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Region-specific changes in 5-HT_{1A} receptor-activated G-proteins in rat brain following chronic buspirone

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Abstract

5-Hydroxytryptamine$_{1A}$ (5-HT_{1A}) receptors, which activate inhibitory G-proteins, are implicated in psychiatric disorders including anxiety and depression. Studies suggest that chronic 5-HT_{1A} receptor agonist administration alters 5-HT_{1A} receptor function, but the effect of chronic treatment on 5-HT_{1A} receptor-activated G-proteins is unclear. In this study, agonist-stimulated [^{35}S]guanylyl-5′-*O*-(γ-thio)-triphosphate (GTPγS) binding was examined following chronic administration of buspirone. Brains were processed for [^{35}S]GTPγS autoradiography using *R*(+)-8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) for 5-HT_{1A} receptors or baclofen for $GABA_B$ receptors. Net 8-OH-DPAT-stimulated [^{35}S]GTPγS binding was decreased by 25–30% in the septum and dorsal raphe nucleus of buspirone-treated animals. No significant changes in 8-OH-DPAT-stimulated [^{35}S]GTPγS binding were found in the prefrontal, entorhinal or cingulate cortices or hippocampus in buspirone-treated rats. $GABA_B$ receptor-stimulated [^{35}S]GTPγS binding was increased by 25% in the hippocampus, with no significant changes in any other region examined. These results demonstrate region-specific alterations in 5-HT_{1A} and $GABA_B$ receptor-activated G-proteins following chronic buspirone treatment, which may contribute to the clinical effects of this drug.

Keywords: G-protein; [^{35}S]GTPγS autoradiography; 5-HT_{1A} receptor; $GABA_B$ receptor; Anxiolytic; Antidepressant

1. Introduction

5-Hydroxytryptamine$_{1A}$ (5-HT_{1A}) receptor agonists constitute an important class of psychotherapeutic drugs. The azapirone buspirone, a partial agonist at 5-HT_{1A} receptors (Smith and Peroutka, 1986; Bockaert et al., 1987; Meller et al., 1990), is used clinically to treat anxiety (Goldberg and Finnerty, 1979; Rickels et al., 1982; Cadieux, 1996; Pecknold, 1997). Recent evidence indicates that buspirone may be also be efficacious in the augmentation of treatment for depression (Harrington et al., 1988; Sussman, 1998; Thase et al., 1998) and alcoholism (Malec et al., 1996). Experimental manipulation of the 5-HT_{1A} receptor system also influences measures of depression, anxiety and alcohol consumption in animal models (Detke et al., 1995; Hedlund and Wahlstrom, 1996; Angrini et al., 1998). Although buspirone is clinically effective, the biochemical mechanisms underlying its therapeutic effects and the consequences of chronic administration of this drug are not clear. It is, therefore, critical to understand the effects of chronic buspirone treatment on 5-HT_{1A} receptor function.

The 5-HT_{1A} receptor belongs to the superfamily of G-protein-coupled receptors and elicits its effects via inhibitory G-proteins that increase K^+ conductance and inhibit adenylyl cyclase (De Vivo and Maayani, 1986; Clarke et al., 1987; Sprouse and Aghajanian, 1987; Harrington et al., 1988; Fargin et al., 1989). The functional activation of G-proteins by 5-HT_{1A} receptors can be measured using agonist-stimulated [^{35}S]guanylyl-5′-O-(γ-thio)-triphosphate ([^{35}S]GTPγS) binding in membranes prepared from brain or cells transfected with the 5-HT_{1A} receptor (Newman-Tancredi et al., 1996; Sim et al., 1997b). The autoradiographic application of the [^{35}S]GTPγS binding assay allows the visualization of receptor-activated

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G-proteins in an anatomically specific way, thus providing a regional measure of receptor-coupled G-protein activity (Sim et al., 1995, 1997a). The applicability of [^{35}S]GTPγS autoradiography to chronic drug studies has previously been demonstrated in studies on the effects of chronic opiate and cannabinoid drugs (Sim et al., 1996a,b).

Previous studies on the effects of chronic azapirone administration have examined 5-HT$_{1A}$ receptor-mediated activity at the effector level, and have been restricted to examination of receptors in the dorsal raphe nucleus and hippocampus. Electrophysiological data has shown that chronic administration of azapirone drugs leads to desensitization of 5-HT$_{1A}$ autoreceptors in the dorsal raphe nucleus, with no change in 5-HT$_{1A}$ receptor activity in the hippocampus (Blier and De Montigny, 1987; Schechter et al., 1990). This data is consistent with studies showing no effect of chronic buspirone treatment on 5-HT synthesis, which suggests desensitization of 5-HT autoreceptors in the dorsal raphe nucleus (Okazawa et al., 1999). The effects of chronic azapirones on 5-HT$_{1A}$ receptor-mediated inhibition of adenylyl cyclase have also been investigated. However, these studies were restricted to the hippocampus and produced varying results, with reports showing both a reduction in inhibition of adenylyl cyclase (Newman et al., 1992) and no change in activity (Schechter et al., 1990; Varrault et al., 1991). Although these data suggest that chronic azapirone administration leads to uncoupling of the 5-HT$_{1A}$ receptor and G-protein, resulting in desensitization, this hypothesis has not been tested directly. Thus, the present study was designed to examine 5-HT$_{1A}$ receptor-coupled G-protein activity throughout the brain following chronic buspirone administration. Since 5-HT$_{1A}$ and GABA$_B$ receptors converge on the same effectors through coupling to separate pools of G-proteins (Andrade et al., 1986; Innis et al., 1988; Odagaki and Fuxe, 1995), GABA$_B$ receptor activity was examined in the same regions to determine whether chronic buspirone effects were specific to 5-HT$_{1A}$ receptors or also resulted in alteration of GABA$_B$ receptor function. This question is also important because some studies have suggested that the antidepressant effects of serotonergic drugs involve GABA-ergic mechanisms (Nakagawa et al., 1996), and that chronic antidepressant treatment alters GABA$_B$ receptor number (Lloyd et al., 1985) and receptor-mediated responses (Beck et al., 1997).

2. Materials and methods

2.1. Materials

Male Sprague–Dawley rats (200 g) were purchased from Zivic-Miller (Zelienople, PA). [^{35}S]GTPγS (1250 Ci/mmol) was purchased from New England Nuclear (Boston, MA). Baclofen and *R*(+)-8-hydroxy-2-(di-*n*-propylamino)tetralin HBr (8-OH-DPAT) were purchased from RBI/Sigma. Adenosine deaminase and guanosine 5′-diphosphate (GDP) were obtained from Sigma (St. Louis, MO). Reflections® autoradiography film was purchased from New England Nuclear (Boston, MA). All other reagent grade chemicals were obtained from Sigma or Fisher.

2.2. Methods

Rats were injected intraperitoneally with 5 mg/kg buspirone or saline vehicle once daily for 21 days. Seven control and seven treated animals were used, because previous studies have shown that 7–8 animals per group allows reliable statistical analysis, while maintaining a manageable number of slides for processing (Sim et al., 1996b). Animals were sacrificed by decapitation on day 22, and brains were removed and immediately frozen in isopentane at −30°C. All animal procedures were performed in accordance with the animal care and use committee of Wake Forest University School of Medicine. Coronal sections were cut on a cryostat maintained at −20°C and mounted on gelatin-subbed slides. Sections were collected at five levels to include regions which are known to contain 5-HT$_{1A}$ receptors, including (1) prefrontal cortex; (2) cingulate and frontal cortices and septum; (3) hippocampus, amygdala and hypothalamus; (4) entorhinal cortex; and (5) dorsal raphe nucleus. Sections were collected on paired slides for basal and agonist-stimulated [^{35}S]GTPγS binding, and each slide in the pair contained alternate consecutive sections in triplicate. Slides were desiccated overnight at 4°C and stored at −80°C until use. On the day of the assay, slides were removed from the freezer and brought to room temperature under a cool dryer. Slides were incubated in assay buffer (50 mM Tris–HCl, 3 mM $MgCl_2$, 0.2 mM EGTA, 100 mM NaCl, pH 7.4) for 10 min at 25°C, then in assay buffer containing 2 mM GDP and 9.5 mU/ml adenosine deaminase for 15 min at 25°C. Adenosine deaminase was included to reduce basal [^{35}S]GTPγS binding by decreasing [^{35}S]GTPγS binding stimulated by endogenous adenosine (Sim et al., 1998; Moore et al., 1999). Slides were then incubated for 2 h at 25°C in assay buffer with 0.04 nM [^{35}S]GTPγS, GDP and adenosine deaminase with either 5 μM 8-OH-DPAT for 5-HT$_{1A}$ receptors or 300 μM baclofen for GABA$_B$ receptors. Concentrated stock solutions of agonists were made in deionized H_2O and diluted in assay buffer for incubation. The final concentrations of agonists have previously been shown to produce maximal stimulation of [^{35}S]GTPγS binding (Sim et al., 1995, 1997b). Basal binding was assessed in the absence of agonist. Slides were rinsed twice for 2 min each in Tris buffer (50 mM Tris–HCl, pH 7.4) and once for 30 s in deionized H_2O at 4°C. Slides were dried under cool air, then exposed to Reflections® autoradiography film for 72 h. Film cassettes contained a [^{14}C] microscale for densitometric analysis. Films were digitized with a Sony XC-77 video camera and analyzed densitometrically using the NIH IMAGE pro-

gram for Macintosh computers. Resulting values are expressed as nCi [^{35}S]GTPγS/g tissue, and were corrected for [^{35}S] based upon incorporation of [^{35}S] into sections of frozen brain paste, as previously described (Sim et al., 1997a). Data are reported as mean values ± standard error (SE) of one or two repetitions of the assay on triplicate sections in each region from seven animals in each group.

3. Results

The highest levels of 5-HT$_{1A}$ receptor-stimulated [^{35}S]GTPγS binding were found in the lateral septum, hippocampus, prefrontal cortex, cingulate cortex, entorhinal cortex and dorsal raphe nucleus, as previously reported (Sim et al., 1997b). Thus, these regions were selected for densitometric analysis. Several other regions previously reported to contain 5-HT$_{1A}$ receptors, such as frontal cortex, amygdala and hypothalamus (Pazos and Palacios, 1985; Sijbesma et al., 1991), also showed detectable levels of 5-HT$_{1A}$ receptor-stimulated [^{35}S]GTPγS binding, although the levels were low (see below). To determine the effect of chronic agonist treatment on 5-HT$_{1A}$ receptor-stimulated [^{35}S]GTPγS binding, rats were treated with buspirone for 21 days (see Section 2.2). Visual inspection of brain sections revealed apparent decreases in 8-OH-DPAT-stimulated [^{35}S]GTPγS binding in the dorsal raphe nucleus and septum, with no obvious changes in the hippocampus, from brains of chronic buspirone-treated animals compared to controls (Fig. 1). Densitometric analysis confirmed these observations (Table 1). Net 8-OH-DPAT-stimulated [^{35}S]GTPγS binding in the dorsal raphe nucleus was decreased by approximately 25% in brain sections from buspirone-treated animals. Similar results were obtained in the septum where net 8-OH-DPAT-stimulated [^{35}S]GTPγS binding was decreased 27% by chronic buspirone treatment. Although a similar decrease in net 8-OH-DPAT-stimulated [^{35}S]GTPγS binding was also measured in the cingulate cortex, this change was slightly below the level of statistical significance ($P = 0.07$) and reflects increased variability in the measurements from control sections. In contrast, chronic buspirone treatment produced no significant changes in net 8-OH-DPAT-stimulated [^{35}S]GTPγS binding in the prefrontal or

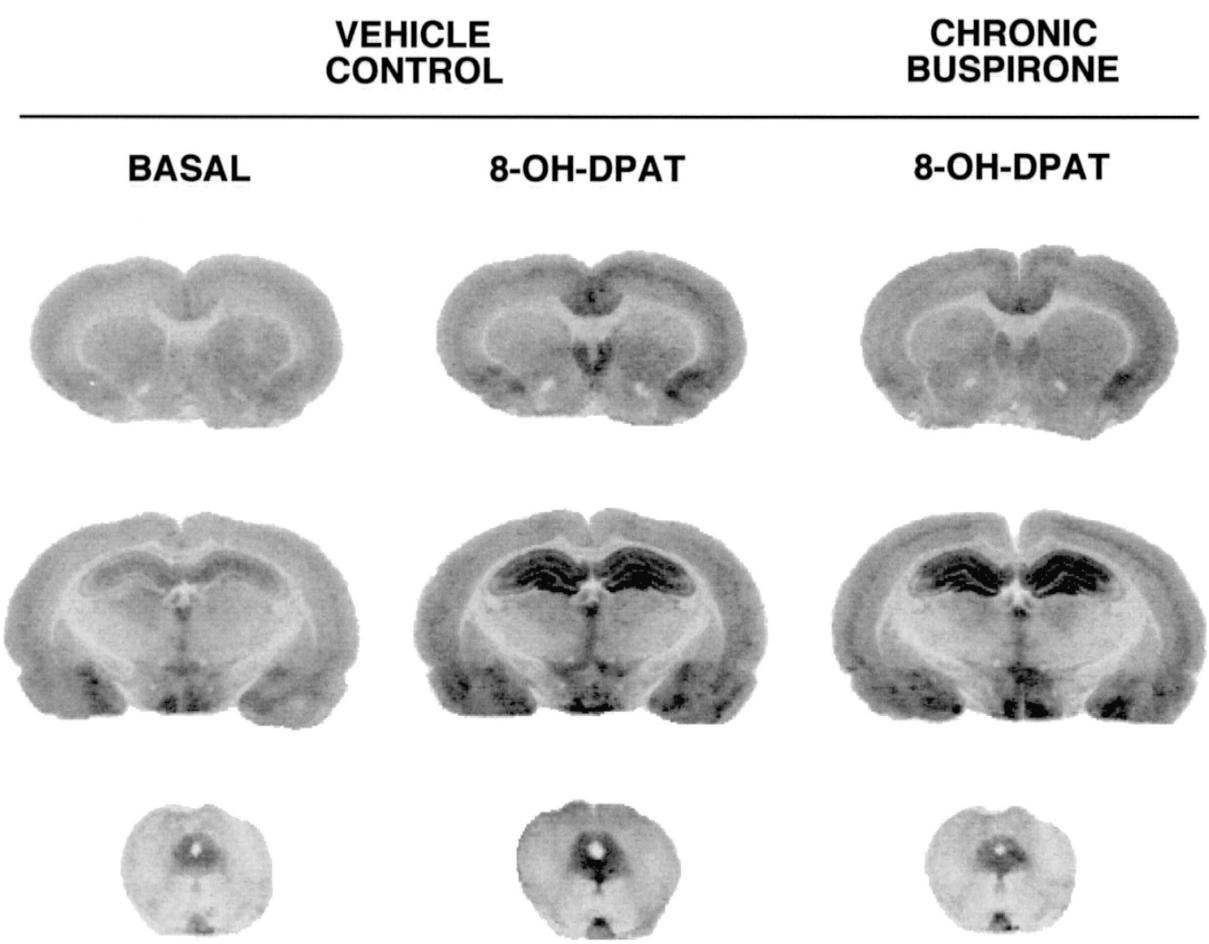

Fig. 1. Brain sections showing basal and 5-HT$_{1A}$ receptor-stimulated [^{35}S]GTPγS binding in control and 5-HT$_{1A}$ receptor-stimulated [^{35}S]GTPγS binding in buspirone-treated rats. Sections were collected at the level of the septum and cingulate cortex (top), hippocampus, amygdala and hypothalamus (center) and dorsal raphe nucleus (bottom), and processed as described in Section 2.2.

entorhinal cortices or hippocampus. Since 5-HT_{1A} receptor binding and 5-HT_{1A} receptor-stimulated [^{35}S]GTPγS binding vary regionally within the hippocampus, 8-OH-DPAT-stimulated [^{35}S]GTPγS binding was measured in the dentate gyrus and CA1 and CA2/3 of Ammon's horn. The same results were obtained whether the hippocampus was measured by regions or as a whole; no differences were found in net 8-OH-DPAT-stimulated [^{35}S]GTPγS binding between control and buspirone-treated animals (data not shown). The data are summarized in Fig. 2, in which net 8-OH-DPAT-stimulated [^{35}S]GTPγS binding in buspirone-treated animals is plotted as percent of net 8-OH-DPAT-stimulated [^{35}S]GTPγS binding in control animals. In addition to areas with high levels of 8-OH-DPAT-stimulated [^{35}S]GTPγS binding, several regions with lower levels of activity were also analyzed. Net 8-OH-DPAT stimulated [^{35}S]GTPγS binding did not significantly differ between saline- and buspirone-treated animals in the frontal cortex (47 ± 10 vs. 42 ± 8 nCi/g), amygdala (28 ± 6 vs. 34 ± 6 nCi/g) or hypothalamus (27 ± 7 vs. 20 ± 5 nCi/g). However, since these levels of agonist-stimulated [^{35}S]GTPγS binding are low and found in regions that generally express higher levels of basal [^{35}S]GTPγS binding (i.e., hypothalamus and amygdala, Fig. 1), these numbers should be interpreted with caution.

To determine whether chronic buspirone treatment affected $GABA_B$ receptor-stimulated [^{35}S]GTPγS binding in these same brain regions, alternate sections were processed using baclofen as an agonist. As shown in Table 1, the only significant difference in baclofen-stimulated [^{35}S]GTPγS binding between control and buspirone-treated animals was found in the hippocampus ($P = 0.05$), where chronic buspirone treatment produced a 26% increase in $GABA_B$ receptor-stimulated [^{35}S]GTPγS binding.

Basal [^{35}S]GTPγS binding was also examined in the same brain regions described above (data not shown). Densitometric analysis showed that basal values ranged from 365 nCi/g in the entorhinal cortex to 519 nCi/g in the dorsal raphe nucleus. No significant differences in basal [^{35}S]GTPγS binding were measured between control and buspirone-treated animals in any region.

Table 1
Effect of chronic buspirone treatment on net 8-OH-DPAT- and $GABA_B$-stimulated [^{35}S]GTPγS binding in the rat brain
Sections were processed and analyzed as described in Section 2.2. Data are expressed as net stimulated [^{35}S]GTPγS binding in nCi [^{35}S]/g tissue and represent mean values ± SE of triplicate sections from seven animals in each group.

Region	8-OH-DPAT-stimulated		$GABA_B$-stimulated	
	Control	Buspirone	Control	Buspirone
Prefrontal cortex	163 ± 11	151 ± 19		
Cingulate cortex	124 ± 27	91 ± 12	261 ± 25	263 ± 27
Entorhinal cortex	95 ± 11	91 ± 16	252 ± 19	276 ± 23
Septum	231 ± 15	168 ± 8[a]	198 ± 17	215 ± 27
Hippocampus	225 ± 16	233 ± 13	118 ± 9	149 ± 11[a]
Dorsal raphe N.	212 ± 10	160 ± 17[a]	93 ± 23	95 ± 20

[a] $P < 0.05$ different from control.

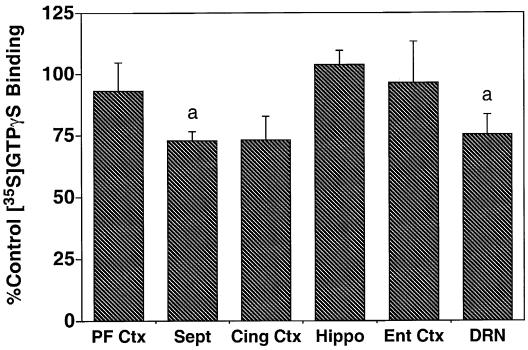

Fig. 2. Net 5-HT_{1A} receptor-stimulated [^{35}S]GTPγS binding in buspirone-treated animals calculated as percent of net 5-HT_{1A} receptor-stimulated [^{35}S]GTPγS binding in control animals. ([a] $P < 0.05$ different from control).

4. Discussion

The results of this study demonstrate region-specific decreases in 5-HT_{1A} receptor-stimulated [^{35}S]GTPγS binding following 21 days of buspirone administration. The two regions that showed the greatest sensitivity to chronic buspirone treatment were the dorsal raphe nucleus and septum, in which net 8-OH-DPAT-stimulated [^{35}S]GTPγS binding decreased by approximately 25%. Interestingly, a comparable increase in baclofen-stimulated [^{35}S]GTPγS binding was found in the hippocampus, an area where 8-OH-DPAT-stimulated [^{35}S]GTPγS binding did not change. Although these effects are rather modest, similar results have been reported for opioid receptor desensitization, where similar magnitude of changes in agonist-stimulated [^{35}S]GTPγS binding were found in only specific brain regions (Sim et al., 1996b). In addition, a somewhat conservative dose of buspirone was chosen for the present study compared to other published reports. Finally, the fact that buspirone is a partial agonist at 5-HT_{1A} receptors may affect the degree of desensitization produced by chronic drug treatment.

The finding that desensitization of 5-HT_{1A} receptors occurs in the dorsal raphe nucleus is consistent with previous studies at the effector level. Schechter et al. (1990) found that chronic treatment with ipsapirone produced desensitization of 5-HT_{1A} autoreceptor-mediated electrophysiological responses in the dorsal raphe nucleus. However, chronic ipsapirone treatment did not alter postsynaptic 5-HT_{1A} receptor-mediated inhibition of adenylyl cyclase in the hippocampus (Schechter et al., 1990; Varrault et al., 1991). Similar results were reported by Blier and De Montigny (1990) in studies which showed that chronic gepirone treatment produced desensitization of 5-HT_{1A} autoreceptors in the dorsal raphe nucleus, but no change in the electrophysiological responsiveness of post-

synaptic 5-HT_{1A} receptors in the hippocampus. It has been suggested that the therapeutic effects of 5-HT_{1A} receptor agonists result from increased 5-HT release in terminal field regions that occurs due to desensitization of somatodendritic autoreceptors in the dorsal raphe nucleus, since no changes in 5-HT_{1A} receptor-mediated activity were found in the hippocampus (Blier and De Montigny, 1990; Schechter et al., 1990; Varrault et al., 1991). However, these previous studies did not examine other terminal field regions, such as the septum or cortex, which also have relatively high levels of 5-HT_{1A} receptors. In the present study, the same degree of desensitization was measured in the septum as in the dorsal raphe nucleus. It would therefore appear from these results that 5-HT_{1A} receptors in the hippocampus, as well as the entorhinal and prefrontal cortices, are resistant to desensitization produced by chronic buspirone administration.

Regional differences in the development of desensitization to chronic agonist treatment have previously been found for both opioid and cannabinoid receptors (Sim et al., 1996a,b). In fact, both the rate and magnitude of desensitization following chronic drug treatment appear to vary by brain region (Breivogel et al., 1999). Further evidence for regional differences in the development of desensitization is provided by behavioral studies, which have demonstrated differential sensitivity of various 5-HT_{1A} receptor-mediated behaviors to repeated agonist pretreatment (O'Connell and Curzon, 1996). It is currently not clear which factors influence the differential regional development of desensitization. There may be regional anatomical differences in 5-HT_{1A} receptors that influence the development of desensitization. 5-HT_{1A} receptors are localized presynaptically in the dorsal raphe nucleus and postsynaptically in regions such as the hippocampus, septum and cortex (Verge et al., 1986). However, this does not explain the present results because desensitization of post-synaptic 5-HT_{1A} receptors was observed only in some terminal field regions, which is not consistent with the results showing the development of tolerance to 5-HT_{1A} receptor-mediated behavioral effects (O'Connell and Curzon, 1996). Electron-microscopic studies on the ultrastructural anatomy of 5-HT_{1A} receptors have shown that 5-HT_{1A} receptors in the dorsal raphe nucleus and septum are localized to both dendritic processes and somata, whereas in the dorsal hippocampus 5-HT_{1A} receptors are found primarily on dendritic spines (Kia et al., 1996). However, it is not clear what effect morphological localization may have in the development of desensitization. Another important factor may be the type of G-protein α and/or $\beta\gamma$ subunits to which the receptor is coupled. The [^{35}S]GTPγS binding assay does not distinguish among different subtypes of α subunits, and it is not known which specific $\beta\gamma$ subunits are associated with 5-HT_{1A} receptor-coupled G-proteins in these regions. It is interesting that the hippocampus, which appears resistant to the effects of chronic 5-HT_{1A} receptor-agonist treatment in this study is an area that shows a rapid, large degree of cannabinoid receptor desensitization to the partial agonist Δ^9-tetrahydrocannabinol (Sim et al., 1996a; Breivogel et al., 1999). Thus, it appears that specificity in the receptor/G-protein complex contributes to the degree of desensitization that is produced in each region.

An unexpected finding was increased $GABA_B$ receptor-stimulated [^{35}S]GTPγS binding in the hippocampus, since no changes were detected in 5-HT_{1A} receptor-stimulated activity in this region. It is possible that the increase in $GABA_B$ receptor-activated G-proteins in this region was due to changes in the activity of a non-5-HT_{1A} serotonergic receptor. Since desensitization of 5-HT_{1A} autoreceptors in the dorsal raphe nucleus affects 5-HT release throughout the brain, the activity of other types of 5-HT receptors may be altered in diverse terminal field regions. In fact, several other 5-HT receptor types (or their mRNA) are found in the hippocampus, including subtypes of 5-HT_1, 5-HT_2, 5-HT_3, 5-HT_4, 5-HT_5, 5-HT_6 and 5-HT_7 receptors (Palacios et al., 1990; Hoyer et al., 1994). Thus, the increase in $GABA_B$ receptor-mediated G-protein activation may result from chronic buspirone-induced alterations in 5-HT neurotransmission. In addition to its serotonergic properties, buspirone can also act as an antagonist at dopamine and noradrenaline receptors (Hjorth and Carlsson, 1982; Enberg, 1989). The major metabolite of buspirone, 1-(2-pyrimidinyl-piperazine), has been shown to increase the firing of locus coeruleus neurons via its α_2 receptor antagonistic properties (Enberg, 1989), which could affect noradrenergic activity throughout the brain.

These results are important to consider in regard to the clinical use of 5-HT_{1A} receptor agonists for the treatment of anxiety and depression, although it is not clear whether the chronic effects of buspirone would be the same in depressed as control subjects. The therapeutic effects of 5-HT_{1A} receptor drugs are known to have a delayed onset of action (Goldberg and Finnerty, 1979; Rickels et al., 1982), which suggests that desensitization develops slowly in the 5-HT_{1A} receptor system. Desensitization may occur with varying rates among different brain regions (Breivogel et al., 1999), so that longer treatment duration may produce desensitization of postsynaptic 5-HT_{1A} receptors in regions that appear resistant to treatment at earlier time points. Electrophysiological studies have demonstrated similar results with serotonin reuptake inhibitors that are used as antidepressant drugs as with chronic azapirones: desensitization of 5-HT_{1A} autoreceptors in the dorsal raphe nucleus occurs in the absence of a change in the effector response of 5-HT_{1A} receptors in the hippocampus (Blier et al., 1988; Jolas et al., 1994). Thus, in future studies it will be of interest investigate the effects of dose and duration of treatment on 5-HT_{1A} receptor-mediated activity throughout all regions containing 5-HT_{1A} receptors in order to determine the contribution of altered 5-HT_{1A} receptor-mediated G-protein activation to the therapeutic effects of serotonergic anti-anxiety and anti-depressant drugs.

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