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Differential Regulation of 5-HT_{1A} Receptor-G Protein Interactions in Brain Following Chronic Antidepressant Administration

Julie G. Hensler, Ph.D.

Changes in 5-HT_{1A} receptor function or sensitivity following chronic antidepressant treatment may involve changes in receptor-G protein interaction. We have examined the effect of chronic administration of the SSRI fluoxetine or the tricyclic antidepressant amitriptyline on 5-HT_{1A} receptor-stimulated [^{35}S]GTPγS binding in serotonergic cell body areas, and cortical and limbic structures using quantitative autoradiography. Treatment of rats with fluoxetine, but not amitriptyline, resulted in an attenuation of 5-HT_{1A} receptor-stimulated [^{35}S]GTPγS binding in the dorsal and median raphe nuclei. The binding of the antagonist radioligand [^{3}H]MPPF to 5-HT_{1A} receptor sites was not altered, suggesting that the observed changes in 5-HT_{1A} receptor-stimulated [^{35}S]GTPγS binding were not due to changes in receptor number. Thus, the desensitization of somatodendritic 5-HT_{1A} autoreceptors in the dorsal and median raphe following chronic SSRI treatment appears to be due to a reduced capacity of the 5-HT_{1A} receptor to activate G protein. By contrast, no significant change in postsynaptic 5-HT_{1A} receptor-stimulated [^{35}S]GTPγS binding was observed in any of the forebrain areas examined following chronic antidepressant treatment. Thus, changes in postsynaptic 5-HT_{1A} receptor-mediated responses reported to follow chronic SSRI or tricyclic antidepressant administration most likely occur distal to receptor-G protein interaction, perhaps at the level of effector, or involving changes in neuronal function at the system or circuit level.

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Of the multiple types of receptors for serotonin (5-hydroxytryptamine; 5-HT) present in brain, the 5-HT_{1A} receptor in particular has been implicated in affective disorders such as anxiety and depression. Adaptive changes in the serotonergic system are believed to underlie the therapeutic effectiveness of the azapirone anxiolytics and a variety of antidepressant drugs. Thus, studies of the regulation of 5-HT_{1A} receptor function may have important implications for our understanding the role of this receptor in the mechanism of action of these therapeutic agents.

Chronic administration of selective serotonin reuptake inhibitors (SSRIs) results in the desensitization of 5-HT_{1A} somatodendritic autoreceptor function in the dorsal raphe (see Blier and de Montigny 1994; Kreiss and Lucki 1995; Le Poul et al. 2000). Chronic SSRI treatment also results in the desensitization of physiological responses mediated by postsynaptic 5-HT_{1A} receptors

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(Goodwin et al. 1987; Hensler et al. 1991; Li et al. 1996). Electrophysiological and neurochemical studies indicate, however, that in hippocampus the sensitivity of postsynaptic 5-HT_{1A} receptor-mediated responses is not changed (Blier and de Montigny 1983; Chaput et al. 1986; Varrault et al. 1991; Le Poul et al. 2000).

Unlike the SSRIs, chronic administration of tricyclic antidepressants does not result in the desensitization of the 5-HT_{1A} somatodendritic autoreceptor (Blier and de Montigny 1980; Kreiss and Lucki 1995). Although some investigators have reported an attenuation of behavioral and physiological responses mediated by postsynaptic 5-HT_{1A} receptors (Goodwin et al. 1987; Lesch et al. 1990), this has not been a consistent observation (Lucki and Frazer 1982; Wozniak et al. 1987; Hensler et al. 1991; Gartside et al. 1992). Electrophysiological studies indicate that chronic treatment with tricyclic antidepressants results in the *sensitization* of postsynaptic neurons in hippocampus to 5-HT_{1A} receptor agonists (de Montigny and Aghajanian 1978; Chaput et al. 1991).

These discrepant observations regarding the regulation of postsynaptic 5-HT_{1A} receptor sensitivity following chronic SSRI or tricyclic antidepressant administration may be due to the effects of these drug treatments on complex neuronal circuits, or differences in the regulation of 5-HT_{1A} receptor function in specific brain regions. In general, changes in 5-HT_{1A} receptor number have not been observed following chronic administration of antidepressants (Wieland et al. 1993; Hensler et al. 1991; Le Poul et al. 2000; Hervàs et al. 2001). Because changes in the sensitivity of 5-HT_{1A} receptor-mediated responses do not appear to be mediated by changes in 5-HT_{1A} receptor binding, the basis for changes in 5-HT_{1A} receptor function or sensitivity may involve changes in the capacity of the 5-HT_{1A} receptor to activate G protein. Receptor-stimulated [^{35}S]GTPγS binding is a direct assay of receptor activation of G proteins, as it measures the exchange of GDP for [^{35}S]GTPγS. [^{35}S]GTPγS autoradiography allows the demonstration of receptor-G protein interaction with neuroanatomical resolution. We have used this approach previously to examine regional differences in the regulation of the 5-HT_{1A} receptor at the level of receptor-G protein interaction following chronic agonist administration (Hensler and Durgam 2001).

In the current study we have examined the effect of repeated administration of the SSRI fluoxetine or the tricyclic antidepressant amitriptyline on 5-HT_{1A} receptor-stimulated [^{35}S]GTPγS binding. Using quantitative autoradiography, this analysis was performed for postsynaptic 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 [^{35}S]GTPγS autoradiography to study the regulation of 5-HT_{1A} receptor-G protein interaction in brain following repeated antidepressant administration.

METHODS

Animals

Male Sprague-Dawley rats (250–300 g; Harlan, Indianapolis, IN) 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

Rats were injected intraperitoneally (i.p.) with saline (n = 12), fluoxetine (10 mg/kg) (n = 8), or amitriptyline (10 mg/kg) (n = 8) once a day for 14 days. Amitriptyline (υ = 1 ml/kg) and fluoxetine (υ = 2 ml/kg) were dissolved in water and injected according to body weight. These doses of amitriptyline and fluoxetine were chosen from the literature to correspond to clinically relevant doses (de Montigny and Aghajanian 1978; Czachura and Rasmussen 2000). Fresh fluoxetine and amitriptyline solutions were made each day. Animals were injected at the same time each day, specifically between 11:00 A.M. and 1:00 P.M. Animals were sacrificed 48 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 at the level of the lateral septum, dorsal hippocampus or dorsal raphe according to the atlas of the rat brain of Paxinos and Watson (1986). Sections were thaw-mounted onto gelatin-coated glass slides, desiccated at 4°C for 18 h under vacuum and then stored at −80°C until use.

[^{35}S]GTPγS Autoradiography

Autoradiography of (±)8-OH-DPAT-stimulated [^{35}S]GTPγS binding in brain sections was performed as previously described (Hensler and Durgam 2001). Because both $MgCl_2$ and GDP affect the interaction of the agonist with the receptor and G protein, as well as basal [^{35}S]GTPγS binding, we have determined in preliminary experiments the optimal concentrations of $MgCl_2$ and GDP to maximize agonist-stimulated [^{35}S]GTPγS binding. The largest increases in 8-OH-DPAT-stimulated [^{35}S]GTPγS binding above basal were obtained with 2 mM GDP

and 3 mM $MgCl_2$ in the assay buffer. Slide-mounted sections were thawed and desiccated at 4°C for 2 h, and then equilibrated in HEPES buffer (50 mM, pH 7.4), supplemented with 3 mM $MgCl_2$, 0.2 mM EGTA, 100 mM NaCl, and 0.2 mM dithiothreitol for 10 min at 30°C. Sections were pre-incubated in HEPES buffer containing GDP (2 mM) for 10 min at 30°C, and then incubated in HEPES buffer containing GDP (2 mM) and 80 pM [^{35}S]GTPγS, either in the absence or in the presence of (±)8-OH-DPAT (1 μM), for 45 min at 30°C. Basal [^{35}S]GTPγS binding was defined in the absence of (±)8-OH-DPAT. Nonspecific [^{35}S]GTPγS binding was defined in the absence of (±)8-OH-DPAT 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 de-ionized 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 (Clarke et al. 2001; Hensler and Durgam 2001). Briefly, slide-mounted sections were thawed and desiccated at 4°C for 2 h. Sections were pre-incubated for 30 min at 30°C in assay buffer (170 mM Tris-HCl, pH 7.6), and then incubated in assay buffer containing 10 nM [^{3}H]MPPF for 90 min at 30°C. Nonspecific binding was defined by incubating adjacent sections in the presence of 10 μM WAY 100,635. 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 de-ionized water. Sections were dried on a slide warmer and exposed to [^{3}H]-sensitive Hyperfilm film (Amersham) for a period of three 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). Tissue sections were stained with thionin and the brain areas identified using the atlas of the rat brain of Paxinos and 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) which had been calibrated using brain-mash sections according to the method of Geary and Wooten (Geary and 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 (±)8-OH-DPAT-stimulated [^{35}S]GTPγS binding were quantified by the use of simultaneously exposed [^{14}C] standards (ARC-146, American Radiochemicals, St. Louis, MO). Standard curves were fit to pixel data obtained from [^{14}C] standards and tissue equivalent values (nCi/g) 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 (±)8-OH-DPAT. Specific, (±)8-OH-DPAT-stimulated binding was expressed as % above basal.

Data Analysis

Statistical comparisons were made by ANOVA. F values reaching significance ($p < .05$) 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).

Materials

[^{35}S]GTPγS (1250 Ci/mmol) and [^{3}H]MPPF (70.5 Ci/mmol) were purchased from Dupont/NEN (Boston, MA). GDP (disodium salt) was purchased from ICN (Costa Mesa, CA). GTPγS (tetralithium salt) was purchased from Roche/Boehringer-Manheim (Indianapolis, IN). (±)8-OH-DPAT hydrobromide was purchase from Tocris Cookson (Ballwin, MO). WAY 100,635 maleate, fluoxetine hydrochloride, and amitriptyline hydrochloride were purchased from Sigma/RBI (St. Louis, MO).

RESULTS

In confirmation of earlier studies (Waeber and Moskowitz 1997; Sim et al. 1997; Meller et al. 2000; Hensler and Durgam 2001), 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 to the basal condition in many brain regions, specifically serotonergic cell body areas (dorsal and median raphe nuclei), as well as in cortical and limbic areas (frontal cortex, entorhinal cortex, hippocampus, septum). We and others have shown that the 5-HT_{1A} receptor antagonist WAY 100,635 completely blocks the stimulation of [^{35}S]GTPγS binding by (±)8-OH-DPAT (Meller et al. 2000; Hensler and Durgam 2001). Autoradiograms of the binding of [^{35}S]GTPγS to sections rat brain taken at the level of the dorsal and median raphe nuclei are shown in Figure 1.

To determine the effect of chronic antidepressant treatment on 5-HT_{1A} receptor-stimulated [^{35}S]GTPγS binding, rats were administered the SSRI fluoxetine or

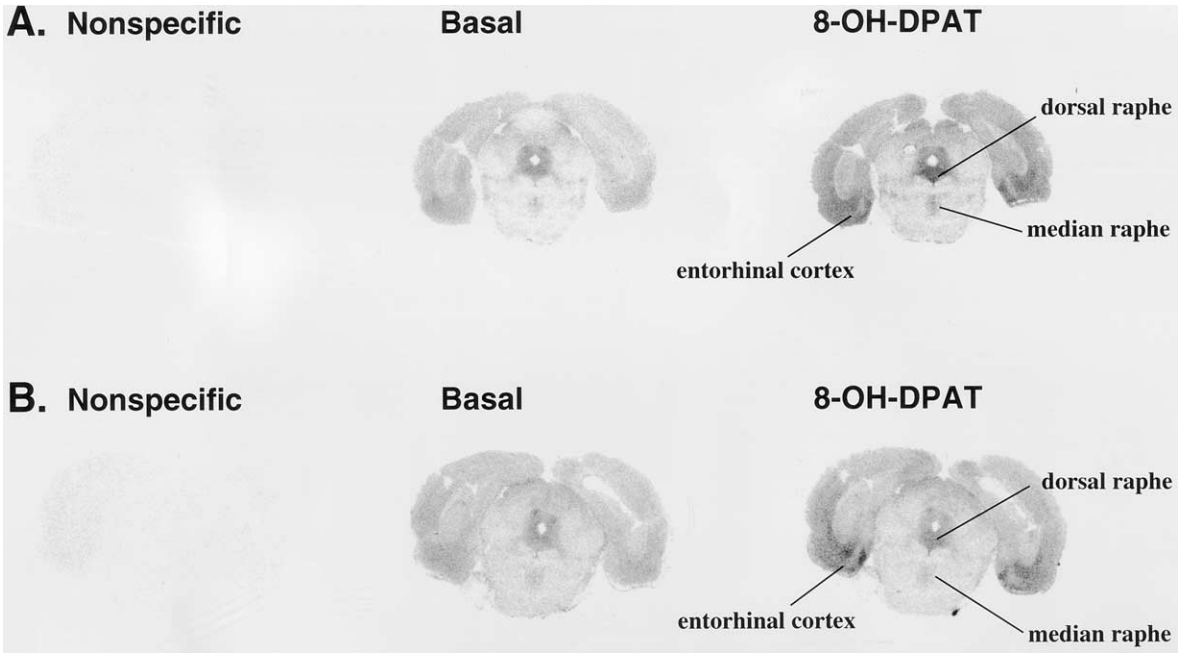

Figure 1. Autoradiograms of [^{35}S]GTPγS binding to sections of rat brain treated for 14 days with (A) saline or (B) fluoxetine (10 mg/kg, i.p.). Coronal sections at the level of the dorsal raphe nucleus were incubated with [^{35}S]GTPγS (80 pM). Nonspecific binding was defined in the presence of 10 μM GTPγS. The binding of [^{35}S]GTPγS was stimulated by (±)8-OH-DPAT (1 μM).

the tricyclic antidepressant amitriptyline for 14 days. The binding of [^{35}S]GTPγS stimulated by a maximal concentration of (±)8-OH-DPAT (1 μM) (Meller et al. 2000; Hensler and Durgam 2001) was quantitated in sections taken at the level of the lateral septum, dorsal hippocampus, or dorsal raphe nucleus. Autoradiograms of the binding of [^{35}S]GTPγS to sections taken at the level of the dorsal and median raphe nuclei from a fluoxetine-treated rat are shown in Figure 1, panel B. The effect of chronic fluoxetine or amitriptyline administration on 5-HT$_{1A}$ receptor-stimulated [^{35}S]GTPγS binding in serotonergic cell body areas was quantitated and is shown in Figure 2. (±)8-OH-DPAT-stimulated [^{35}S]GTPγS binding was significantly attenuated in the dorsal raphe nucleus following fluoxetine treatment. Although there was a decrease in (±)8-OH-DPAT-stimulated [^{35}S]GTPγS binding in the median raphe, this did not reach statistical significance. Chronic amitriptyline administration did not change (±)8-OH-DPAT-stimulated [^{35}S]GTPγS binding in either the dorsal or median raphe nuclei. These data suggest that in serotonergic cell body areas, the regulation of presynaptic 5-HT$_{1A}$ receptor sensitivity or function following chronic treatment with fluoxetine occurs at the level of receptor-G protein interaction.

To confirm that changes in (±)8-OH-DPAT-stimulated [^{35}S]GTPγS binding observed in the dorsal and median raphe nuclei were not due to changes in the density or expression of 5-HT$_{1A}$ receptors, experiments were performed using a single, saturating concentration of the 5-HT$_{1A}$ receptor antagonist radioligand [^{3}H]MPPF. The binding of [^{3}H]MPPF (10 nM) to 5-HT$_{1A}$ receptor sites was not altered following administration of fluoxetine or amitriptyline in the dorsal or median raphe nuclei (Table 1). These data indicate that the observed changes in (±)8-OH-DPAT-stimulated [^{35}S]GTPγS binding in serotonergic cell body areas are not due to changes in 5-HT$_{1A}$ receptor number.

The effect of chronic administration of fluoxetine or amitriptyline on (±)8-OH-DPAT-stimulated [^{35}S]GTPγS binding in terminal field areas of serotonergic innervation is shown in Figure 3. Surprisingly, (±)8-OH-DPAT-stimulated [^{35}S]GTPγS binding was not altered in any of the forebrain areas examined, suggesting that the regulation of postsynaptic 5-HT$_{1A}$ receptor sensitivity or function following chronic treatment with either of these antidepressant drugs is *not* at the level of receptor-G protein interaction.

The effect of chronic administration of fluoxetine or amitriptyline on 5-HT$_{1A}$ receptor number in forebrain regions was determined. The binding of a single, saturating concentration of the 5-HT$_{1A}$ receptor antagonist radioligand [^{3}H]MPPF (10 nM) to 5-HT$_{1A}$ receptor sites was not altered in any forebrain area examined following chronic administration of fluoxetine. A significant increase in the binding of [^{3}H]MPPF to 5-HT$_{1A}$ receptor sites, however, was observed in dentate gyrus and CA$_1$ region of the hippocampus following chronic amitriptyline treatment (Table 1). [^{3}H]MPPF binding to 5-HT$_{1A}$ receptor sites was not altered in lateral septum or in the

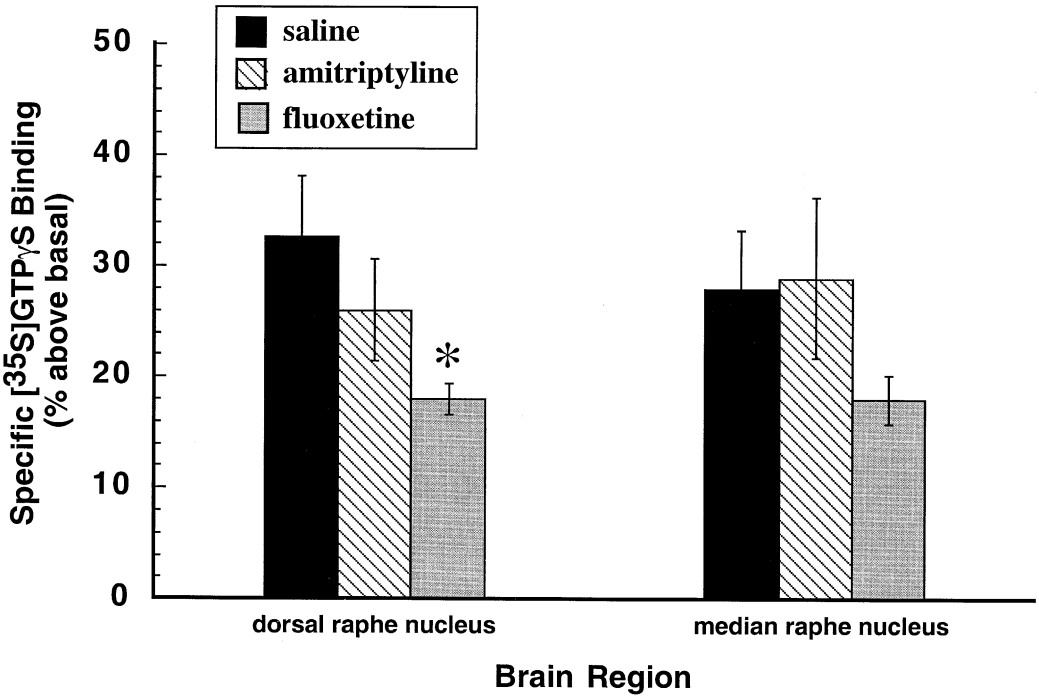

Figure 2. Effect of repeated administration of fluoxetine or amitriptyline on 5-HT$_{1A}$ receptor-stimulated [^{35}S]GTPγS binding in serotonergic cell body areas. Rats were administered either saline, amitriptyline, or fluoxetine (10 mg/kg, once daily, i.p.) for 14 days. Coronal sections were incubated 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 % above basal. Shown are the mean ± S.E.M. Saline-treated, n = 12, amitriptyline-treated, n = 8, fluoxetine-treated, n = 8, per experimental group. *$p < .05$.

cortical areas examined following chronic amitriptyline administration (Table 1). An increase in 5-HT$_{1A}$ receptor number in hippocampus following chronic amitriptyline treatment, in the absence of an increase in (±)8-OH-DPAT-stimulated [^{35}S]GTPγS binding, suggests that these additional 5-HT$_{1A}$ receptors are uncoupled from G protein.

DISCUSSION

In the current study we have examined the effect of chronic administration of the SSRI fluoxetine or the tricyclic antidepressant amitriptyline on 5-HT$_{1A}$ receptor-stimulated [^{35}S]GTPγS binding in serotonergic cell body areas, as well as in cortical and limbic areas, using quantitative autoradiography. Chronic treatment of rats with amitriptyline did not alter 5-HT$_{1A}$ receptor-stimulated [^{35}S]GTPγS binding in the dorsal or median raphe nuclei. Chronic administration of fluoxetine resulted in an attenuation of 5-HT$_{1A}$ receptor-stimulated [^{35}S]GTPγS binding in the dorsal and median raphe nuclei, with no change in the number of 5-HT$_{1A}$ receptor binding sites. Our data suggest that the capacity of the 5-HT$_{1A}$ receptor to activate G protein is reduced in serotonergic cell body areas following chronic fluoxetine administration. No significant change in postsynaptic 5-HT$_{1A}$ receptor-stimulated [^{35}S]GTPγS binding was observed in any of the forebrain areas examined following chronic treatment with either antidepressant. Thus changes in postsynaptic 5-HT$_{1A}$ receptor-mediated responses reported to follow chronic SSRI or tricyclic antidepressant administration appear to occur more distal to receptor-G protein interaction, perhaps at the level of effector, or involving changes in neuronal function at the system or circuit level.

Desensitization of 5-HT$_{1A}$ somatodendritic autoreceptor function in the dorsal raphe has been demonstrated in electrophysiological studies to follow chronic administration of the SSRI fluoxetine (Czachura and Rasmussen 2000; Le Poul et al. 2000). In vivo microdialysis studies have confirmed these observations (Kreiss and Lucki 1995). Chronic inactivation of 5-HT reuptake, by injection of antisense coding sequence of the 5-HT transporter gene into the dorsal raphe of the rat, results in a decrease in 5-HT$_{1A}$ receptor-stimulated [^{35}S]GTPγS binding in this brain region (Fabre et al. 2000b). Similar observations have been made in knock-out mice lacking the 5-HT transporter, although in these mice 5-HT$_{1A}$ receptor density in the dorsal raphe is significantly reduced (Fabre et al. 2000a). In the current study, (±)8-OH-DPAT-stimulated [^{35}S]GTPγS binding was attenuated in

Table 1. Effect of Chronic Administration of Fluoxetine or Amitriptyline on the Binding of [^{3}H]MPPF to 5-HT$_{1A}$ Receptors

	Treatment		
Brain Region	**Saline (fmol/mg protein)**	**Amitriptyline (fmol/mg protein)**	**Fluoxetine (fmol/mg protein)**
Anterior cingulate cortex	405 ± 22	395 ± 28	415 ± 32
Lateral septum	694 ± 54	738 ± 72	735 ± 44
Hippocampus			
CA_1 region	941 ± 44	1299 ± 67*	865 ± 58
dentate gyrus	1272 ± 39	1523 ± 52*	1088 ± 114
Dorsal raphe nucleus	1399 ± 68	1365 ± 62	1390 ± 42
Median raphe nucleus	593 ± 58	560 ± 44	580 ± 65
Entorhinal cortex	1459 ± 78	1473 ± 79	1571 ± 66

Rats were administered either saline, or fluoxetine or amitriptyline (10 mg/kg, once daily, i.p.) for 14 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 protein. Shown are the mean ± S.E.M. Saline-treated, n = 12, amitriptyline-treated, n = 8, fluoxetine-treated, n = 8, per experimental group.

* $p < 0.05$

the dorsal and median raphe nuclei following chronic administration of fluoxetine. In agreement with previous studies (Le Poul et al. 2000; Hervàs et al. 2001), we observed no change in the number of 5-HT$_{1A}$ receptor sites following chronic fluoxetine treatment. Our data suggest that changes in the capacity of the 5-HT$_{1A}$ receptor to activate G protein underlie the desensitization of somatodendritic 5-HT$_{1A}$ autoreceptors in the dorsal and median raphe following chronic SSRI treatment.

Using the agonist radioligand [^{3}H]8-OH-DPAT, at a concentration near the Kd value, we and others have observed no change in 5-HT$_{1A}$ receptor binding sites in the dorsal raphe following chronic SSRI administration (Hensler et al. 1991; Hervàs et al. 2001). [^{3}H]8-OH-DPAT binding to 5-HT$_{1A}$ receptors, at a concentration near the Kd value, is expected to label only the coupled, high affinity state of the receptor. Thus, the observation that [^{3}H]8-OH-DPAT binding in the dorsal raphe is not changed following chronic SSRI administration (Hensler et al. 1991; Hervàs et al. 2001) suggests that the number of 5-HT$_{1A}$ receptor sites in the coupled, high affinity state is not altered by this drug treatment. By measuring 8-OH-DPAT-stimulated [^{35}S]GTPγS binding in the current study, we are measuring the ability of the agonist 8-OH-DPAT to stimulate the exchange of GTPγS for GDP. Thus, this is a direct assay of receptor activation of G protein as a result of agonist binding. With chronic SSRI administration the number of receptors in the coupled, high affinity state is unchanged (Hensler et al. 1991; Hervàs et al. 2001), but the ability of agonist to stimulate the exchange of GTPγS for GDP is decreased, an indication that the activation of G protein by the receptor as a result of agonist binding is reduced. A reduced capacity of the 5-HT$_{1A}$ receptor to activate G protein may be due to regulatory processes (e.g. phosphorylation) at the level of the G protein (see Lohse 1993).

Unlike the SSRIs, chronic administration of tricyclic antidepressants (i.e. desipramine, imipramine and iprindole) does not result in the desensitization of the 5-HT$_{1A}$ somatodendritic autoreceptor (Blier and de Montigny 1980; Kreiss and Lucki 1995). Our findings in the current study are consistent with this, in that we observed no change in (±)8-OH-DPAT-stimulated [^{35}S]GTPγS binding in the dorsal and median raphe nuclei following chronic administration of amitriptyline. Thus, following chronic amitriptyline treatment 5-HT$_{1A}$ receptor-G protein interaction in the dorsal and median raphe appears to be unaltered.

Although some investigators have reported an attenuation of neuroendocrine or temperature responses mediated by postsynaptic 5-HT$_{1A}$ receptors following chronic amitriptyline administration (Goodwin et al. 1987; Lesch et al. 1990), this has not been a consistent observation (Gartside et al. 1992; Yamada et al. 1994). Neuroendocrine and temperature responses mediated by postsynaptic 5-HT$_{1A}$ receptors are also attenuated following chronic administration of fluoxetine (Li et al. 1996; Lerer et al. 1999) or a variety of SSRIs (see Introduction). However, we are unable to examine the regulation of 5-HT$_{1A}$ receptor-G protein interaction in hypothalamus following chronic administration of antidepressant drugs due to the high level of basal [^{35}S]GTPγS binding in this brain region which prevents us from obtaining a statistically significant increase in [^{35}S]GTPγS binding with the addition of agonist (Hensler and Durgam, unpublished observations).

As has been observed for a variety of SSRIs (see Introduction), chronic treatment with fluoxetine does not alter 5-HT$_{1A}$ receptor-evoked electrophysiological responses in hippocampus (Le Poul et al. 2000). Chronic inactivation of 5-HT reuptake, by injection of antisense coding sequence of the 5-HT transporter gene into the

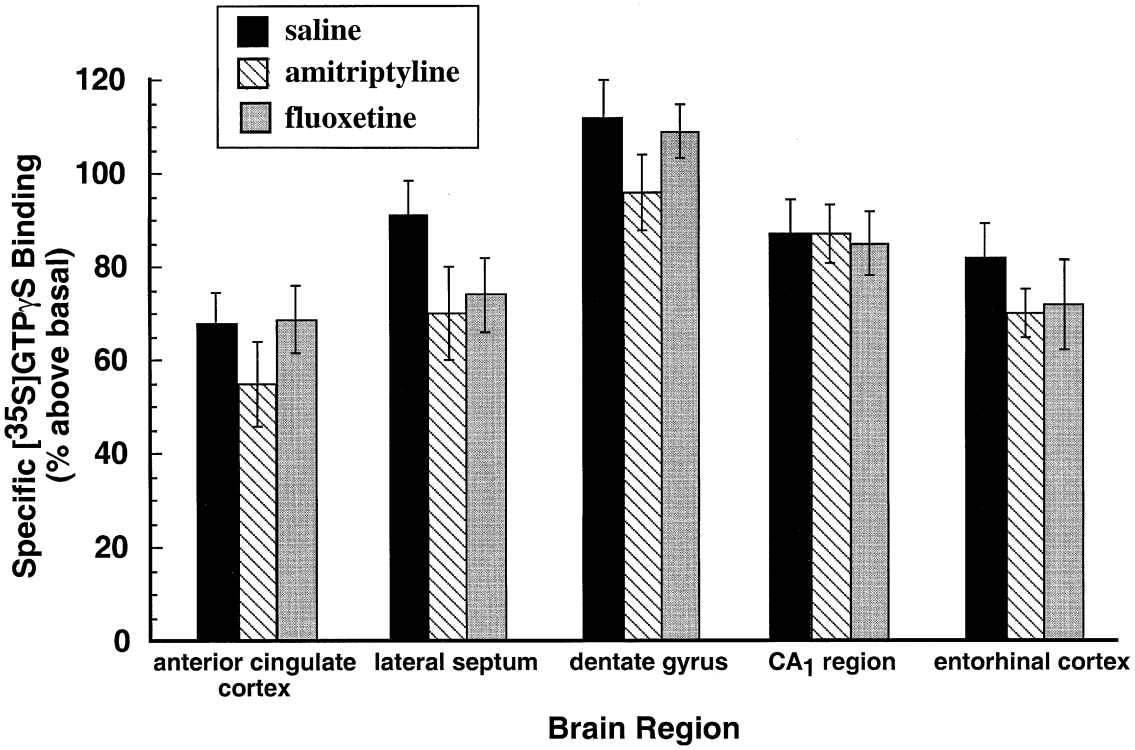

Figure 3. Effect of repeated administration of fluoxetine or amitriptyline on 5-HT$_{1A}$ receptor-stimulated [^{35}S]GTPγS binding in terminal field areas of serotonergic innervation. Rats were administered either saline, amitriptyline, or fluoxetine (10 mg/kg, once daily, i.p.) for 14 days. Coronal 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 % above basal. Saline-treated, n = 12, amitriptyline-treated, n = 8, fluoxetine-treated, n = 8, per experimental group.

dorsal raphe of the rat (Fabre et al. 2000b) or in knockout mice lacking the 5-HT transporter (Fabre et al. 2000a), does not alter 5-HT$_{1A}$ receptor-stimulated [^{35}S]GTPγS binding in hippocampus. Our findings in the current study are consistent with these previous studies in that we observed no change in 5-HT$_{1A}$ receptor-stimulated [^{35}S]GTPγS binding in hippocampus. Our data suggest that following chronic fluoxetine treatment, the capacity of the 5-HT$_{1A}$ receptor to activate G protein in the hippocampus is unaltered. Thus, changes in hippocampal 5-HT$_{1A}$ receptor-mediated inhibition of adenylyl cyclase activity observed following chronic fluoxetine treatment (Newman et al. 1992) may be the result of changes at the level of effector (i.e. adenylyl cyclase).

Postsynaptic 5-HT$_{1A}$ receptors may also play a role in the regulation of serotonergic neuronal firing rate and serotonin release through neuronal feedback loops between projection areas and raphe nuclei. Postsynaptic 5-HT$_{1A}$ receptor-mediated feedback has been demonstrated in several terminal field areas of serotonergic innervation, including cortex (Ceci et al. 1994; Casanovas et al. 1999) and amygdala (Bosker et al. 1997). Bosker et al. (2001) have shown that postsynaptic 5-HT$_{1A}$ receptor-mediated feedback in the amygdala is diminished following chronic treatment with the SSRI citalopram. Our data suggest that following chronic fluoxetine treatment, 5-HT$_{1A}$ receptor-G protein coupling in the cortical and limbic structures is unaltered. It is possible that the regulation of 5-HT$_{1A}$ receptor function in amygdala occurs at the level of 5-HT$_{1A}$ receptor-G protein interaction. However, because of the high level of basal [^{35}S]GTPγS binding in the amygdala we are unable to reproducibly quantitate 5-HT$_{1A}$ receptor-stimulated [^{35}S]GTPγS binding and to examine the regulation of 5-HT$_{1A}$ receptor-G protein interaction in this brain area following chronic antidepressant administration. Alternatively, regulation of postsynaptic 5-HT$_{1A}$ receptor function in amygdala, as well as in hippocampus or cortex involves changes in neuronal function at the system or circuit level.

Electrophysiological studies indicate that chronic treatment with amitriptyline results in the *sensitization* of postsynaptic neurons to serotonin in the hippocampus, where this response has been shown to be mediated by the 5-HT$_{1A}$ receptor (de Montigny and Aghajanian 1978; Gallager and Bunney 1978; Chaput et al. 1991). In the current study, however, we did not observe an increase in 5-HT$_{1A}$ receptor-stimulated [^{35}S]GTPγS binding in hippocampus, or in any of the other forebrain ar-

eas examined. Our data suggest that changes in the capacity of the 5-HT_{1A} receptor to activate G protein do not underlie this sensitization observed following chronic amitriptyline treatment. The sensitization of 5-HT_{1A} receptor-mediated electrophysiological responses in hippocampus may be due to changes in channel function. We did observe an increase in the number of 5-HT_{1A} receptor binding sites in hippocampus following chronic amitriptyline treatment, in confirmation of previous studies (Welner et al. 1989). However, because we did not observe a concomitant increase in 5-HT_{1A} receptor-stimulated [^{35}S]GTPγS binding, this increase in the binding of the antagonist radioligand [^{3}H]MPPF to 5-HT_{1A} receptor sites in hippocampus is most likely to be due to an increase in the total number of 5-HT_{1A} receptors, receptors which are uncoupled to G protein.

5-HT_{1A} receptors are coupled to the Gi family of G proteins, which include G_{i1}, G_{i2}, G_{i3}, G_o and G_z (Raymond et al. 1993; Barr et al. 1997). Chronic administration of tricyclic antidepressants and the monoamine oxidase inhibitor clorgyline has been reported to decrease Giα in several brain regions, while Goα was increased by tricyclic antidepressants but not clorgyline (Lesch et al. 1991). Chronic administration of the SSRI fluoxetine produces a gradual reduction in the levels of G_{i1}, G_{i3} and G_z protein in hypothalamus that matches the time course of desensitization of hypothalamic 5-HT_{1A} receptors (Li et al. 1996; Raap et al. 1999). Thus, fluoxetine-induced desensitization of hypothalamic postsynaptic 5-HT_{1A} receptor systems may be caused in part by reductions in Gz, which mediates hypothalamic 5-HT_{1A} receptor-stimulated ACTH and oxytocin secretion (Serres et al. 2000). Thus, antidepressant efficacy and the regulation of 5-HT_{1A} receptor function may be based on compensatory changes distal to the receptor, such as regulation of G protein expression or as discussed above reduced capacity of the receptor to activate G protein due to regulatory processes (e.g. phosphorylation) at the level of the G protein.

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