and NaCl was reduced to 87 mM to maintain iso-osmotic conditions) was introduced into the buffer flow line. The amount of evoked neurotransmitter release (over baseline) was calculated as the area under the stimulation curve (AUC) from fractions 3–8 for S1 and from fractions 13–18 for S2 (Fig. 1). Baseline was defined as the least-squares regression line through fractions 1, 2, 9, 10, 11, 12, 19, and 20. To test the effects of agonist on K^+-stimulated release, 2C-I was introduced to the slices at the beginning of fraction 9 and was present continuously throughout the rest of the experiment. Antagonists, when tested, were introduced at the beginning of fraction 7 and were present throughout the rest of the experiment. To quantify drug effects, the *S2/S1* ratio

(AUC for fractions 13–18/AUC for fractions 3–8) was calculated and compared to the (control) ratio in the absence of drugs.

2.4. Depletion of endogenous serotonin

Some rats were treated with the tryptophan hydroxylase inhibitor *para*-chlorophenylalanine to deplete endogenous stores of serotonin (Koe and Weissman, 1966; Miller et al., 1970). *Para*-chlorophenylalanine methyl ester HCl was dissolved in 0.9% saline to a final concentration of 80 mg/ml. Rats were given two intraperitoneal injections of 200 mg/kg *para*-chlorophenylalanine 12 h apart, for a total dose of 400 mg/kg. The animals were killed 48 h after the last dose and their brain tissue was used to prepare brain slices for superfusion experiments as previously described, and for high-pressure liquid chromatography analysis of serotonin content as described below.

In some serotonin depletion experiments, animals were treated with both *para*-chlorophenylalanine and the serotonergic neurotoxin *para*-chloroamphetamine. This was to ensure that serotonin biosynthesis was inhibited and to denervate specific serotonergic pathways (Mamounas and Molliver, 1988; Miller et al., 1970; Pletscher et al., 1964). *Para*-chloroamphetamine HCl was dissolved in 0.9% saline to a final concentration of 10 mg/ml. Rats were treated with two intraperitoneal injections of 10 mg/kg *para*-chloroamphetamine 24 h apart, for a total dose of 20 mg/kg. Approximately 6.5 days after the last dose of *para*-chloroamphetamine, the rats were treated with 2 × 200 mg/kg *para*-chlorophenylalanine 12 h apart for a total dose of 400 mg/kg *para*-chlorophenylalanine. These animals were killed 48 h after the last dose of *para*-chlorophenylalanine and their brain tissue was used for brain slice superfusion experiments as previously described.

2.5. Analysis of tissue serotonin

In experiments in which endogenous serotonin was depleted by pretreatment with *para*-chlorophenylalanine, the slices that were not selected for superfusion were retained for analysis of serotonin content using high-pressure liquid chromatography with electrochemical detection (HPLC-EC). These slices were placed in 1.5 ml Eppendorf tubes, weighed, and quickly frozen in liquid N_2. The frozen slices were then stored at −70°C until HPLC analysis using the following system.

HPLC system. The HPLC system consisted of a refrigerated autosampler (TosoHaas model TSK-6080), a reverse-phase Brownlee octadecylsilane analytical cartridge column (4.6 mm × 250 mm, 5 μm particle size; Anspec) supplied with mobile phase consisting of (mM): NaH_2PO_4 (50), citric acid (30), disodium ethylenediaminetetraacetate (0.1), sodium octyl sulfate (1.46), and CH_3OH, 25% v/v; pH 2.75. Mobile phase was supplied by a Rainin pump at a flow rate of 0.7 ml/min; an electrochemical detector (EG&G Princeton Applied Research Corporation model 400) with a dual electrode potential set at $E_1 = -200$ mV and $E_2 = 850$ mV against a Ag/AgCl reference electrode was used to detect serotonin. Automatic sample injection and data acquisition were controlled with an Apple Macintosh SE computer running commercial software (Dynamax Methods Manager; Rainin).

For serotonin assessment, the frozen brain tissue was thawed and homogenized in 5 μl/mg tissue HPLC mobile phase (minus sodium octyl sulfate) in 1.5 ml Eppendorf tubes with a motor-driven Teflon pestle at 0°C. The homogenate was then centrifuged with a table-top centrifuge (American Scientific Products Biofuge A) at 13000 rpm for 5 min. 50 μl aliquots of the supernatant were used for determination of serotonin content by HPLC-EC. Serotonin standard solutions were prepared from a stock solution of serotonin creatinine sulfate in HPLC mobile phase diluted to the appropriate concentration with additional mobile phase. The serotonin standards were then analyzed by HPLC-EC and standard curves were generated by fitting the serotonin peak areas (μV-s) to a computer-generated least-squares regression line. The derived regression equation was used to calculate serotonin concentrations in tissue samples from saline-treated and *para*-chlorophenylalanine-treated animals. The identity of the serotonin peak in tissue extracts was verified by spiking the samples with authentic serotonin.

2.6. Data analysis

For spontaneous release experiments with ketanserin, and spontaneous and K^+-stimulated release experiments with various concentrations of 2C-I, individual slices were exposed to single concentrations of test drug. Each '*n*' represents one slice from one animal. Stimulated release experiments with other antagonists and in *para*-chlorophenylalanine- and *para*-chloroamphetamine plus *para*-chlorophenylalanine-treated animals were performed in triplicate, and the mean of these three determinations represents one '*n*'. Data from the release experiments were transformed from decays per minute to percentage tritium released; for stimulated release experiments the *S2/S1* value was calculated and used to compare drug effects, as described above. Multiple comparisons of the various 2C-I concentrations to controls in drug-naive rats were performed using one-way analysis of variance (ANOVA) followed by Dunnett's *t*-test; pairwise comparisons of the different antagonists to controls were made using Student's *t*-test. Pairwise comparisons of drug effects to controls in *para*-chlorophenylalanine- and *para*-chloroamphetamine plus *para*-chlorophenylalanine-treated rats were made using Student's *t*-test.

3. Results

2C-I, tested at concentrations up to 10 μM, had no effect on GABA release under resting conditions (data not

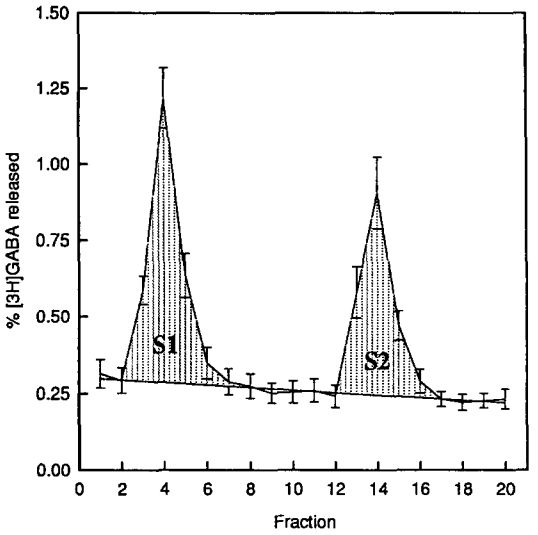

Fig. 1. K^+-stimulated [^{3}H]GABA release from rat cortical slices. [^{3}H]GABA release was evoked from superfused slices of rat frontal cortex by replacing normal KR buffer with 40 mM K^+ KR during fractions 3 and 13. Data are the means ± S.E.M. of six singular determinations. Shaded areas were used to calculate *S2/S1*.

shown). Ketanserin, at 10 μM, also did not affect spontaneous release of the neurotransmitter (data not shown).

Stimulated GABA release, evoked by 40 mM K^+ KR, was robust and readily reproducible (Fig. 1). Under K^+ stimulation, 2C-I had no effect on GABA release (data not shown). Ketanserin (10 μM), however, decreased release by 27% (Table 1). Three other 5-HT_{2A} receptor antagonists, MDL 100,907, ritanserin, and spiperone, also significantly reduced K^+-stimulated GABA release (Table 1). MDL 100,907, at 1 μM and 10 μM, reduced GABA release by 12% and 31%, respectively. Ritanserin (10 μM) depressed GABA release by 16% and spiperone (10 μM) reduced GABA efflux by 22%.

HPLC-EC analysis of the serotonin level in frontal cortex from a control (saline-treated) rat revealed a serotonin content of 218 pg/mg wet weight, in excellent agreement with previously reported results (Johnson and Nichols, 1990). Treatment of rats with *para*-chlorophenylalanine two days prior to killing resulted in the complete loss of detectable serotonin by HPLC-EC in all animals. Despite this loss of endogenous serotonin, 10 μM 2C-I alone still had no effect on stimulated GABA release (Table 2). The depressant effect of 10 μM ketanserin was unaffected by the *para*-chlorophenylalanine treatment; the *S2/S1* ratio was still 22.5% lower than controls (Table 2). When 2C-I and ketanserin (both at 10 μM) were simultaneously present, the reduction in GABA release approached significance ($P < 0.052$).

Para-chloroamphetamine treatment followed by *para*-chlorophenylalanine treatment 6.5 days later did partially reverse the reduction in stimulated GABA release produced by 10 μM ketanserin (Table 3); release was still reduced, but this was no longer significantly different from controls. There was also a small, nonsignificant reduction in GABA release in the presence of 10 μM 2C-I. When both drugs were simultaneously present in the superfusion

Table 1
S2/S1 values for K^+-evoked [^{3}H]GABA release: antagonists [a]

Treatment	*S2/S1*	*n* [b]
Control	0.87 ± 0.02	13
Ketanserin, 100 nM	0.89	1
Ketanserin, 1 μM	0.83	1
Ketanserin, 10 μM	0.63 ± 0.06 [e]	9
MDL 100,907, 1 μM	0.76 ± 0.04 [c]	5
MDL 100,907, 10 μM	0.60 ± 0.02 [f]	5
Ritanserin, 10 μM	0.72 ± 0.02 [d]	4
Spiperone, 10 μM	0.68 ± 0.01 [e]	3

[a] The ability of various antagonists to affect K^+-stimulated [^{3}H]GABA release was examined in slices of rat frontal cortex. *S2/S1* values ± S.E.M. were calculated based on areas under the stimulation curves and pairwise comparisons to controls were made using Student's *t*-test. [b] *n* is the number of animals assessed (triplicate determinations) at each concentration of drug. [c] $P < 0.05$. [d] $P < 0.01$. [e] $P < 0.005$. [f] $P < 0.0001$.

Table 2
S2/S1 values for K^+-evoked [^{3}H]GABA release: *para*-chlorophenylalanine pretreatment [a]

Treatment	*S2/S1*	*n* [b]
Control	0.77 ± 0.04	6
2C-I, 10 μM	0.81 ± 0.02	3
Ketanserin, 1 μM	0.78 ± 0.06	3
Ketanserin, 10 μM	0.60 ± 0.04 [c]	3
2C-I + ketanserin, 10 μM	0.62 ± 0.02	3

[a] The ability of 2C-I and ketanserin to affect K^+-stimulated [^{3}H]GABA release was examined in slices of rat frontal cortex harvested from animals that had been treated with *para*-chlorophenylalanine 48 h previously. *S2/S1* values ± S.E.M. were calculated based on areas under the stimulation curves and pairwise comparisons to controls were made using Student's *t*-test. [b] *n* is the number of animals assessed (triplicate determinations) at each concentration of drug. [c] $P < 0.05$ compared to controls.

Table 3
S2/S1 values for K^+-evoked [^{3}H]GABA release: *para*-chloroamphetamine and *para*-chlorophenylalanine pretreatment [a]

Treatment	*S2/S1*	*n* [b]
Control	0.80 ± 0.03	3
2C-I, 10 μM	0.73 ± 0.01	3
Ketanserin, 10 μM	0.63 ± 0.03	3
2C-I + ketanserin, 10 μM	0.57 ± 0.08 [c]	3

[a] The ability of 2C-I and ketanserin to affect K^+-stimulated [^{3}H]GABA release was examined in slices of rat frontal cortex harvested from animals that had been treated with *para*-chloroamphetamine 8.5 days previously and with *para*-chlorophenylalanine 48 h previously. *S2/S1* values ± S.E.M. were calculated based on areas under the stimulation curves and were compared to controls using one-way ANOVA followed by Dunnett's *t*-test. [b] *n* is the number of animals assessed (triplicate determinations) at each concentration of drug. [c] $P < 0.05$ compared to controls.

buffer, GABA release was depressed 30% relative to controls (Table 3).

4. Discussion

The antagonist data from drug-naive rats support the hypothesis that serotonin regulates GABA function through 5-HT_{2A} receptors in cerebral cortex. The regulation by serotonin is facilitory, since receptor blockade results in decreased GABA release. All four 5-HT_{2A} receptor antagonists significantly depressed K^+-evoked GABA release, with MDL 100,907 showing effects at 1 μM (Table 1). While none of these drugs is absolutely specific for the 5-HT_{2A} receptor, this subtype is the only one known for which all of these drugs share high affinity (Hoyer et al., 1985; Johnson et al., 1990; Leysen et al., 1982, 1985; Middlemiss et al., 1986; Sorensen et al., 1992). Furthermore, the rank order of potencies for inhibition of GABA release at equimolar drug concentrations parallels the drugs' affinities for 5-HT_{2A} receptors: MDL 100,907 > ketanserin > spiperone > ritanserin (Table 1). The 5-HT_{2A} receptor affinities decrease by less than 3-fold within this ranking. As expected, this results in a relatively narrow range for GABA release inhibition (31–16%). In contrast, the test compounds' range of affinities at other sites including α_1- and α_2-adrenoceptors, 5-HT_{1A}, 5-HT_{1B}, D_2 dopamine, and H_1 histamine receptors varies from about 50-fold at α_1 sites to a range of over 75 000-fold at D_2 sites and there is no correlation between the drugs' potencies for GABA release inhibition and their affinities at these other receptors. The fact that these compounds have different chemical structures makes it improbable that they share some other common molecular recognition site, but the possibility that these drugs are blocking another, unidentified component of the GABA release mechanism cannot be ruled out. It is unlikely that the drug concentrations used in these experiments are causing nonspecific membrane effects resulting in depressed neuronal function. For example, the thermodynamic activities (Ferguson, 1939) of the drugs used in this study are on the order of 10^{-4}; nonspecific drugs generally have higher thermodynamic activities, between 0.01 and 1 (Nogrady, 1985).

The failure of *para*-chlorophenylalanine pretreatment to reverse the ketanserin-induced depression of GABA release (Table 2), even though serotonin levels were undetectable, is puzzling. However, there is evidence for a large functional reserve in the serotonergic system. For example, Kuhn et al. (1985) were able to elicit serotonin-mediated behaviors (head weaving, reciprocal forepaw treading, hindlimb abduction, salivation, wet-dog shakes) after reducing brain serotonin levels by 90–95% with reserpine. Chaput et al. (1990) showed that even when serotonin content in the dorsal hippocampus was reduced by 90% with *para*-chlorophenylalanine treatment, the efficiency of serotonergic neurotransmission to CA3 pyramidal cells was unaltered in most animals tested, as assessed electrophysiologically. Postsynaptic receptor supersensitivity or increases in 5-HT_{2A} receptor numbers following *para*-chlorophenylalanine treatment (Brunello et al., 1982; Roth and Chaung, 1987) may provide mechanisms for maintaining normal function. Another possibility is that ketanserin may be acting as an inverse agonist, as discussed below.

To address the possibility that residual stores of serotonin were able to furnish enough serotonin to activate 5-HT_{2A} receptors which could be antagonized by ketanserin, some rats were treated with both *para*-chloroamphetamine and *para*-chlorophenylalanine. Because *para*-chloroamphetamine causes serotonergic axonal degeneration (Mamounas and Molliver, 1988; Mamounas et al., 1988), this treatment should eliminate any remaining serotonin stores that are unaffected by *para*-chlorophenylalanine alone. While the degree of axonal degeneration was not specifically tested in these experiments, numerous studies have shown that the treatment protocol used here is an effective means of lesioning the serotonergic axons innervating precisely those areas of cerebral cortex containing the highest densities of 5-HT_{2A} sites (Mamounas and Molliver, 1988; Miller et al., 1970; Molliver et al., 1990; O'Hearn et al., 1988; Pletscher et al., 1964). With this treatment, the ketanserin-induced inhibition of GABA release, while still depressed, was no longer significantly different from controls (Table 3). This result indirectly supports the hypothesis that serotonin increases GABA release via 5-HT_{2A} receptors in rat cerebral cortex and, examined in the light of the *para*-chlorophenylalanine results, indicates that this serotonergic system requires aggressive manipulations to decrease its efficiency. These data, together with the data from the *para*-chlorophenylalanine experiments, also raise the possibility that under potassium stimulation ketanserin may behave as an inverse agonist. It has recently been reported (Barker et al., 1994; Westphal and Sanders-Bush, 1994) that some drugs usually considered to be serotonin antagonists can decrease agonist-independent 5-HT_{2C} receptor activation; these drugs have been termed inverse agonists. Ketanserin, normally regarded as a 5-HT_{2A} receptor antagonist, may actually be an inverse agonist.

If one assumes that the hallucinogen 2C-I is an agonist, the present results are difficult to interpret; no concentration of 2C-I affected spontaneous or evoked GABA release. Based on the antagonist data, one might expect an agonist to increase GABA release under K^+ stimulation, but this was not observed. There are several explanations for this finding.

It is possible that under K^+ stimulation, GABA release is already maximal and an agonist cannot increase release over this 'basal' level. Antagonists, however, could still decrease this release.

It may also be that 2C-I is not an agonist at 5-HT_{2A} receptors, but is instead a partial agonist or even an antagonist. It seems unlikely that 2C-I is an antagonist

because it did not inhibit GABA release in drug-naive animals, as seen with the antagonists used in this study (which are not hallucinogenic) (Arriaga et al., 1984; Ceulemans et al., 1984; De Cree et al., 1981; Demoulin et al., 1981; Gelders et al., 1986).

It is more likely that 2C-I is a partial agonist. The psychological effects of 2C-I in humans are known to be qualitatively similar to the effects of other 5-HT_{2A} partial agonists such as the structural homolog (2,5-dimethoxy-4-iodophenyl)-2-aminopropane (Shulgin and Shulgin, 1991). Also, Pierce and Peroutka (1988) and Sanders-Bush et al. (1988) showed that (2,5-dimethoxy-4-substituted-phenyl)-2-aminopropane hallucinogens which are structurally similar to 2C-I exhibited efficacies lower than serotonin itself at stimulating phosphatidylinositol hydrolysis in rat cortex. Finally, Seggel et al. (1987) reported the 5-HT_{2A} partial agonist effect of (2,5-dimethoxy-4-halo-phenyl)-2-aminopropane hallucinogens on platelet aggregation.

Related to the putative partial agonist activity of 2C-I is the possibly confounding effect of endogenous serotonin. The full-efficacy agonist effect of endogenous serotonin, which is also released under K^+ stimulation and which possibly reaches millimolar concentrations in the synapse, might obscure any agonist effect of 2C-I, especially if 2C-I is of low efficacy. In experiments conducted with serotonin-depleted cortical slices harvested from rats that were pretreated with both *para*-chloroamphetamine and *para*-chlorophenylalanine (Table 3), 10 μM ketanserin or 2C-I alone each reduced GABA release (22% and 9%, respectively) but this did not reach significance. This suggests that 5-HT function has been impaired, but still retains some residual capacity. When both drugs at the same concentrations were simultaneously present in the superfusion buffer, an additive inhibitory effect was observed, and the *S2/S1* ratio was 30% less than the control ratio (Table 3). Together, these results are consistent with what one would expect of a partial agonist: at high concentrations or in the presence of a full-efficacy agonist the partial agonist appears to be an antagonist. Implicit in this analysis is the assumption that some residual serotonin function remains in the tissue even after rather extreme pharmacological manipulations, but this assumption is not unreasonable in light of evidence reported elsewhere (Brunello et al., 1982; Chaput et al., 1990; Kuhn et al., 1985; Roth and Chaung, 1987).

In summary, the results suggest that serotonin, acting on 5-HT_{2A} receptors, facilitates GABA release in the cerebral cortex. This action of serotonin may be involved in regulating mental functions, such as sensory perception and cognition, that are modified by hallucinogenic drugs. Although a drug effect for the hallucinogen 2C-I was not detectable in this study, GABAergic neurons may nevertheless be involved in the drug's mechanism of action, based on the antagonist data. More sensitive assessment methods such as tissue or single cell electrophysiology may answer this question. In addition, the serotonin system that regulates GABA release was seen to be very resistant to treatments designed to impair its function, such as *para*-chlorophenylalanine- and *para*-chloroamphetamine plus *para*-chlorophenylalanine-induced serotonin depletion.

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