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Regulation of 5-HT_{1A} receptor-stimulated [^{35}S]-GTPγS binding as measured by quantitative autoradiography following chronic agonist administration

*,[1]Julie G. Hensler & [1]Hymavathi Durgam

[1]Department of Pharmacology, MC 7764, University of Texas Health Science Center-San Antonio, 7703 Floyd Curl Drive, San Antonio, Texas, TX 78229-3900, U.S.A.

1 Because changes 5-HT_{1A} receptor number do not occur following repeated agonist treatment, we hypothesized that the basis for 5-HT_{1A} receptor desensitization involves changes in receptor-G protein coupling. We measured the effect of repeated agonist administration on 5-HT_{1A} receptor-stimulated [^{35}S]-GTPγS binding in forebrain areas, (i.e. anterior cingulate cortex, lateral septum, hippocampus, entorhinal cortex), and serotonergic cell body areas, the dorsal and median raphe nuclei.

2 Following treatment of rats with (±)8-OH-DPAT (1 mg kg^{-1}, s.c.) for 7 or 14 days, 5-HT_{1A} receptor-stimulated [^{35}S]-GTPγS binding was significantly attenuated in both the dorsal and median raphe nuclei.

3 5-HT_{1A} receptor-stimulated [^{35}S]-GTPγS binding was significantly attenuated in the CA_1 region of the hippocampus after 7, but not 14 days of 8-OH-DPAT administration. 5-HT_{1A} receptor-stimulated [^{35}S]-GTPγS binding was not altered in other forebrain areas examined.

4 The binding of [^{3}H]-MPPF to 5-HT_{1A} receptor sites was not altered in any brain region examined following repeated agonist administration, suggesting that the observed changes in (±)8-OH-DPAT-stimulated [^{35}S]-GTPγS binding were not due to changes in 5-HT_{1A} receptor number.

5 Our data indicate that in serotonergic cell body areas the regulation of presynaptic 5-HT_{1A} receptor function following repeated agonist administration occurs at the level of receptor-G protein interaction. In forebrain areas, however, the regulation of postsynaptic 5-HT_{1A} receptor sensitivity appears not to be at the level of receptor-G protein coupling.

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Abbreviations: GDP, guanosine-5′-diphosphate; GTPγS, guanosine-5′-O-(3-thio)triphosphate; HEPES, (N-[2-hydroxyethyl]-piperazine-N′-[2-ethanesulphonic acid); [^{3}H]-MPPF, 4-(2′-methoxy)-phenyl-1-[2′-(N-2″-pyridinyl)-p-fluorobenzamido]ethyl-piperzin; 8-OH-DPAT, 8-hydroxy-dipropylaminotetralin hydrobromide: WAY 100635, N-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-N-2-pyridinyl-cyclohexanecarboxamide maleate

Introduction

In the central nervous system, cell bodies of serotonergic neurons are localized to discrete groups or nuclei in the brainstem. Serotonergic cell bodies send a dense plexus of serotonergic processes throughout the brain. The majority of ascending serotonergic processes to the forebrain arise from cell bodies in the dorsal and median raphe nuclei (see Molliver, 1987).

The serotonin-1A (5-HT_{1A}) receptor is present in high density in serotonergic cell body areas, in particular the dorsal and median raphe nuclei, as well as in cortical and limbic areas (i.e. frontal cortex, entorhinal cortex, hippocampus, septum) (Vergé *et al*., 1986; Hensler *et al*., 1991). In serotonergic cell body areas the 5-HT_{1A} receptor is located on serotonergic cell bodies and dendrites (Sotelo *et al*., 1990), and functions as the somatodendritic autoreceptor (de Montigny *et al*., 1984; see Aghajanian *et al*., 1990). In terminal field areas of serotonergic innervation, the 5-HT_{1A} receptor is located postsynaptically (Vergé *et al*., 1986; Hensler *et al*., 1991). 5-HT_{1A} receptors are coupled *via* pertussis toxin-sensitive G proteins to the inhibition of adenylyl cyclase, or to the opening of potassium channels (De Vivo & Maayani, 1986; Andrade *et al*., 1986; Markstein *et al*., 1986; Clarke *et al*., 1987). In the hippocampus, the 5-HT_{1A} receptor is coupled to both effector systems. By contrast, in the dorsal raphe 5-HT_{1A} receptors are not coupled to the inhibition of adenylyl cyclase (Clarke *et al*., 1996).

The 5-HT_{1A} receptor has been implicated in psychiatric illnesses, such as anxiety disorders and major depression. The azapirone compounds, such as buspirone and its analogues gepirone and ipsapirone, comprise a new class of psychoactive agents with both anxiolytic and antidepressant effects. These agents, which are agonists with high affinity for the 5-HT_{1A} receptor (Gozlan *et al*., 1983; Traber & Glaser, 1987), have anxiolytic and antidepressant effects in animal models (Traber & Glaser, 1987; Lucki, 1991; File *et al*., 1996) and are currently used in the treatment of anxiety disorders (Keppel Hesselink, 1992).

*Author for correspondence; E-mail: hensler@uthscsa.edu

Furthermore, antagonists of the 5-HT$_{1A}$ receptor have been suggested to improve the efficacy of certain antidepressant drugs (Artigas *et al*., 1996; Blier & Bergeron, 1998). 5-HT$_{1A}$ receptor knockout mice display decreased exploratory activity and increased fear of aversive environments (open or elevated spaces), and exhibit decreased immobility in the forced swim test, an effect commonly associated with antidepressant treatment (Ramboz *et al*., 1998). Because therapeutic effects are not attained in patients until 2–3 weeks after the beginning of treatment, adaptive changes in the serotonergic system may underlie the therapeutic effectiveness of antidepressants and anxiolytics. Thus, studies of the regulation of the 5-HT$_{1A}$ receptor may have important implications for our understanding the role of this receptor in the action of antidepressant drugs or azapirone anxiolytics.

Chronic administration of 5-HT$_{1A}$ receptor agonists, including the azapirone anxiolytics, results in the desensitization of behavioural responses mediated by postsynaptic 5-HT$_{1A}$ receptors (Larsson *et al*., 1990; Wieland *et al*., 1993). Desensitization of 5-HT$_{1A}$ somatodendritic autoreceptor function in the dorsal raphe has been demonstrated in electrophysiological studies to follow chronic administration of 5-HT$_{1A}$ receptor agonists (Blier & de Montigny, 1987; Schechter *et al*., 1990; Dong *et al*., 1997). *In vivo* microdialysis studies have confirmed these observations. The ability of 5-HT$_{1A}$ autoreceptors of the dorsal raphe to modulate serotonin release in the striatum is attenuated following 7 days of 8-OH-DPAT administration (Kreiss & Lucki, 1997). In general, changes in 5-HT$_{1A}$ receptor number have not been observed following chronic 5-HT$_{1A}$ receptor agonist administration (Larsson *et al*., 1990; Schechter *et al*., 1990; Wieland *et al*., 1993), although decreased receptor density has been reported by some investigators (Fanelli & McMonagle-Strucko, 1992).

Because changes in the sensitivity of 5-HT$_{1A}$ receptor-mediated responses following chronic administration of 5-HT$_{1A}$ receptor agonists do not appear to be mediated by changes in 5-HT$_{1A}$ receptor binding, we hypothesized that the basis for changes in 5-HT$_{1A}$ receptor function or sensitivity involves post-receptor events, specifically changes in receptor-G protein coupling. Receptor-stimulated [^{35}S]-GTPγS binding is a direct assay of receptor activation of G proteins, as it measures the exchange of GDP for GTPγS. The development of [^{35}S]-GTPγS autoradiography to identify receptor-activated G proteins in brain sections (Sim *et al*., 1995) allows the demonstration of functional activity at the level of receptor-G protein coupling with anatomical resolution. This approach offers a unique opportunity to examine regional differences in the regulation of the 5-HT$_{1A}$ receptor at the level of receptor-G protein interaction. In the current study we have examined the effect of repeated agonist administration on 5-HT$_{1A}$ receptor-stimulated [^{35}S]-GTPγS binding using quantitative autoradiography. This analysis was performed for post-synaptic 5-HT$_{1A}$ receptors in forebrain areas, which serve as terminal field areas of serotonergic innervation, and presynaptic 5-HT$_{1A}$ receptors located on the soma and dendrites of serotonergic cell bodies in the dorsal and median raphe nuclei. The present study is the first to report the use of this technique to study the regulation of 5-HT$_{1A}$ receptor function in brain following repeated agonist administration.

Methods

Animals

Male Sprague-Dawley rats (250–300 g; Harlan, Indianapolis, IN, U.S.A.) were group-housed and maintained on a 14 : 10 h day/night cycle, with constant access to food and water. These studies were carried out in accordance with the *Guide for the Care and Use of Laboratory Animals* as adopted and promulgated by the National Institutes of Health.

Drug treatment

In some experiments, rats were injected once daily with vehicle ($n=8$) or the 5-HT$_{1A}$ receptor agonist (±)8-OH-DPAT (1 mg kg^{-1}, s.c.) ($n=8$) for 7 days. In a second set of experiments, rats were injected once daily with vehicle ($n=8$) or the 5-HT$_{1A}$ receptor agonist (±)8-OH-DPAT (1 mg kg^{-1}, s.c.) ($n=8$) for 14 days. Animals were injected at the same time each day, specifically between 10 : 00 and 11 : 00 h. Animals were sacrificed 24 h after the last injection.

Tissue preparation

Rat brains were rapidly removed and frozen on powdered dry ice. Brains were stored at −80°C until sectioning. Coronal sections of 20 μm thickness were cut at −17°C in a cryostat microtome and thaw-mounted onto gelatin-coated glass slides. The sections were desiccated at 4°C for 18 h under vacuum and then stored at −80°C until use.

[^{35}S]-GTPγS autoradiography

Autoradiography of agonist-stimulated [^{35}S]-GTPγS binding in brain sections was performed as described by Dupuis *et al*. (1999) with some modifications. Slide-mounted sections were thawed quickly at room temperature for 5 min and then equilibrated in HEPES buffer (50 mM, pH 7.4), supplemented with (mM): $MgCl_2$ 3, EGTA 0.2, NaCl 100 and dithiothreitol 0.2, at room temperature for 10 min. Sections were then pre-incubated in HEPES buffer containing GDP (2 mM) for 20 min. Sections were subsequently incubated in pre-warmed HEPES assay buffer containing GDP (2 mM) and 80 pM [^{35}S]-GTPγS either in the absence or in the presence of agonist for 1 h at 30°C. Basal [^{35}S]-GTPγS binding was defined in the absence of agonist. Nonspecific [^{35}S]-GTPγS binding was defined in the absence of agonist and in the presence of 10 μM GTPγS. The incubation was stopped by two washes for 2 min each in ice-cold 50 mM Tris-HCl buffer (pH 7.4), followed by a brief immersion in ice-cold deionized water. Sections were dried on a slide-warmer and exposed to Kodak Biomax MR film (Amersham) for 24 h.

[^{3}H]-MPPF autoradiography

Autoradiography of the binding of [^{3}H]-MPPF to 5-HT$_{1A}$ receptors in brain sections was performed as described by Clarke *et al*. (2001). Briefly, slide-mounted sections were thawed in a dessicator at 4°C for 30 min. Sections were preincubated for 30 min at room temperature in assay buffer (170 mM Tris-HCl, pH 7.6 at room temperature). Sections were subsequently incubated in assay buffer containing 10 nM

[^{3}H]-MPPF for 90 min at room temperature. Nonspecific binding was defined by incubating adjacent sections in the presence of 10 μM WAY-100635. Incubation was terminated by two washes for 5 min each in ice-cold 170 mM Tris-HCl buffer (pH 7.6), followed by a dip in ice-cold deionized water. Sections were dried on a slide warmer and exposed to [^{3}H]-sensitive Hyperfilm film (Amersham) for a period of 3 weeks to generate autoradiograms.

Image analysis

Analysis of the digitized autoradiograms was performed using the image analysis program NIH Image, version 1.47 (NIH, Bethesda, MD, U.S.A.). Tissue sections were stained with thionin and the brain areas identified using the atlas of the rat brain of Paxinos & Watson (1986). Autoradiograms of [^{3}H]-MPPF binding were quantified by the use of simultaneously exposed [^{3}H] standards (ART-123, American Radiochemicals, St. Louis, MO, U.S.A.) which had been calibrated using brain-mash sections according to the method of Geary & Wooten (Geary & Wooten 1983; Geary *et al*., 1985). The amount of ligand bound was determined by converting optical density measurements to femtomoles per milligram of protein. Specific binding was calculated by subtracting nonspecific binding from total binding on adjacent sections.

Autoradiograms of 5-HT$_{1A}$ receptor-stimulated [^{35}S]-GTPγS binding were quantified by the use of simultaneously exposed [^{14}C] standards (ARC-146, American Radiochemicals, St. Louis, MO, U.S.A.). Standard curves were fit to pixel data obtained from [^{14}C] standards and tissue equivalent values (nCi g^{-1}) provided by American Radiochemicals, and were used to transform the actual regional densitometric values into relative radioactivity measures. Nonspecific binding of [^{35}S]-GTPγS was subtracted from basal binding and from binding in the presence of agonist. Specific, agonist-stimulated binding was expressed as per cent above basal.

Data analysis

Individual dose-response curves for specific, agonist-stimulated [^{35}S]-GTPγS binding were fit by nonlinear regression using KaleidaGraph software (version 3.0, Synergy Software, Reading, PA, U.S.A.) to the model: $E = E_{max}/(1 + (EC_{50}/[A])^n$, where E is the response at the agonist concentration [A], E_{max} is the maximal response, EC_{50} is the concentration of drug producing the half-maximal response and n is the slope factor. Statistical comparisons were made by ANOVA. F values reaching significance ($P < 0.05$) were evaluated further by *post hoc* analysis using Fisher's Protected Least Significant Difference test. Statistical tests were performed using Statistica software (version 4.1, Statsoft, Tulsa, OK, U.S.A.).

Materials

[^{35}S]-GTPγS (1250 Ci mmol^{-1}) and [^{3}H]-MPPF (66.2 Ci mmol^{-1}) were purchased from Dupont/NEN (Boston, MA, U.S.A.). GDP (disodium salt) was purchased from ICN (Costa Mesa, CA, U.S.A.). (±)8-OH-DPAT hydrobromide and WAY 100635 maleate were purchased from Sigma/RBI (Natick, MA, U.S.A.). GTPγS (tetralithium salt) was purchased from Roche/Boehringer-Mannheim (Indianapolis, IN, U.S.A.).

Results

Figure 1 shows autoradiograms of the binding of [^{35}S]-GTPγS to rat brain sections taken at the level of the lateral septum, dorsal hippocampus or dorsal raphe nucleus. As expected (Waeber & Moskowitz, 1997; Sim *et al*., 1997; Meller *et al*., 2000), application of the 5-HT$_{1A}$ receptor agonist (±)8-OH-DPAT (1 μM) resulted in an increase in the binding of [^{35}S]-GTPγS in comparison with the basal condition in many brain regions. Dose-response analyses for (±)8-OH-DPAT-stimulated [^{35}S]-GTPγS binding indicated that the maximal stimulation of [^{35}S]-GTPγS binding by 8-OH-DPAT was greater in forebrain areas (E_{max} values of 70–100% above basal) than in serotonergic cell body areas (E_{max} values of approximately 44% above basal) (see Table 1). In agreement with previous studies (Meller *et al*., 2000), the 5-HT$_{1A}$ receptor antagonist WAY 100635 (100 nM) completely blocked the stimulation of [^{35}S]-GTPγS binding by (±)8-OH-DPAT (1 μM) in all areas examined (Table 2).

To determine the effect of repeated agonist administration on 5-HT$_{1A}$ receptor-stimulated [^{35}S]-GTPγS binding, rats were treated for 7 or 14 days with (±)8-OH-DPAT (1 mg kg^{-1}, s.c.). For these studies a maximal dose of (±)8-OH-DPAT was used. The binding of [^{35}S]-GTPγS to rat brain sections taken at the level of the lateral septum, dorsal hippocampus or dorsal raphe nucleus was quantitated. The effect of repeated administration of (±)8-OH-DPAT on 5-HT$_{1A}$ receptor-stimulated [^{35}S-GTPγS binding in terminal field areas of serotonergic innervation is shown in Figure 2. (±)8-OH-DPAT-stimulated [^{35}S]-GTPγS binding was significantly attenuated only in the CA$_1$ region of the hippocampus after 7, but not 14 days of agonist administration (Figure 2). (±)8-OH-DPAT-stimulated [^{35}S]-GTPγS binding was not altered in other forebrain areas examined. These data indicate that in forebrain areas the regulation of postsynaptic 5-HT$_{1A}$ receptor sensitivity or function following repeated agonist administration appears not to be at the level of receptor-G protein interaction.

The effect of administration of (±)8-OH-DPAT for 7 or 14 days on 5-HT$_{1A}$ receptor-stimulated [^{35}S]-GTPγS binding in serotonergic cell body areas is shown in Figure 3. (±)8-OH-DPAT-stimulated [^{35}S]-GTPγS binding was significantly attenuated in the dorsal and median raphe nuclei. These data indicate that in serotonergic cell body areas the regulation of presynaptic 5-HT$_{1A}$ receptor sensitivity and function occurs at the level of receptor-G protein interaction.

To confirm that repeated agonist administration did not result in changes in 5-HT$_{1A}$ receptor number, experiments were performed measuring the binding of a single saturating concentration of the 5-HT$_{1A}$ receptor antagonist [^{3}H]-MPPF (10 nM). As shown in Table 3, the binding of [^{3}H]-MPPF to 5-HT$_{1A}$ receptor sites was not altered by repeated agonist administration in any brain region examined. These data indicate that changes in 8-OH-DPAT-stimulated [^{35}S]-GTPγS binding were not due to changes in 5-HT$_{1A}$ receptor number.

Discussion

We have examined the effect of repeated administration of the agonist (±)8-OH-DPAT on 5-HT$_{1A}$ receptor-stimulated [^{35}S]-GTPγS binding using quantitative autoradiography. The

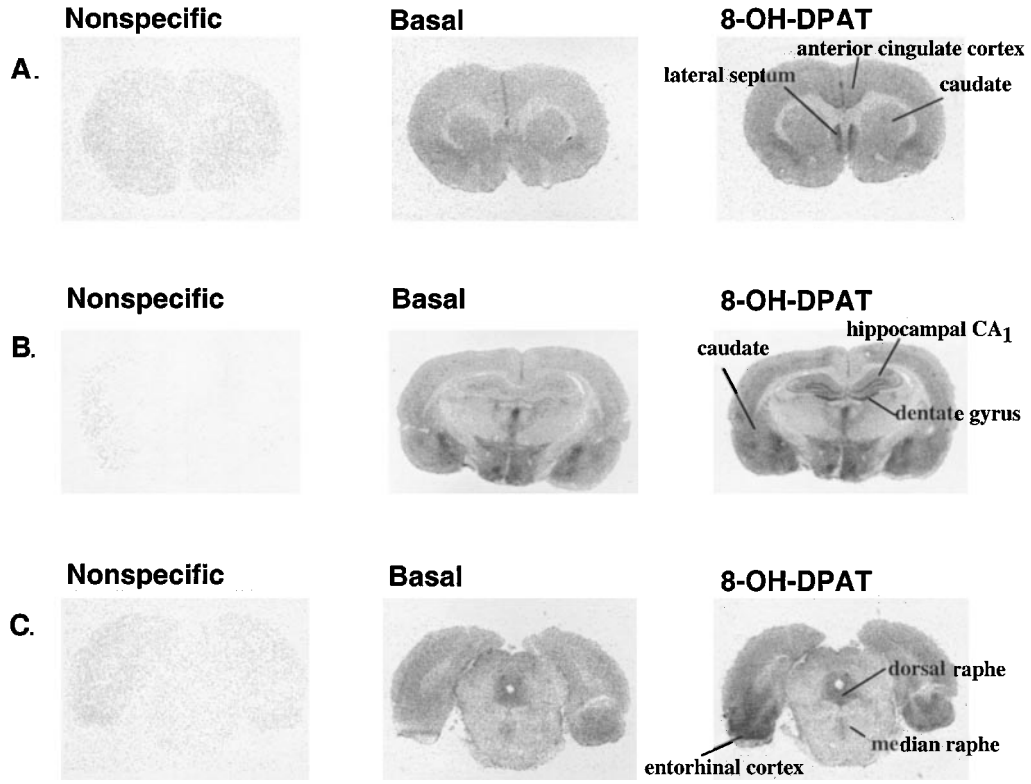

Figure 1 Autoradiograms of [^{35}S]-GTPγS binding to sections of rat brain. Coronal sections at the level of (A) the lateral septum, (B) the dorsal hippocampus, and (C) the dorsal raphe nucleus, were incubated with [^{35}S]-GTPγS (80 pM). Nonspecific binding was defined in the presence of 10 μM GTPγS. The binding of [^{35}S]-GTPγS was stimulated by (±)8-OH-DPAT (1 μM).

Table 1 Stimulation of [^{35}S]-GTPγS binding by (±)8-OH-DPAT

Brain region	*EC_{50}* (nM)	*E_{max}* (44% above basal)
Anterior cingulate cortex	28±6.8	70±3.9
Lateral septum	27±4.2	75±11
Hippocampus:		
CA_1 region	26±2.0	91±7.3
dentate gyrus	26±3.9	106±5.9
Dorsal raphe nucleus	13±4.0*	44±5.5
Median raphe nucleus	6.2±2.4**	43±4.3
Entorhinal cortex	33±7.9	84±8.7

EC_{50} and E_{max} values were derived from the analysis individual dose-response curves using eight concentrations of (±)8-OH-DPAT (1 nM – 10 μM) as described in Methods. Shown are the mean±s.e.mean of four individual experiments carried out in duplicate. *$P<0.05$ when compared to anterior cingulate cortex, entorhinal cortex. **$P<0.05$ when compared to anterior cingulate cortex, lateral septum, CA_1 region, dentate gyrus, entorhinal cortex.

Table 2 The binding of [^{35}S]-GTPγS stimulated by (±)8-OH-DPAT (1 μM) in the absence and presence of the 5-HT_{1A} receptor antagonist WAY 100635 (100 nM)

Brain region	*8-OH-DPAT-stimulated [^{35}S]-GTPγS binding* (% above basal)	*8-OH-DPAT-stimulated [^{35}S]-GTPγS binding in the presence of WAY 100635* (% above basal)
Anterior cingulate cortex	67±5.6	5.7±2.3
Lateral septum	62±5.8	6.6±3.4
Hippocampus:		
CA_1 region	85±8.5	1.0±1.0
dentate gyrus	96±13	2.3±2.3
Dorsal raphe nucleus	38±7.6	6.5±2.3
Median raphe nucleus	32±8.5	5.3±2.3
Entorhinal cortex	79±10	8.4±6.7

Brain sections were incubated with [^{35}S]-GTPγS (80 pM). Nonspecific binding of [^{35}S]-GTPγS was determined in the presence of 10 μM GTPγS. Specific binding of [^{35}S]-GTPγS is expressed as per cent above basal. Shown are the mean±s.e.mean of four individual experiments carried out in duplicate.

present study is the first to report the use of this technique to study the regulation of 5-HT_{1A} receptor function in brain following repeated agonist administration. Our data indicate that in serotonergic cell body areas the regulation of presynaptic 5-HT_{1A} receptor sensitivity following repeated agonist administration occurs at the level of receptor-G protein interaction. In forebrain areas, however, the regulation of postsynaptic 5-HT_{1A} receptor sensitivity or function following repeated agonist treatment appears not to be at the level of receptor-G protein interaction, but may occur more distally, for example at the level of the effector system or signalling cascade.

The distribution of (±)8-OH-DPAT-stimulated [^{35}S]-GTPγS binding in rat brain as measured by quantitative autoradiography (Waeber & Moskowitz, 1997; Sim *et al*., 1997; Meller *et al*., 2000; current study), is in agreement with

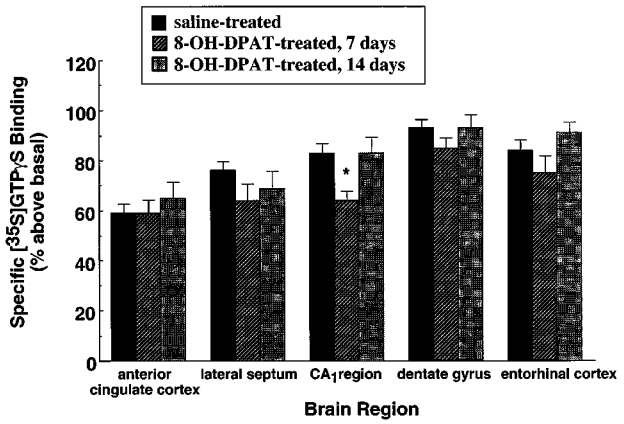

Figure 2 Effect of repeated administration of 8-OH-DPAT on 5-HT$_{1A}$ receptor-stimulated [^{35}S]-GTPγS binding in terminal field areas of serotonergic innervation. Rats were administered either saline vehicle or (±)8-OH-DPAT (1 mg kg^{-1}, once daily, s.c.) for 7 or 14 days. Coronal sections were incubated with [^{35}S]-GTPγS (80 pM). Nonspecific binding was defined in the presence of 10 μM GTPγS. [^{35}S]-GTPγS binding was stimulated by (±)8-OH-DPAT (1 μM). Specific binding of [^{35}S]-GTPγS is expressed as per cent above basal. Shown are the mean±s.e.mean saline-treated, $n=16$; 8-OH-DPAT-treated, $n=8$ per experimental group. *$P<0.05$.

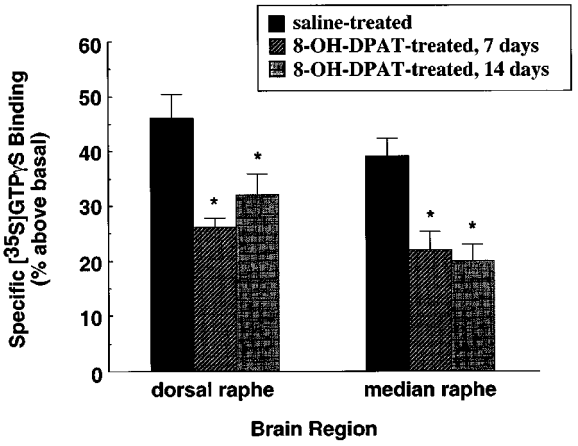

Figure 3 Effect of repeated administration of 8-OH-DPAT on 5-HT$_{1A}$ receptor-stimulated [^{35}S]-GTPγS binding in serotonergic cell body areas. Rats were administered either saline vehicle or 8-OH-DPAT (1 mg kg^{-1}, once daily, s.c.) for 7 or 14 days. Coronal sections were incubated with [^{35}S]-GTPγS (80 pM). Nonspecific binding was defined in the presence of 10 μM GTPγS. [^{35}S]-GTPγS binding was stimulated by 8-OH-DPAT (1 μM). Specific binding of [^{35}S]-GTPγS is expressed as per cent above basal. Shown are the mean±s.e.mean. Saline-treated, $n=16$; 8-OH-DPAT-treated, $n=8$ per experimental group. *$P<0.05$.

the distribution of 5-HT$_{1A}$ receptor sites observed with the binding of [^{3}H]-8-OH-DPAT or the 5-HT$_{1A}$ receptor antagonist radioligand [^{3}H]-WAY 100635 (Hensler *et al*., 1991; Gozlan *et al*., 1995; Khawaja, 1995). Our experiments using the 5-HT$_{1A}$ receptor antagonist WAY 100635 (100 nM) are in agreement with previous studies (Waeber & Moskowitz, 1997; Sim *et al*., 1997; Meller *et al*., 2000) and indicate that (±)8-OH-DPAT stimulated [^{35}S]-GTPγS binding is mediated by activation of 5-HT$_{1A}$ receptors.

Dose-response analyses for (±)8-OH-DPAT-stimulated [^{35}S]-GTPγS binding indicated that the maximal stimulation

Table 3 Effect of repeated administration of 8-OH-DPAT on the binding of [^{3}H]-MPPF to 5-HT$_{1A}$ receptors

Brain region	*Vehicle-treated* (fmol mg^{-1} protein)	*8-OH-DPAT-treated* (fmol mg^{-1} protein)
Anterior cingulate cortex	590±28	634±26
Lateral septum	1035±58	1080±63
Hippocampus:		
CA$_1$ region	1102±70	1118±74
dentate gyrus	1458±67	1388±30
Dorsal raphe nucleus	1264±68	1230±47
Median raphe nucleus	263±24	294±24
Entorhinal cortex	1563±92	1615±79

Rats were administered either saline vehicle or (±)8-OH-DPAT (1 mg kg^{-1}, once daily, s.c.) for 7 days. Coronal sections of rat brain were incubated with [^{3}H]-MPPF (10 nM). Nonspecific binding was defined in the presence of 10 μM WAY100635 and ranged from 11% of total binding in the dentate gyrus to 30% of total binding in the median raphe nucleus. Specific binding is expressed as fmol mg^{-1} protein. Shown are the mean±s.e.mean, $n=8$ animals per experimental group.

of [^{35}S]-GTPγS binding by (±)8-OH-DPAT was greater in forebrain areas than in serotonergic cell body areas (Table 1). These data are in agreement with the observations of Meller *et al*. (2000) that R-(+)8-OH-DPAT is more efficacious to stimulate [^{35}S]-GTPγS binding in hippocampus and lateral septum than in the dorsal raphe. The E_{max} values of (±)8-OH-DPAT to stimulate [^{35}S]-GTPγS binding in various brain regions in the current study do not necessarily correlate with the density of 5-HT$_{1A}$ receptor sites (Table 3; Hensler *et al*., 1991; Khawaja, 1995). For example, although the density of 5-HT$_{1A}$ receptors in the dorsal raphe is comparable to that in the lateral septum or CA$_1$ region of hippocampus, the maximal stimulation of [^{35}S]-GTPγS binding in the dorsal raphe is approximately half of that observed in these forebrain areas. We are uncertain as to why (±)8-OH-DPAT is less efficacious to stimulate [^{35}S]-GTPγS binding in the dorsal raphe nucleus. Differences in the efficacy of (±)8-OH-DPAT to stimulate [^{35}S]-GTPγS binding in forebrain areas and the dorsal raphe may be due to regional differences in the ratio of 5-HT$_{1A}$ receptors to G proteins, or in the availability of G protein subunits for coupling.

Following treatment of rats with 8-OH-DPAT (1 mg kg^{-1}, s.c.) for 7 or 14 days, 5-HT$_{1A}$ receptor-stimulated [^{35}S]-GTPγS binding was not altered in anterior cingulate cortex, lateral septum, dentate gyrus or entorhinal cortex. Of the forebrain areas examined, 5-HT$_{1A}$ receptor-stimulated [^{35}S]-GTPγS binding was significantly attenuated only in the CA$_1$ region of the hippocampus after 7, but not 14 days of chronic agonist treatment. The apparent transient decrease in 5-HT$_{1A}$ receptor-stimulated [^{35}S]-GTPγS binding in this region may reflect a region specific regulatory phenomenon and highlights the importance of time-course studies. Repeated administration of 8-OH-DPAT results in the desensitization of behavioural responses mediated by postsynaptic 5-HT$_{1A}$ receptors (Larsson *et al*., 1990). The regulation of postsynaptic 5-HT$_{1A}$ receptor sensitivity or function following repeated 8-OH-DPAT administration appears *not* to be at the level of receptor-G protein interaction, but may occur more distally, for example at the level of the effector system or signalling cascade, or in

the case of 5-HT$_{1A}$ receptor-mediated behaviours, may involve complex neuronal circuits.

Electrophysiological and neurochemical responses mediated by postsynaptic 5-HT$_{1A}$ receptors in hippocampus are not altered following chronic administration of the azapirone anxiolytics (i.e. buspirone, gepirone and ipsapirone) (Blier & de Montigny, 1987; Dong *et al*., 1997; Schechter *et al*., 1990; Varrault *et al*., 1991). Because these agents are partial agonists at postsynpatic 5-HT$_{1A}$ receptors (Smith & Peroutka, 1986; Andrade & Nicoll, 1987; Bockaert *et al*., 1987; Martin & Mason, 1987; Sprouse & Aghajanian, 1988), we have speculated that the apparent resistance of postsynaptic 5-HT$_{1A}$ receptors to regulation by the azapirone anxiolytics may be related to the low intrinsic efficacy of these agonists at postsynaptic 5-HT$_{1A}$ receptor sites. Postsynaptic 5-HT$_{1A}$ receptors may be more readily desensitized following treatment with the more efficacious agonist 8-OH-DPAT. Data from the current study indicate however that postsynaptic 5-HT$_{1A}$ receptors are resistant to regulation by repeated 8-OH-DPAT treatment.

5-HT$_{1A}$ receptor-stimulated [^{35}S]-GTPγS binding was significantly attenuated in the dorsal and median raphe nuclei following treatment of rats with 8-OH-DPAT (1 mg kg^{-1}) for 7 and 14 days. Desensitization of 5-HT$_{1A}$ somatodendritic autoreceptor function in the dorsal raphe has been demonstrated in electrophysiological studies to follow chronic administration of 5-HT$_{1A}$ receptor agonists gepirone or ipsapirone (Blier & de Montigny, 1987; Schechter *et al*., 1990; Dong *et al*., 1997). *In vivo* microdialysis studies have shown that following 7 days of 8-OH-DPAT administration the ability of 5-HT$_{1A}$ autoreceptors of the dorsal raphe to modulate serotonin release in the striatum is attenuated (Kreiss & Lucki, 1997), an indication of receptor desensitization. In these studies however, 5-HT$_{1A}$ autoreceptors of the median raphe appeared to be more resistant to regulation by 5-HT$_{1A}$ receptor agonists than those of the dorsal raphe. Although the ability of median raphe 5-HT$_{1A}$ autoreceptors to modulate serotonin release in the hippocampus was reduced after 14 days of 8-OH-DPAT administration, this trend was not statistically significant (Kreiss & Lucki, 1997). It is interesting to note that while 5-HT$_{1A}$ receptor–G protein interactions are decreased in both the dorsal and median raphe nuclei by repeated 8-OH-DPAT administration (current study), cells in the median raphe may be functionally less sensitive to this apparent decrease in autoreceptor-G protein coupling.

Following repeated treatment of rats with 8-OH-DPAT, the binding of [^{3}H]-MPPF to 5-HT$_{1A}$ receptor sites was not altered in any brain region examined, suggesting that the observed changes in (±)8-OH-DPAT-stimulated [^{35}S]-GTPγS binding were not due to changes in 5-HT$_{1A}$ receptor number. In general, changes in 5-HT$_{1A}$ receptor number have not been observed following chronic 5-HT$_{1A}$ receptor agonist administration (Larsson *et al*., 1990; Schechter *et al*., 1990; Wieland *et al*., 1993), although decreased receptor density has been reported by some investigators (Fanelli & McMonagle-Strucko, 1992). The data from the present study indicate that in serotonergic cell body areas the regulation of presynaptic 5-HT$_{1A}$ receptor sensitivity and function following repeated agonist administration occurs at the level of receptor-G protein interaction. In many forebrain areas, however, the regulation of postsynaptic 5-HT$_{1A}$ receptor sensitivity or function following repeated agonist administration appears not to be at the level of receptor-G protein coupling. These observations raise interesting questions as to the different cellular processes or mechanisms underlying regional differences in the regulation of 5-HT$_{1A}$ receptor responsiveness.

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