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Characterization of a novel effect of serotonin 5-HT_{1A} and 5-HT_{2A} receptors: increasing cGMP levels in rat frontal cortex

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Abstract

Elucidating the mechanisms of action of hallucinogens has become an increasingly important area of research as their abuse has grown in recent years. Although serotonin receptors appear to play a role in the behavioral effects of the phenethylamine and indoleamine hallucinogens, the signaling pathways activated by these agents are unclear. Here it is shown that administration of serotonin (5-hydroxytryptamine, 5-HT) increased cyclic guanosine monophosphate (cGMP) production in frontal cortical slices of rat brain. The effect of 5-HT was greater than that of N-methyl-D-aspartate (NMDA), a stimulant of cGMP formation in the central nervous system. The 5-$HT_{2A/2C}$ receptor phenethylamine agonist, 2,5-dimethoxy-4-methylamphetamine (DOM), increased cGMP content in the slices. Additionally 8-hydroxy-2-(di-*n*-propylamino)tetralin (DPAT), a 5-$_{HT1A/7}$ receptor agonist also increased cGMP production. Stimulation of cGMP formation by DOM was prevented by a 5-$HT_{2A/2C}$ receptor antagonist, pirenperone, as well as by a 5-HT_{2A} receptor selective antagonist, MDL100907. A 5-HT_{2C} receptor antagonist, SB242084, did not block the effect of DOM. Stimulation of cGMP production by DPAT was blocked by the 5-HT_{1A} receptor antagonist, WAY100635. Stimulation of cGMP formation by serotonin could be prevented by pirenperone orWAY100635. In summary, activation of serotonin 5-HT_{1A} and 5-HT_{2A} receptors increase brain cGMP levels.

Keywords: 5-HT_{1A}; 5-HT_{2A}; NMDA; cGMP; Frontal cortex; Brain slices

1. Introduction

The use of hallucinogens by adolescents in this country has been subject to widespread and increasing abuse; these compounds are abused more frequently than tranquilizers, inhalants, and sedatives combined (SAMHSA, 1999). Evidence of this trend is apparent in that lysergic acid (LSD) was recently identified as a 'club drug' in NIDA's campaign to alert the public to the hazards of these agents (Leshner, 1999). However, even though it has been nearly 50 years since Hofmann first observed the remarkable behavioral effects of this indoleamine hallucinogen (Hofmann, 1959), its cellular and molecular mechanisms are still being elucidated. The importance of understanding the mechanisms of action of hallucinogens speaks not only to ameliorating the burden of illicit use, but also to providing keys that may unlock the mysteries of psychosis.

Using the powerful tool of drug-induced stimulus control (DISC) to study psychoactive drugs, the serotonergic system was shown to mediate the stimulus effects of the indoleamine and phenethylamine hallucinogens (Browne and Ho, 1975; Winter, 1978; Winter, 1975). There are currently 14 5-HT receptor subtypes which fall into seven families, 5-HT_{1-7} (Raymond et al., 2001). The 5-HT_2 receptor family was implicated in hallucinogenesis based on the high degree of correlation between affinities of the indoleamine and phenethylamines for these receptors and both potency in substitution for 2,5-dimethoxy-4-methylamphetamine (DOM)-induced stimulus control as well as hallucinogenic potentcy in man (Glennon et al., 1983; Glennon et al., 1984). Subsequent studies in our laboratory using antagonist correlational analysis established that the 5-HT_{2A} receptor rather than the 5-HT_{2C} receptor was necessary for the stimulus effects of

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the indoleamine and phenethylamine hallucinogens (Fiorella et al., 1995). This was in agreement with Schreiber et al. (1994) who reported that the 5-HT_{2A} receptor antagonist MDL100907, but not the 5-HT_{2C} receptor antagonist SB200646, blocked the stimulus effects of the DOM cogener, DOI. Furthermore, psychotic syndromes caused by LSD and psilocybin, another indoleamine hallucinogen, can be prevented or reduced by antagonists of the 5-HT_{2A} receptor (Vollenweider and Geyer, 2001; Vollenweider et al., 1998). Interestingly, the 5-HT_{1A} receptor as well as the 5-HT_{2A} receptor mediate the stimulus effects of the indoleamine hallucinogen 5-methoxy-N,N-dimethyltryptamine (5-MeO-DMT; Winter et al., 2000).

Although activation of the 5-HT_{2A} receptors is necessary for the behavioral effects of these psychoactive drugs, the intracellular changes associated with binding of the hallucinogens to this receptor are unknown. Stimulation of the 5-HT_{2A} receptor through activation of $G_{q/11}$ increases phospholipase C activity with the subsequent production of inositol trisphosphate, which can release intracellular calcium stores, and diacylglycerol which increases protein kinase C activity (Raymond et al., 2001). However, it is unlikely that the increase in phospholipase activity is involved in the stimulus effects of LSD and DOM, as the ability of a series of compounds to stimulate inositol trisphosphate production does not correlate with the degree of generalization of LSD and DOM in animals trained to discrminate those stimuli from saline (Rabin et al., 2001). Although stimulation of the 5-HT_{2A} receptor exerts other intracellular actions (Raymond et al., 2001), preliminary studies revealed that serotonin increased cyclic guanosine monophosphate (cGMP) content in rat brain slices. Interestingly, cGMP has been implicated in CNS plasticity and appears to mediate some of the cellular actions of nitric oxide (NO; Miyawaki et al., 1997; Kiss and Vizi, 2001). Additionally, cGMP analogues as well as phenethylamine and indoleamine hallucinogens facilitate spontaneous excitatory postsynaptic currents in rat brain tissue (Wei et al., 2002; Arvanov et al., 1999; Aghajanian and Marek, 1997).

The purpose of the current study was to characterize the increase in cGMP content of brain slices induced by 5-HT and to identify the serotonin receptor subtypes responsible for increasing cGMP production. These studies were carried out in rat frontal cortex slices because it has been suggested that the frontal and prefrontal cortex play a major role in the pathophysiology of psychosis and hallucination (Goldman-Rakic, 1999).

2. Methods

All experimental protocols were approved by the Laboratory Animal Care Committee of SUNY at Buffalo, and animals were maintained in accordance with the Guide for Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council. Male Fischer 344 rats, aged 3–4 weeks, were anesthetized with halothane and sacrificed by decapitation. The entire brain was rapidly removed and submerged in an ice cold sucrose solution (234 mM sucrose, 2.5 mM KCl, 1 mM Na_2HPO_4, 4 mM $MgSO_4.7H_2O$, 0.1 mM $CaCl_2$, 15 mM HEPES, 11 mM glucose, pH 7.4). The frontal half of the brain was mounted with epoxy in a vibrotome and sliced coronally (400 μm). Frontal cortical slices were defined as sections taken from the most rostral point of the brain through and including sections where the white matter of the forceps major became grossly apparent. For each rat, sectioning produces a total of 12 coronal hemisections which are each defined as a slice. Slices were rinsed in ice cold isethionate buffer (132 mM sodium isethionate, 2 mM KCl, 4 mM $MgCl_2$, 0.1 mM $CaCl_2$, 15 mM HEPES, 23 mM glucose, pH 7.4) and transferred to vials containing artificial cerebrospinal fluid bubbled with 95% O_2/5% CO_2 (ACSF; 130 mM NaCl, 26 mM $NaHCO_3$, 3 mM KCl, 5 mM $MgCl_2$, 1.25 mM NaH_2PO_4, 1 mM $CaCl_2$, 10 mM glucose, pH 7.4). Slices were preincubated at 37 °C for 30 min in ACSF followed by 30 min in Mg-free ACSF with continued oxygenation of the buffer.

At the end of the preincubation period, appropriate drugs were added directly into the Mg-free ACSF. Stimulation with serotonergic agonists or antagonists lasted a total of 13 min; where indicated N-methyl-D-aspartate (NMDA, 100 μM) and/or glycine (20 μM) were added for the final 3 min. A monoamine oxidase inhibitor, pargyline (10 μM) was used in experiments with serotonin (5-HT), DOM and 8-hydroxy-2-(di-*n*-propylamino) tetralin (DPAT). Each condition/drug tested was carried out using triplicate measurements, i.e. the slices from an individual animal were divided into groups of three. The reaction was terminated by probe sonication of the slices in 10% trichloroacetic acid (TCA), and homogenates were centrifuged for 5 min at 760 *g*. Resulting supernatant was extracted three times with 1.25 ml of water-washed ether to remove the TCA, and cGMP content of each slice was determined by radioimmunoassay according to the method of Brooker et al. (1979). The pellet was dissolved in 0.1 N NaOH, and protein content was determined using the colormetric Bio-Rad protein dye binding procedure with bovine serum albumin (fraction V) as a standard. Data were expressed as picomoles cGMP/mg protein.

Statistical significance was determined by one-way repeated measures ANOVA with post-hoc analysis utilizing the Student–Newman–Keuls test. Statistical tests were performed using SigmaStat version 2.03 (Access Softek, Inc., San Rafael, CA). Results from concentration–response experiments were analyzed using non-

linear regression analysis (SigmaPlot 2001, Access Softek, Inc., San Rafael, CA) to fit the logistic equation to the data.

Anti-cGMP antibody (polyclonal) was purchased from OED Bioscience, Inc (San Diego, CA), and radiolabeled (I^{125}) cGMP was purchased from Perkin Elmer Life Science (Boston, MA). Protein dye reagent was purchased from BioRad (Hercules, CA). Serotonin, pirenperone, pargyline, WAY100635, and NMDA were purchased from Sigma (St. Louis, MO). Spiperone, and DPAT were purchased from RBI (Natick, MA). DOM was generously provided by the National Institute on Drug Abuse (Rockville, MD). All other reagents were obtained from common commercial suppliers.

3. Results

Similar to studies involving the cerebellum and hippocampus (Garthwaite, 1982; Strosznajder et al., 1996), NMDA increased cGMP content in cortical slices of rat brain. As shown in Fig. 1, addition of 100 μM NMDA to the slices caused a 60% increase in cGMP formation. This was a maximally effective concentration of NMDA as no further stimulation of cGMP production was observed with higher concentrations (data not shown). Serotonin caused a significantly greater increase in cGMP production compared to NMDA, and the combination of 5-HT + NMDA produced an increase in cGMP formation that was of the same magnitude as 5-HT alone. The response to 5-HT was concentration-dependent (Fig. 2); the calculated concentration which resulted in half-maximal stimulation (EC_{50}) was 8.4 ± 2.6 μM, while the maximum stimulation of cGMP formation by 5-HT (V_{max}) was 58.7 ± 4.9%.

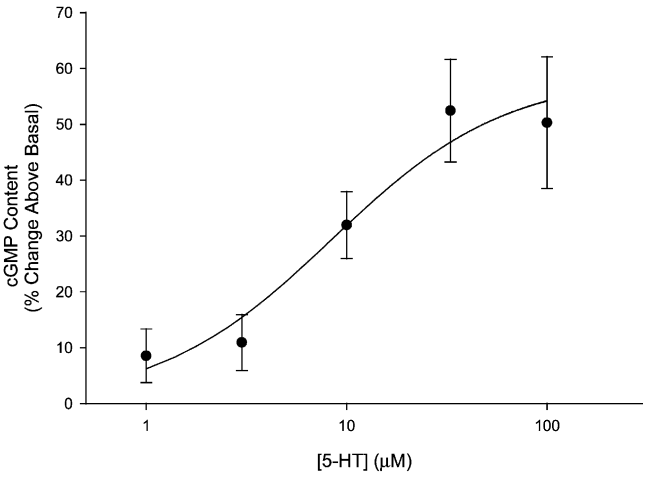

Fig. 2. Concentration–response relationship for 5-HT stimulation of cGMP formation. Slices from the rat frontal cortex were incubated with various concentrations of 5-HT. Cyclic GMP content is expressed as the percent increase above basal levels (2.08 ± 0.41 pmol/mg). Data are plotted as the means ± SEM of triplicate determinations from between three and six animals. The line represents a computer-fit of the logistic equation to the data.

Various 5-HT receptors, including the 5-HT_{1A}, 5-HT_{2A}, and 5-HT_{2C} subtypes, are found in the frontal cortex (Raymond et al., 2001). Therefore, experiments were undertaken to identify which serotonin receptors could be responsible for increasing cGMP content. Fig. 3 shows the relative increase in cGMP content induced by 100 μM DOM. This 5-$HT_{2A/2C}$ receptor agonist significantly elevated cGMP content from 1.76 ± 0.29 to 2.98 ± 0.93 pmol/mg. This was a maximally effective concentration of DOM, as addition of 100 and 333 μM DOM produced comparable stimulation of cGMP formation (in separate experiments stimulation of cGMP for-

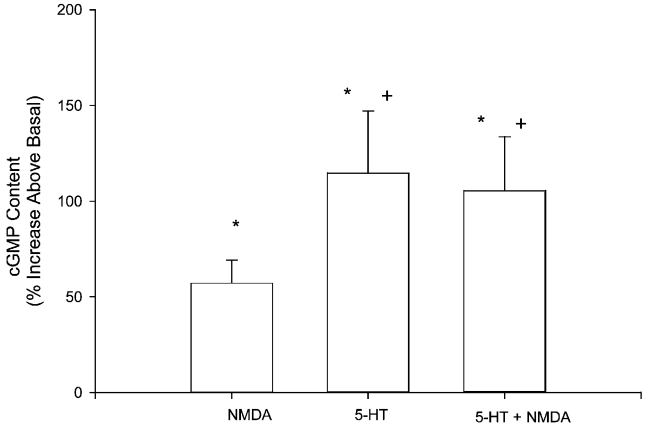

Fig. 1. Serotonin increases cGMP content in slices of the rat frontal cortex. Brain slices were incubated in the absence (i.e. basal) or presence of 100 μM 5-HT, 100 μM NMDA, and 5 - HT + NMDA. Cyclic GMP content is expressed as the percent increase above basal levels (2.49 ± 0.70 pmol/mg). Data are plotted as the mean ± SEM of triplicate determinations from seven animals. $*P < 0.05$, compared to basal; $+P < 0.05$, compared to NMDA alone.

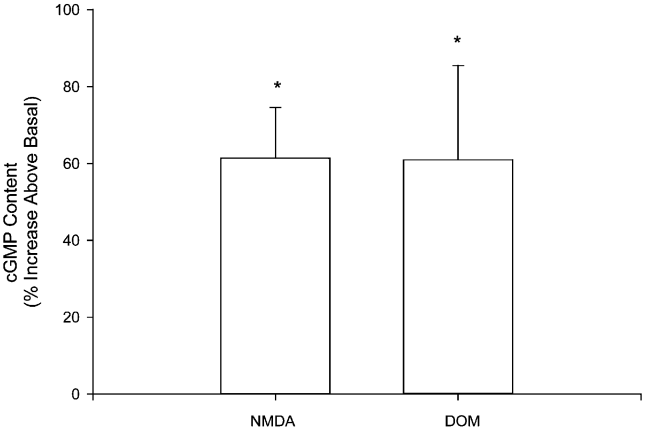

Fig. 3. The 5-$HT_{2A/2C}$ agonist DOM increases cGMP content in slices of the rat frontal cortex. Coronal slices from the rat frontal cortex were incubated in the absence (i.e. basal) or the presence of 100 μM DOM or 100 μM NMDA. Cyclic GMP content is expressed as the percent increase above basal levels (1.76 ± 0.29 pmol/mg). Data are plotted as the mean ± SEM of triplicate determinations from between four and five animals. $*P < 0.05$, compared to basal.

mation by 100 μM and 333 μM DOM was 32.75 ± 8.98% and 36.45 ± 13.96%, respectively, n = 3–4). As noted by Leysen et al. (1989), DOM can activate other types of receptors including adrenergic and dopaminergic receptors. To determine the specificity of the effect of DOM, pirenperone, a 5-$HT_{2A/2C}$ receptor-selective antagonist, was used (Fig. 4). While having no effect on basal cGMP levels, pirenperone completely blocked DOM-mediated stimulation of cGMP formation.

Because DOM and pirenperone bind to the 5-HT_{2A} and the 5-HT_{2C} receptors, additional experiments were undertaken to determine the role of each of these receptor subtypes. MDL100907 is an antagonist that exhibits a 300 times greater affinity for blockade of the 5-HT_{2A} receptor than the 5-HT_{2C} receptor (Meneses and Terron, 2001). Inclusion of 10 μM MDL100907 alone did not alter basal cGMP content, however DOM stimulation of cGMP content was antagonized (Fig. 5). In agreement with these results, incubation with spiperone, an antagonist with 2600 times greater affinity for the 5-HT_{2A} receptor over the 5-HT_{2C} receptor (Fiorella et al., 1995), had no effect on basal cGMP content of the slices, but abolished DOM stimulation of cGMP formation (data not shown.) Use of 10 μM SB242084, a 5-HT_{2C}-selective antagonist (Kennett et al., 1997) produced no inhibition of the DOM-mediated increase in cGMP content (Fig. 6).

As 5-HT_{1A} receptors are also found in the frontal cortex (Bonasera and Tecott, 2000), experiments were carred out to determine if these receptors also play a role in regulating cGMP production. Addition of 100 μM DPAT, a 5-$HT_{1A/7}$ selective agonist (Meneses and Terron, 2001), increased cGMP content (Fig. 7). This concentration of DPAT was found to be maximally effective at stimulating cGMP formation, as 100 and 333 μM DPAT produced comparable increases in cGMP content (in separate experiments stimulation of cGMP formation by 100 μM and 333 μM DPAT was 34.85 ± 2.83% and 29.50 ± 3.81%, respectively, n=3–4). Additionally, the selective 5-HT_{1A} receptor antagonist, WAY100635 (10 μM), had no effect on basal cGMP levels, but prevented the DPAT-mediated increases in cGMP forma-

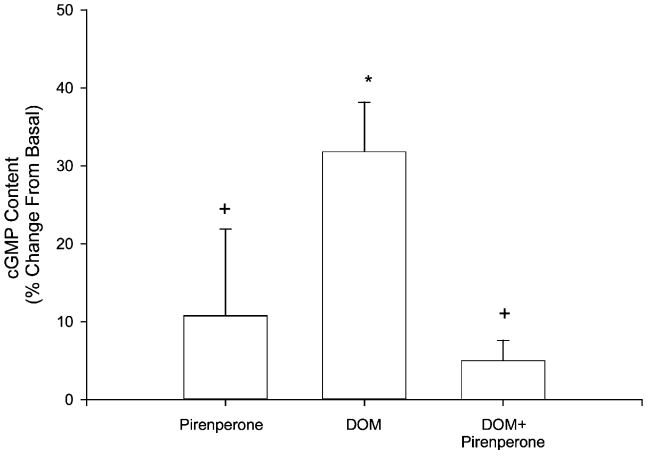

Fig. 4. The 5-$HT_{2A/2C}$ selective antagonist pirenperone blocks the DOM-Mediated Increase in cGMP Formation. Slices of the rat frontal cortex were incubated in the absence (i.e. basal) or presence of 10μM pirenperone, 100μM DOM, and pirenperone + DOM. Data are expressed as the percent change from basal levels (1.70 ± 0.26 pmol/mg) and are plotted as the mean ± SEM of triplicate determinations from 5 animals. The data show that coapplication of pirenperone blocks cGMP formation by DOM. ∗ P<0.05, compared to basal; + P<0.05, compared to DOM.

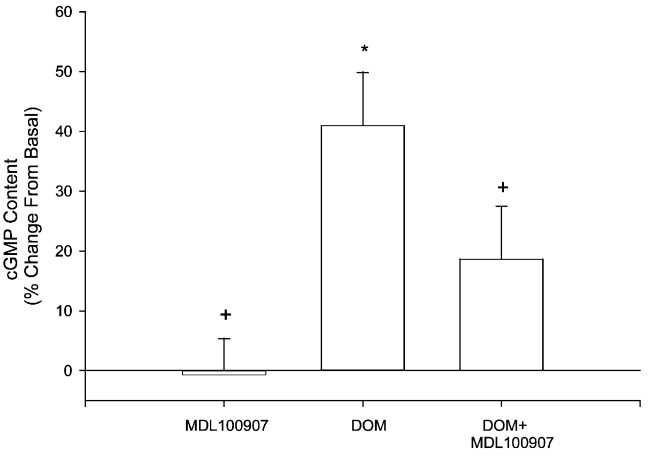

Fig. 5. The 5-HT_{2A} selective antagonist MDL100907 blocks the DOM-mediated increase in cGMP formation. Slices of the rat frontal cortex were incubated in the absence (i.e. basal) or presence of 10 μM MDL100907, 100 μM DOM, and MDL100907 + DOM. Data are expressed as the percent change from basal levels (4.65 ± 0.30 pmol /mg) and are plotted as the mean ± SEM of triplicate determinations from between four and five animals. The data show that coapplication of MDL100907 blocks cGMP formation by DOM. $*P < 0.05$, compared to basal; $+P < 0.05$, compared to DOM.

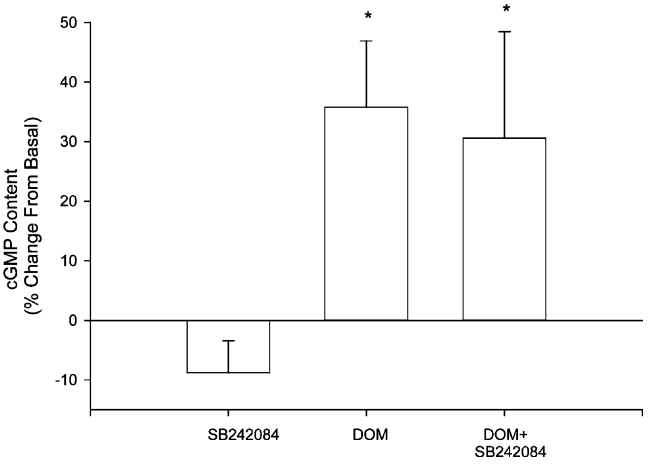

Fig. 6. The 5-HT_{2C} selective antagonist SB242084 does not block the DOM-mediated increase in cGMP formation. Slices of the rat frontal cortex were incubated in the absence (i.e. basal) or presence of 10 μM SB242084, 100 μM DOM, or SB242084 + DOM. Data are expressed as the percent change from basal levels (2.31 ± 0.23 pmol/mg) and are shown as the mean ± SEM of triplicate determinations from six animals. $*P < 0.05$, compared to basal.

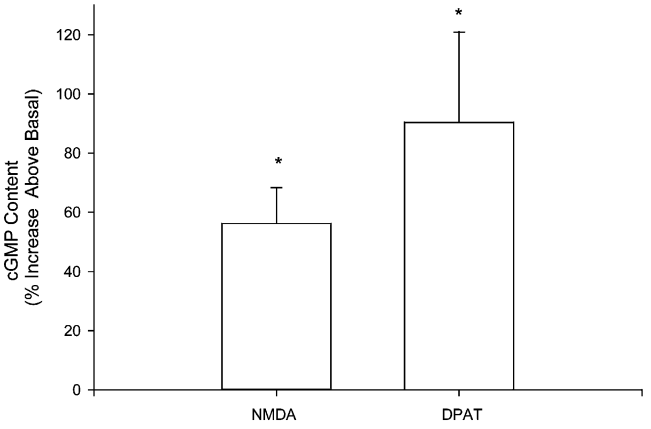

Fig. 7. The 5-$HT_{1A/7}$ receptor agonist DPAT increases cGMP content in slices of the rat frontal cortex. Brain slices were incubated in the absence (i.e. basal) or presence of 100 μM DPAT or 100 μM NMDA, and 5 - HT + NMDA. Cyclic GMP content is expressed as the percent increase above basal levels (2.35 ± 0.63 pmol/mg). Data are plotted as the mean ± SEM of triplicate determinations from four animals. ∗ $P < 0.05$, compared to basal.

tion (Fig. 8). Similarly, incubation of slices with 10 μM DPAT elicited a significant increase in cGMP generation that was inhibited by coapplication of 100 nM WAY100635. Thus, 10 μM DPAT increased cGMP content from a basal level of 0.97 ± 0.20 pmol/mg to 1.91 ± 0.50 pmol cGMP/mg ($P > 0.001$; $n = 3$–4), while cGMP content was 1.19 ± 0.60 pmol/mg in the presence of DPAT plus 100 nM WAY100635 ($n = 3$–4).

Finally, experiments were carried out using 5-HT. Addition of 10 μM pirenperone completed blocked the stimulation of cGMP formation by 100 μM 5-HT, a maximally effective concentration (Fig. 9A). Furthermore, 100 nM WAY100635 also completely blocked 5-HT-stimulated cGMP production (Fig. 9B). Under the conditions used pirenperone was not interacting with 5-HT_{1A} receptors as 10 μM pirenperone did not alter stimulation of cGMP content by 100 μM DPAT (DPAT stimulated cGMP production from 2.66 ± 1.13 pmol/mg to 6.55 ± 3.10 pmol/mg; DPAT + pirenperone cGMP level was 6.23 ± 3.03 pmol/mg; $n = 3$–4, $P < 0.05$). Similarly, WAY100635 was not interacting with 5-$HT_{2A/C}$ receptors as 100 nM WAY100635 had no affect on the stimulation of cGMP formation by 100 μM DOM (DOM stimulated cGMP production from 4.33 ± 1.07 pmol/mg to 7.35 ± 1.54 pmol/mg; DOM + WAY100635 cGMP level was 7.56 ± 1.55 pmol/mg; $n = 5$, $P < 0.05$).

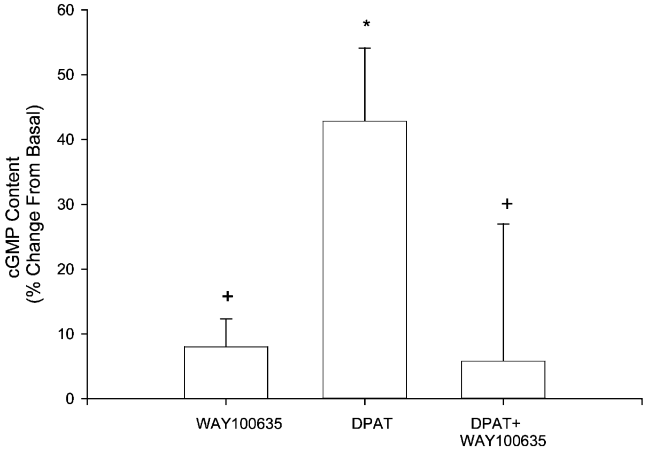

Fig. 8. The 5-HT_{1A} selective antagonist WAY100635 blocks the DPAT-mediated increase in cGMP formation. Slices of the rat frontal cortex were incubated in the absence (i.e. basal) or presence of 10 μM WAY100635, 100 μM DPAT, and WAY100635 + DPAT. Data are expressed as the percent change from basal levels (1.77 ± 0.21 pmol/mg) and are plotted as the mean ± SEM of triplicate determinations from between four and five animals. The data show that coapplication of WAY100635 blocks cGMP formation by DPAT. ∗$P < 0.05$, compared to basal; +$P < 0.05$, compared to DPAT.

4. Discussion

The present study demonstrates an increase in cGMP production in rat frontal cortex after exposure to serotonergic agonists. This serotonergic mediated increase in cGMP content involved activation of the 5-HT_{2A} and 5-HT_{1A} receptors, but not the 5-HT_{2C} receptor. Thus, an increase in cGMP production was observed with the 5-$HT_{2A/C}$ agonist DOM as well as the 5-$HT_{1A/7}$ agonist DPAT. The response to DOM was blocked by the 5-HT_{2A} antagonists spiperone and MDL 100907, while the 5-HT_{1A} antagonist WAY100635 prevented the stimulation of cGMP formation by DPAT. Conversely, the 5-

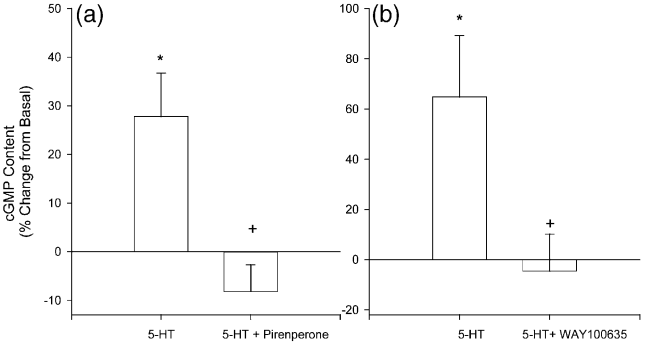

Fig. 9. Blockade of 5-HT-stimulated cGMP formation by 5-$HT_{2A/2C}$ and 5-HT_{1A} selective antagonists. (A) Brain slices were incubated with 100 μM 5-HT in the absence or presence of 10 μM pirenperone. Data are expressed as the percent change from basal levels (7.97 ± 3.38 pmol/mg), and are plotted as the mean ± SEM of triplicate determinations from five animals. The data show that pirenperone blocks stimulation of cGMP content by 5-HT. ∗$P < 0.05$, compared to basal; +$P < 0.05$, compared to 5-HT. (B) Brain slices were incubated with 100 μM 5-HT in the presence or absence of 100 nM WAY100635. Data are expressed as the percent change from basal levels (1.54 ± 0.21 pmol/mg), and are plotted as the mean ± SEM of triplicate determinations from between four and five animals. The data show that WAY100635 blocks stimulation of cGMP content by 5-HT. ∗$P < 0.05$, compared to basal; +$P < 0.05$, compared to 5-HT.

HT_{2C} antagonist SB242084 did not alter DOM-stimulated cGMP production.

The increase in cGMP production by 5-HT was concentration-dependent with an EC_{50} of 8 μM. In the cortex a spatial mismatch exists between 5-HT containing varicosities and 5-HT_{2A} receptors (Jansson et al., 2001) indicating that receptor activation occurs via volume transmission. As postsynaptic 5-HT_{1A} receptors are found both associated with synapses and extrasynaptically (Kia et al., 1996a; Riad et al., 2000), volume transmission also may be involved in activation of these receptors. However, based on experimentally predicted synaptic concentrations and diffusion distances, it would appear that sufficient serotonin is present (i.e. micromolar quantities) at the 5-HT_{1A} and 5-HT_{2A} receptors to cause stimulation of cGMP production in vivo (Bunin and Wightman, 1998). In addition, the observed EC_{50} value for cGMP production is comparable to the EC_{50} for 5-HT stimulation of phosphoinositide hydrolysis (Kendall and Nahorski, 1985; Sanders-Bush et al., 1988), which is the prototypic response to activation of 5-HT_{2A} receptors (Raymond et al., 2001).

Contrary to the present findings, serotonin was reported to inhibit NMDA-stimulated cGMP production in the hippocampus and cerebellum through activation of 5-HT_{1A} and 5-HT_{2C} receptors (Strosznajder et al., 1996; Marcoli et al., 1998; Marcoli et al., 1997). The reason for this discrepancy is unclear, but may be methodological. Alternatively, these differences may reflect tissue specific effects of the serotonin receptors. Meller et al. (2000) have shown that the response to 5-HT_{1A} receptor activation is tissue specific and depends on the extent of the receptor reserve. Interestingly, Maura et al. (2000) reported that in human neocortex stimulation of 5-HT_{1A} and 5-HT_{2C}, but not 5-HT_{2A}, receptors, also inhibited NMDA-stimulated cGMP formation. It is important to bear in mind that human samples are often obtained from subjects with underlying pathologies which are potential confounders in the results. Furthermore, serotonin enhances the amplitude of the NMDA current in cortical slices from rodents and this involves the 5-HT_{2A} receptor and not the 5-HT_{2C} receptor (Arvanov and Wang, 1998). Although Arvanov et al. (1999) also observed an inhibition of the NMDA current, 5-HT_{2A} rather than 5-HT_{2C} receptors were involved, and this inhibition, in contrast to the results of Maura et al. (2000), required higher concentration and/or long incubations with the serotonergic agonists.

Interestingly, the addition of either pirenperone or WAY100635 alone completely prevented 5-HT stimulation of cGMP production. This observation does not appear to be consistent with the 5-HT_{1A} and 5-HT_{2A} receptors being on different cells and/or activating separate pools of guanylate cyclase. In the cortex 5-HT_{2A} receptors are found on GABAergic interneurons and on pyramidal cells (Willins et al., 1997; Jakab and Goldman-Rakic, 1998, 2000; Jansson et al., 2001), while the 5-HT_{1A} receptors are located on neuronal perikarya and dendrites (Kia et al., 1996a,b). However, the same layers of the neocortex display heavy immunoreactivity for 5-HT_{1A} and 5-HT_{2A} receptor antibodies (Kia et al., 1996a,b; Xu and Pandey, 2000; Jansson et al., 2001), and the 5-HT_{2A} and 5-HT_{1A} receptors are co-localized in some pyramidal neurons (Feng et al., 2001; Martin-Ruiz et al., 2001). Although 5-HT_{2A} receptors also are found on astrocytes (Xu and Pandey, 2000), the presence of 5-HT_{1A} receptors on glia is uncertain. Whitaker-Azmitia et al. (1993) reported 5-HT_{1A} receptor immunoreactivity on astrocytes, while Kia et al. (1996a,b) observed only scattered glia in the spinal cord contained 5-HT_{1A} receptors and other studies found no astrocytic 5-HT_{1A} receptors in the CNS (Kia et al., 1996a,b; Riad et al., 2000). Thus, the most parsimonious explanation appears to be that the 5-HT_{1A} and 5-HT_{2A} receptors increase cGMP content in cortical pyramidal cells through overlapping mechanisms.

At the present time the molecular mechanism by which activation of the 5-HT_{1A} and 5-HT_{2A} receptors increase cGMP production is unclear. The finding that stimulation of cGMP content by NMDA and 5-HT was not additive suggests these compounds were acting through a common mechanism. Activation of the NMDA receptor-ionophore complex allows an influx of calcium which can binds to calmodulin and stimulate nitric oxide synthase. The resulting increase in nitric oxide subsequently stimulates soluble guanylate cyclase to increase production of cGMP (Garthwaite, 1991). Activation of the 5-HT_{2A} receptor might directly enhance soluble guanylate cyclase activity as activation of protein kinase C, which would occur after stimulation of the 5-HT_{2A} receptor (Raymond et al., 2001), was reported to increase the activity of soluble guanylate cyclase (Louis et al., 1993). Similarly, 5-HT_{1A} receptor activation also has been reported to increase PKC activity although this effect is cell specific (Liu and Albert, 1991; Raymond et al., 2001). Furthermore, it is also possible that the 5-HT_{1A} and 5-HT_{2A} receptor-mediated stimulation of cGMP formation could occur through an increase in nitric oxide synthase activity, as activation of these receptors has been reported to increase nitric oxide synthase activity in rodent prostate and canine renal arteries, respectively (Tian et al., 2002; Carmena et al., 1998). Alternatively, stimulation of the 5-HT_{1A} and 5-HT_{2A} receptors could alter the NMDA receptor-ionophore complex resulting in an enhanced NMDA-induced calcium influx. Stimulation of 5-HT_{2A} receptors with the phenethylamine hallucinogen DOB enhanced NMDA-activated currents in cortical neurons and this effect was prevented by a protein kinase C inhibitor (Arvanov et al., 1999). Similarly, in oocytes expressing both 5-HT_2 and NMDA receptors, serotonin enhanced NMDA responses through a PKC-dependent process (Blank et

al., 1996). Furthermore, NMDA receptor function is often enhanced by tyrosine phosphorylation and it has been shown that this process can occur via a PKC-dependent mechanism (Grosshans and Browning, 2001; Ali and Salter, 2001). It is unlikely, however, that the 5-HT_{1A} receptor mediates an increase in cGMP formation through modulation of NMDA receptor-ionophore activity, as this serotonin receptor has actually been shown to decrease NMDA receptor activity in rat brain (Edagawa et al., 1999). Stimulation of the 5-HT_{2A} receptor has been suggested to enhance glutamate release (Aghajanian and Marek, 1997), but this mechanism is not likely to be the sole means responsible for the observed increase in cGMP content as the increase in cGMP content with 5-HT was observed with a maximally effective concentration of NMDA.

The possible role of cGMP in the responses to 5-HT_{1A} and 5-HT_{2A} receptor activation is currently speculative. The 5-HT_{2A} receptors, which are involved in regulating mood and cognition, are responsible for the psychotropic effects of phenethylamine and indoleamine hallucinogens and appear to play a role in the pathology of schizophrenia (Vollenweider and Geyer, 2001; Marek et al., 2000). Interestingly, serotonergic hallucinogens and cGMP analogues share some common electrophysiological effects; activation of 5-HT_{2A} receptors by phenethylamine hallucinogens increases the amplitude of NMDA-evoked EPSPs in cortical pyramidal cells (Aghajanian and Marek, 1997; Arvanov et al., 1999), while cGMP analogues potentiate postsynaptic NMDA responses in the visual cortex (Wei et al., 2002). The 5-HT_{1A} receptors also are involved in a variety of CNS responses and appear to play a role in the pathologies of depression and anxiety (Gingrich and Hen, 2001; Hoyer et al., 2002). Interestingly, it appears as though the 5-HT_{1A} receptor may play a role in formation of memory, as 5-HT_{1A}-knock out mice have impaired memory formation and synaptic plasticity (Sarnyai et al., 2000). Similarly, cGMP may be involved in cellular memory as cGMP analogues have been shown to evoke long-term depression in cerebellar tissue (Shibuki and Okada, 1991; Bliss and Collingridge, 1993; Miyawaki et al., 1997).

In conclusion, we have observed that in frontal cortical slices of rat brain, serotonin increases cGMP content. Moreover, we have determined that this increase in cGMP is mediated specifically by the 5-HT_{1A} and 5-HT_{2A} serotonin receptors.

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