Ionotropic Glutamate Receptor Modulation of 5-HT$_6$ and 5-HT$_7$ mRNA Expression in Rat Brain

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The novel serotonin receptor subtypes, 5-HT$_6$ and 5-HT$_7$, are located in limbic regions and have nanomolar affinities for atypical antipsychotics. These factors have led some to speculate about the involvement of 5-HT$_6$ and 5-HT$_7$ receptors in schizophrenia. However, relatively little is known about these receptor subtypes, including the regulation of their expression in limbic regions. In particular, the regulation of extracellular serotonin levels in the striatum and hippocampal formation by glutamate receptors led us to examine the effects of systemic ionotropic glutamate receptor modulator treatment on 5-HT$_6$ and 5-HT$_7$ receptor expression in these regions. MK-801 treatment induced a dose-dependent decrease in striatal 5-HT$_6$ receptor mRNA levels; similarly, both aniracetam and NBQX treatments also led to decreases in striatal 5-HT$_6$ receptor mRNA levels. Hippocampal 5-HT$_6$ and 5-HT$_7$ receptor expression were not dramatically affected by any of the treatments. To our knowledge, this is the first demonstration of the regulation of striatal 5-HT$_6$ receptor mRNA expression, and provides neurochemical anatomical evidence for the interaction of serotonergic and glutamatergic systems. Furthermore, although these two neurotransmitter systems are separately implicated in schizophrenia, the glutamatergic regulation of the expression of a receptor subtype associated with schizophrenia suggests that alterations in serotonin receptor expression in schizophrenia may result, in part, from altered glutamatergic activity.
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Serotonergic dysfunction is implicated in schizophrenia via converging lines of evidence. Although there are multiple serotonin receptors, psychotic symptoms are most likely associated with 5-HT$_2$-like receptors (Meltzer 1992). 5-HT$_2$ receptors are localized in multiple limbic structures associated with schizophrenia, particularly in the hippocampus and nucleus accumbens (Joyce et al. 1993). Agonists of 5-HT$_2$ receptors, such as lysergic acid, are psychotomimetic, and antipsychotics often have 5-HT$_2$ receptor antagonist properties. Of note, atypical antipsychotics have high 5-HT$_2$ receptor antagonist properties, which is postulated to be a mechanism that confers the unique therapeutic profile of atypical antipsychotics (Meltzer 1992). Abnormalities of 5-HT$_2$ receptor expression have been demonstrated in schizophrenic brain, as well (Burnet et al. 1996; Joyce et al. 1993; Laruelle et al. 1993). Taken together, these findings suggest that schizophrenia is associated with altered 5-HT$_2$-like receptor activity in limbic structures.

Two novel serotonin receptor subtypes, 5-HT$_6$ (Monsma et al. 1993) and 5-HT$_7$ (Shen et al. 1993), have recently been identified; they are positively coupled to adenylate cyclase and are distributed in limbic regions of the brain, similar to 5-HT$_2$-like receptors (Monsma et al. 1993; Shen et al. 1993). Appreciable levels of 5-HT$_6$

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and 5-HT$_7$ receptors are present in hippocampus, and 5-HT$_6$ mRNA has been localized in ventral striatum (Gerard et al. 1996; Gustafson et al. 1996; Ward et al. 1995). Furthermore, an interesting pharmacological property of these receptors is their nanomolar affinities for typical and atypical antipsychotic medications (Roth et al. 1994). These factors have led some to speculate about the involvement of 5-HT$_6$ and 5-HT$_7$ receptors in schizophrenia, vis-à-vis their similarity to 5-HT$_2$-like receptors. Although there is no direct evidence for increased 5-HT$_6$ and 5-HT$_7$ receptor activity, altered expression of these receptor subtypes may be associated with schizophrenia. However, little is known about whether the expression of these novel subtypes can be regulated in limbic areas. One putative regulator of serotonin receptor expression is glutamate, because previous studies have suggested that altered glutamate receptor activity may affect serotonergic systems.

Regulation of 5-HT$_6$ and 5-HT$_7$ receptor expression may be associated with alterations in serotonin release in hippocampus and ventral striatum. The raphe nuclei send topographic serotonergic projections to these regions, with the dorsal raphe nuclei projecting to the ventral hippocampus and striatum, and the median raphe projecting to the dorsal hippocampus (summarized in McQuade and Sharp 1997). Glutamate receptors on these raphe nuclei seem to regulate serotonin release in striatum and hippocampus. Glutamate receptors are divided into four families, including the ionotropic families NMDA, AMPA, and kainate (Hollmann and Heinemann 1994). Several studies have examined the effects of ionotropic glutamate receptor modulators on serotonin release. Local application of glutamate, AMPA, and NMDA modestly increase extracellular serotonin levels in hippocampus and striatum, presumably via glutamate receptors on raphe projections (Maione et al. 1997; Tao and Auerbach 1996; Tao et al. 1997; Whitton et al. 1994a,b). Greater effects are seen when NMDA is applied directly to the raphe, suggesting that glutamate receptors in the raphe, rather than its target structures, regulate serotonin release in these structures (Tao and Auerbach 1996).

The effects of NMDA are blocked by coadministration of NMDA receptor antagonists (Tao and Auerbach 1996). However, systemic administration of such NMDA receptor antagonists as MK-801 and ketamine also increase serotonin levels, although probably via a mechanism separate from presynaptic NMDA receptors located on raphe neurons (Lindefors et al. 1997; Whitton et al. 1992; Yan et al. 1997). Specifically, these antagonists are postulated to block NMDA receptors on inhibitory GABAergic inputs to the raphe, and this disinhibition is believed to lead to the increased serotonin levels. We can infer from this theory that there is tonic GABAergic input to the raphe that may be close to maximally driven, because NMDA does not inhibit serotonin release futher.

In addition to NMDA receptors, AMPA receptor-mediated control of serotonin release has also been examined. The effect of AMPA is time limited, probably because of desensitization of AMPA receptors. Desensitization inhibitors (cyclothiazide and diazoxide) coadministered with AMPA attenuates this decay, supporting the involvement of densensitization in this process (Tao et al. 1997). In fact, diazoxide administered alone also facilitates serotonin release, suggesting tonic glutamatergic input to the raphe that is limited by desensitization of AMPA receptors (Whitton et al. 1994b). In summary, multiple glutamate receptor subtypes seem to mediate serotonin release in hippocampus and striatum at multiple points in serotonergic circuitry.

Studies suggest that 5-HT$_6$ and 5-HT$_7$ receptor expression can be regulated by antipsychotics and the HPA axis (Fredrick and Meador-Woodruff in press; LeCorre et al. 1997; Yau et al. 1997), so altered serotonin release may also lead to altered expression of serotonin receptors. We performed two experiments examining the expression of 5-HT$_6$ and 5-HT$_7$ receptors in hippocampus and ventral striatum after systemic treatment with glutamate receptor modulators. The first experiment consisted of daily injections of MK-801 (0.3, 1.0, or 3.0 mg/kg) for 7 days, and the second experiment included antagonists of AMPA/kainate receptors, modulators of AMPA receptor desensitization, and a glutamate release inhibitor. Our hypothesis is that these treatments, which are known to affect serotonin release, will also result in changes in 5-HT$_6$ and 5-HT$_7$ receptor mRNA levels in limbic brain regions.

METHODS

Adult, male Sprague–Dawley rats (250 gm) were housed three to six to a cage, with food and water *ad libitum.* In the MK-801 experiment (Experiment 1), one set of 10 rats was injected subcutaneously with either 0.3 mg/kg, 1.0 mg/kg, 3.0 mg/kg of the NMDA receptor uncompetitive antagonist MK-801, or sterile H$_2$O vehicle for 7 days. For the AMPA/kainate receptor modulator experiment (Experiment 2), another set of 10 rats was treated with seven daily subcutaneous injections of either DMSO vehicle, the AMPA receptor desensitization inhibitor aniracetam (20 mg/kg), the selective competitive AMPA receptor antagonist NBQX (7 mg/kg), the less selective competitive AMPA/kainate receptor antagonist CNQX (10 mg/kg), the AMPA receptor densensitization facilitator GYKI 52466 (8 mg/kg), or the glutamate release inhibitor riluzole (4 mg/kg). Twenty-four hours after the last injection, the animals were sacrificed, their brains were immediately removed and frozen in isopentane. The brains were stored at −80°C until sectioned.

Each brain was thawed for 30 minutes to a temperature of $-20°C$ and mounted for cryostat sectioning. 10 μm sections were obtained throughout each brain and thaw-mounted on polylysine-subbed microscope slides. The slides were dessicated and stored at $-80°C$ until used for *in situ* hybridization.

In Situ Hybridization

Riboprobes were synthesized from linearized plasmid DNA, as has been previously described (Fredrick and Meador-Woodruff in press). The 5-HT$_6$ insert is 530 base pairs, and the 670-base pair 5-HT$_7$ insert hybridizes with all four 5-HT$_7$ splice variants (Heidmann et al. 1997). Briefly, 10 μl of ^{35}S-UTP was dried and 5.0 μl 5X transcription buffer, 2.0 ml 0.1M DTT, 1.0 μl each of 10 mm ATP, CTP, and GTP, 4.5 μl H$_2$O, 1.0 μl linearized plasmid DNA, 1.0 μl RNase inhibitor, and 1.0 μl RNA polymerase enzyme were added to the tube and incubated for 1.5–2 hours at 37°C. 1.0 μl DNase (RNase free) was then added, and the mixture was incubated for 15 minutes at room temperature. The reaction mixture was sieved through a 1-cc syringe containing G-50 Sephadex equilibrated in Tris buffer (100 mm Tris-HCl, pH 7.5, 12.5 mm EDTA, pH 8.0, 150 mm NaCl, and 0.2% SDS), and 100 μl fractions were eluted.

Four to eight slides per animals were removed from $-80°C$ storage and placed in 4% (vol:vol) formaldehyde at room temperature for 1 hour. The slides were then washed in 2X SSC (300 mm NaCl/30 mm sodium citrate, pH 7.2) three times for 5 minutes each. The slides were then washed in deionized H$_2$O for 1 minute before being placed in 0.1 m triethanolamine, pH 8.0/acetic anhydride, 400:1 (vol:vol), on a stir plate, for 10 minutes. The final wash was in 2X SSC buffer for 5 minutes, followed by dehydration through graded alcohols and air drying. A cover slip with 30 μl of riboprobe (1 million dpm)/75% formamide buffer/0.01 m DTT was placed on each slide. Slides were placed in a covered tray with filter paper saturated with 75% formamide buffer and incubated at 55°C overnight.

The next day the cover slips were removed, and the slides were placed in 2X SSC for 5 minutes, followed by RNase (200 μg/ml in 10 mm Tris-HCl, pH 8.0/0.5 M NaCl) at 37°C for 30 minutes. The slides then underwent the following washes: 2X SSC at room temperature for 10 minutes; 1X SSC for 10 minutes at room temperature; 0.5X SSC at 55°C for 60 minutes; and 0.5X SSC for 10 minutes at room temperature. The slides were dehydrated in graded ethanol solutions and air dried. They were placed in X-ray cassettes and exposed to Kodak XAR-5 film for 30–35 days.
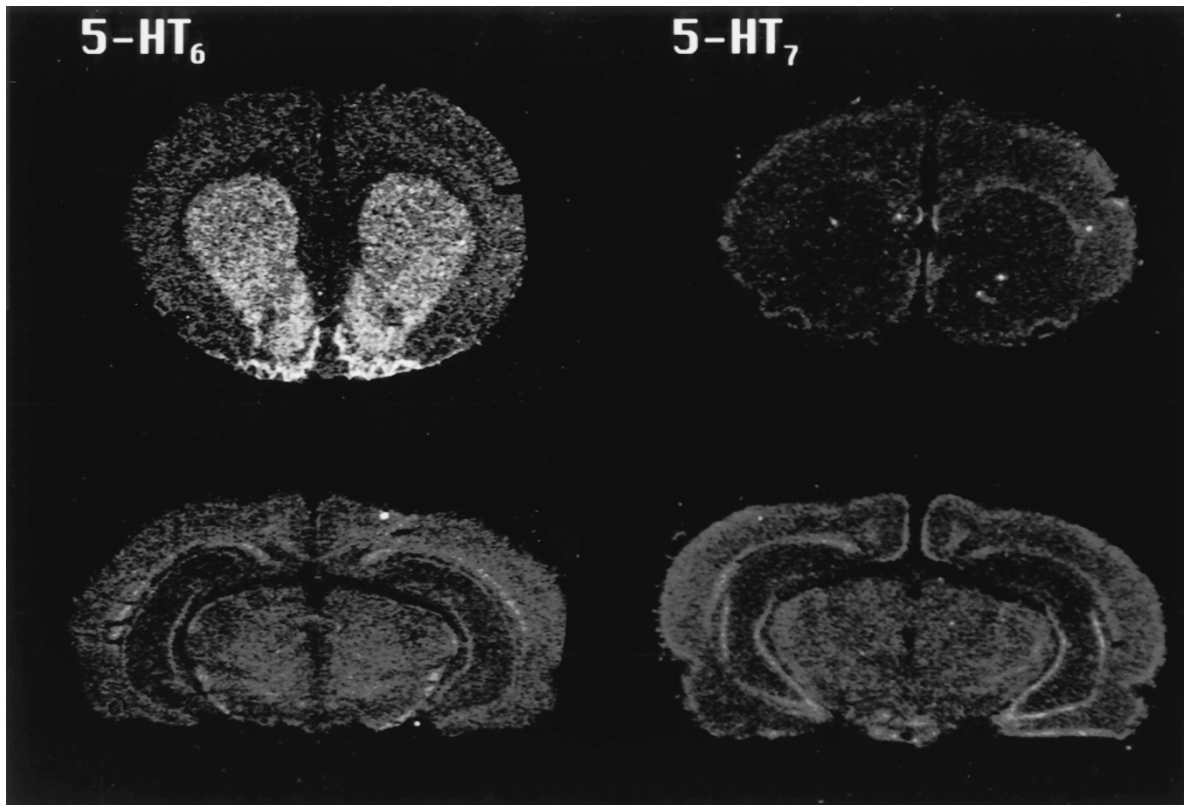

Figure 1. Distribution of 5-HT$_6$ and 5-HT$_7$ receptor mRNAs in rat brain. Note 5-HT$_6$ mRNA labeling in striatum, cortex, and hippocampal formation, and 5-HT$_7$ mRNA labeling in septum, cortex, and hippocampus.

Image and Statistical Analyses

The film was developed and used for quantitative, computer image analysis (with NIH Image 1.56), as previously described (Fredrick and Meador-Woodruff in press). Tissue background was subtracted from total gray scale values (GSV) for the caudate-putamen, nucleus accumbens shell and core, and the hippocampal formation (which included dentate gyrus and CA1-CA4) identified using a defined atlas (Paxinos and Watson 1982). Left and right side values were pooled, as were values for duplicate slides, providing one averaged GSV per region per animal. The corrected average GSV was converted into optical density (OD), and OD values for each region were examined using separate two-way analyses of variance (ANOVAs) for striatum and hippocampal formation. *Post hoc* comparisons were performed using Fisher's PLSD.

RESULTS

The distribution of 5-HT$_6$ and 5-HT$_7$ receptor mRNA in the striatum was consistent with previous reports (Figure 1) (Gerard et al. 1996; Gustafson et al. 1996; Ward et al. 1995). The distribution of 5-HT$_7$ receptor mRNA in the hippocampus was also consistent with previous reports (Figure 1) (Gustafson et al. 1996; LeCorre et al. 1997; Yau et al. 1997). For our exposure times, only CA2 and CA3 subfields yielded consistent 5-HT$_7$ receptor mRNA signals above background.

EXPERIMENT 1. MK-801 TREATMENT

Striatum

There was a dose-dependent decrease in 5-HT$_6$ receptor mRNA levels after MK-801 treatment, with the two
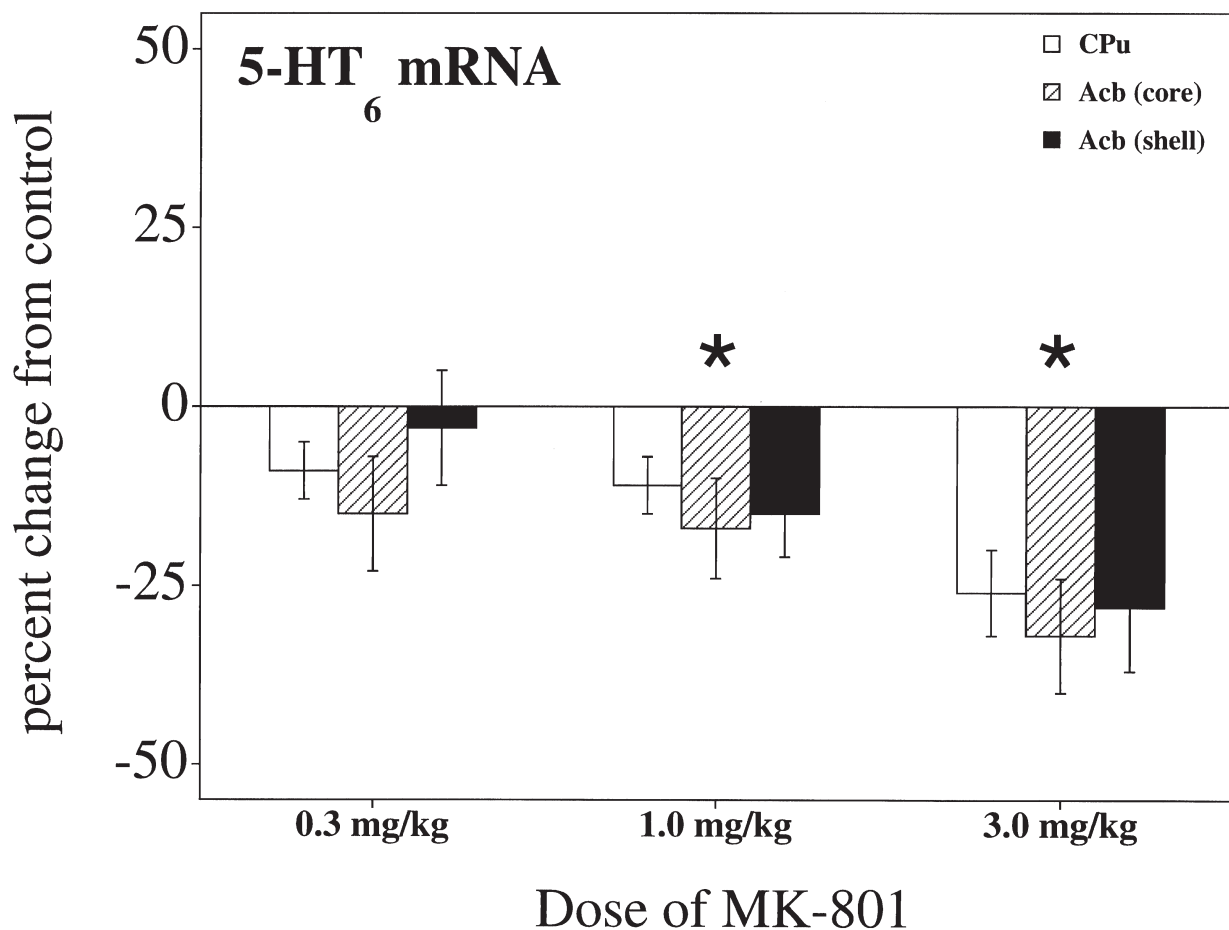

Figure 2. Striatal 5-HT$_6$ receptor mRNA levels after MK-801 treatment (Experiment 1). Values expressed as percentage change normalized to controls ± SEM for 10 animals. * Denotes significant difference from control treatment. CPu = caudate-putamen; Acb (core) = core region of the nucleus accumbens; Acb (shell) = shell region of the nucleus accumbens.

higher doses causing significant decreases of 20–35% in striatal regions (Figure 2). There was no significant difference between dorsal and ventral striatal 5-HT$_6$ mRNA expression. 5-HT$_7$ receptor mRNA was not quantifiable in the striatum.

Hippocampal Formation

MK-801 treatment did not significantly alter the expression of 5-HT$_6$ receptor mRNA in the hippocampal formation (Table 1). Similarly, none of the three treatments resulted in significant changes in 5-HT$_7$ receptor mRNA levels in the hippocampus (Table 2).

EXPERIMENT 2. AMPA/KAINATE RECEPTOR MODULATOR TREATMENT

Striatum

Aniracetam and NBQX treatment caused significant (30–50%) decreases of 5-HT$_6$ receptor mRNA levels; whereas, riluzole and GYKI52466 treatment caused nonsignificant decreases of 10–20% (Figure 3). There was no significant difference between dorsal and ventral striatal 5-HT$_6$ mRNA expression. 5-HT$_7$ receptor mRNA was not quantifiable in the striatum.

Hippocampal Formation

Only GYKI52466 treatment resulted in a signifcant alteration in 5-HT$_6$ receptor mRNA levels (Table 1). None of the five treatments resulted in significant changes in 5-HT$_7$ receptor mRNA levels in the hippocampus (Table 2), although all led to nonsignificant increases.

DISCUSSION

Treatment with MK-801, aniracetam, and NBQX resulted in decreased striatal 5-HT$_6$ receptor mRNA expression, while treatment with GYKI52466 significantly increased 5-HT$_6$ mRNA levels in the hippocampal formation. None of the treatments affected 5-HT$_7$ receptor mRNA expression.

To our knowledge, this is the first demonstration of the regulation of striatal 5-HT$_6$ receptor mRNA. Neither clozapine treatment, which is direct antagonist of the 5-HT$_6$ receptor, nor haloperidol treatment affected striatal 5-HT$_6$ receptor mRNA expression in the striatum (Fredrick and Meador-Woodruff in press). In fact, not even serotonergic deafferentation resulting from 5,7 dihydroxytryptamine treatment caused a change in 5-HT$_6$ receptor mRNA expression in the striatum (Gerard et al. 1996).

EXPERIMENT 1. MK-801 TREATMENT

Striatum

The effects of MK-801 on 5-HT$_6$ receptor mRNA expression may be the result of dose dependent increases in serotonin release, probably mediated via blockade of NMDA receptors on GABAergic inputs to the raphe (summarized in Figure 4). Doses as low as 0.3 mg/kg have been shown to increase serotonin levels acutely, but the effects of subacute doses are not known (Yan et al. 1997). The lack of effect we found with this dose may reflect lower serotonin concentrations that do not result in 5-HT$_6$ receptor mRNA expression alterations (Yan et al. 1997). However, the low micromolar affinity of the dopamine transporter for MK-801 may also help to explain an increase in MK-801-induced serotonin release (Clarke and Reuben 1995). MK-801 has been shown to increase dopamine, serotonin, and norepinephrine lev-

Table 1.   Summary of Effects of Treatment on Hippocampal Formation 5-HT$_6$ Receptor mRNA Expression (Values Expressed as Mean Optical Density for Each of the Five Hippocampal Formation Subfields $\pm$ SEM for 10 Animals)

Drug	CA1	CA2	CA3	CA4	DG
Sterile water vehicle	.066 $\pm$ .005	.070 $\pm$ .007	.072 $\pm$ .007	.070 $\pm$ .008	.056 $\pm$ .008
MK-801 (0.3 mg/kg)	.069 $\pm$ .004	.064 $\pm$ .004	.070 $\pm$ .004	.078 $\pm$ .005	.051 $\pm$ .005
MK-801 (1.0 mg/kg)	.056 $\pm$ .006	.068 $\pm$ .009	.059 $\pm$ .009	.058 $\pm$ .009	.041 $\pm$ .008
MK-801 (3.0 mg/kg)	.066 $\pm$ .006	.068 $\pm$ .008	.072 $\pm$ .006	.070 $\pm$ .007	.056 $\pm$ .006
DMSO vehicle	.023 $\pm$ .003	.030 $\pm$ .003	.033 $\pm$ .005	.022 $\pm$ .002	.016 $\pm$ .001
Aniracetam	.019 $\pm$ .004	.034 $\pm$ .004	.029 $\pm$ .004	.020 $\pm$ .004	.014 $\pm$ .004
CNQX	.021 $\pm$ .003	.027 $\pm$ .005	.031 $\pm$ .004	.023 $\pm$ .004	.019 $\pm$ .004
GYK152466	.028 $\pm$ .006	.030 $\pm$ .005	.043 $\pm$ .006[a]	.028 $\pm$ .006[a]	.023 $\pm$ .004[a]
NBQX	.017 $\pm$ .002	.029 $\pm$ .003	.033 $\pm$ .003	.009 $\pm$ .002	.014 $\pm$ .003
Riluzole	.026 $\pm$ .005	.037 $\pm$ .005	.043 $\pm$ .005	.025 $\pm$ .004	.020 $\pm$ .005

[a]Treatment significantly different from vehicle ($p < .05$)

Table 2. Summary of Effects of Treatment on Hippocampal Formation 5-HT$_7$ Receptor mRNA Expression

Drug	CA2	CA3
Sterile water vehicle	.094 ± .011	.085 ± .012
MK-801 (0.3 mg/kg)	.102 ± .013	.109 ± .016
MK-801 (1.0 mg/kg)	.082 ± .017	.069 ± .009
MK-801 (3.0 mg/kg)	.076 ± .010	.078 ± .009
DMSO vehicle	.080 ± .018	.067 ± .016
Aniracetam	.106 ± .007	.101 ± .010
CNQX	.097 ± .014	.081 ± .011
GYK152466	.101 ± .010	.092 ± .009
NBQX	.113 ± .011	.095 ± .010
Riluzole	.092 ± .010	.085 ± .010

(Values are expressed as mean optical density for CA2 and CA3 ± SEM for 10 animals.)

els, and these three neurotransmitters share uptake transporters (Nishimura et al. 1998; Yan et al. 1997). Therefore, the putative increase in serotonin levels resulting in decreased 5-HT$_6$ receptor mRNA expression may be attributable to a direct effect on serotonin uptake. This would be consistent with the dose-dependent results found in our study, because synaptic concentrations of MK-801 might need to rise to a critical threshold before serotonin levels were high enough to result in altered 5-HT$_6$ receptor mRNA expression.

The regulation of striatal 5-HT$_6$ receptor mRNA levels by the MK-801 also may be interesting vis-à-vis schizophrenia. NMDA receptor hypofunction and serotonin receptor dysfunction have been separately implicated in schizophrenia, but our data suggest that these neurotransmitter systems are functionally linked at the level of receptor expression. Although there has been no direct measurement of 5-HT$_6$ mRNA levels in the striatum of schizophrenics, our current data suggest that disruptions in serotonin receptor expression may not be attributable to a primary defect in the serotonergic system. In addition, it remains to be determined how our data may be related to the putative therapeutic efficacy of 5-HT$_6$ receptor antagonism associated with atypical antipsychotic treatment. Future studies that measure final 5-HT$_6$ receptor protein levels and activity, which are currently technically difficult, are necessary to establish whether NMDA receptor antagonist treatment induces an upregulation or a downregulation of 5-HT$_6$ receptor function. It would be interesting, however, if glutamatergic dysfunction led to altered serotonergic activity mediated by 5-HT$_6$ receptors, which was then ameliorated by atypical antipsychotics.

Hippocampal Formation

The expression of neither 5-HT$_6$ mRNA nor 5-HT$_7$ mRNA was altered by MK-801 treatment. It is surprising that hippocampal 5-HT$_7$ receptor expression was not altered by MK-801 treatment, because this tran-

script is sensitive to regulation; functional adrenalectomy, after metyrapone treatment, or by surgical removal, results in increased 5-HT$_7$ receptor mRNA levels in the hippocampus, with the largest increases seen in the CA3 subfield (LeCorre et al. 1997; Yau et al. 1997). On the other hand, clozapine treatment results in decreased hippocampal 5-HT$_7$ receptor mRNA expression (Fredrick and Meador-Woodruff in press). The lack of effect in the present study may be explained by properties of the median raphe neurons that send serotonergic projections to dorsal hippocampus. Dorsal raphe projections to the striatum are more sensitive than median raphe neurons to the excitatory effects of local NMDA application, which may also explain why changes were seen in the striatum but not the hippocampus (Tao and Auerbach 1996).

EXPERIMENT 2. AMPA/KAINATE RECEPTOR MODULATOR TREATMENT

Striatum

This experiment was performed to determine if the effects of MK-801 were specific for the NMDA receptor subtype or is generalizable to other glutamate receptor subtypes. Striatal 5-HT$_6$ mRNA levels were altered by several, but not all, of the drugs used in this study, suggesting that this phenomenon is generalizable to other subtypes.

Densensitization Modulators

The effect of aniracetam on striatal 5-HT$_6$ receptor mRNA is consistent with previous studies. AMPA receptor desensitization limits tonic glutamatergic regulation of extracellular serotonin levels, and desensitization inhibitors alone increase serotonin levels. The decrease in 5-HT$_6$ receptor mRNA may reflect a compensatory decrease resulting from increased striatal serotonin levels (Figure 4). Interestingly, 5-HT$_{2C}$ mRNA levels did not change with aniracetam treatment (data not shown), suggesting that the effect is specific for 5-HT$_6$ receptors. One substrate for this difference may be the localization of serotonin receptor subtypes on different populations of neurons. 5-HT$_{2C}$ receptor mRNA is relatively enriched in the ventral versus dorsal striatum, while 5-HT$_6$ mRNA is more homogenously distributed; this differential distribution may be related to the disparity in their regulation. A recent study suggests that 5-HT$_{2C}$ receptor mRNA is expressed by striatal GABAergic projection neurons (Eberle-Wang et al. 1997). No such study has been attempted regarding 5-HT$_6$ receptors, but they may be localized on acetylcholine interneurons. Consistent with this speculation, behavioral studies suggest that 5-HT$_6$ receptors mediate a circumscribed behavioral pattern that requires an intact
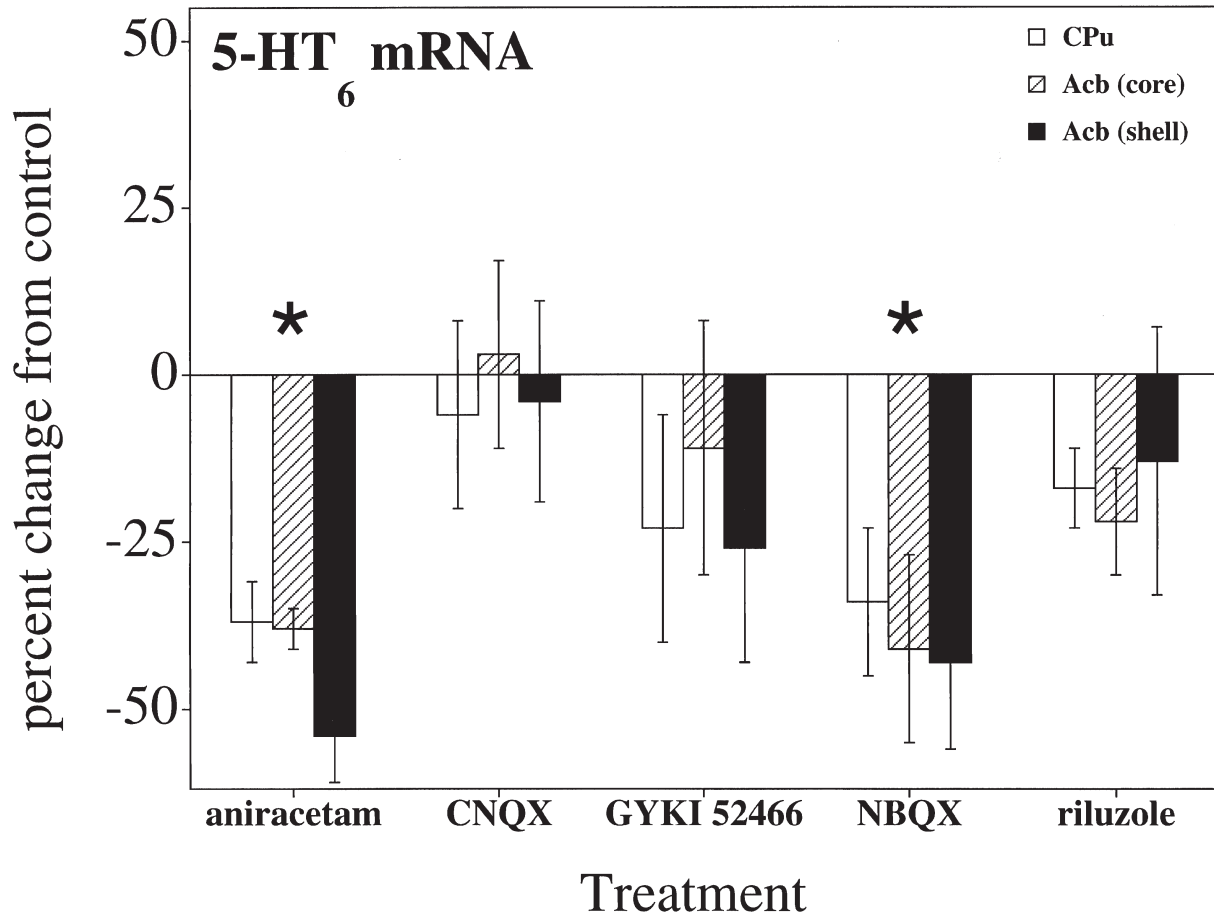
Figure 3. Striatal 5-HT$_6$ receptor mRNA levels after AMPA/kainate receptor modulator treatment (Experiment 2). Values expressed as percentage change normalized to controls ± SEM for 10 animals. * Denotes significant difference from control treatment.

cholinergic system (Bourson et al. 1995; Bourson et al. 1998). Furthermore, aniracetam has been shown to modulate cholinergic systems (Bartolini et al. 1992; Pepeu and Spignoli 1989). It is tempting to speculate that cholinergic, serotonergic, and glutamatergic systems intersect in the striatum, and the present data reflect a disruption in the way these systems interact. Alterations in the cholinergic system induced by aniracetam, with or without increased levels of extracellular serotonin, may lead to a compensatory change in 5-HT$_6$ receptor expression.

There are several potential explanations for the lack of effect of GYKI52466 on striatal 5-HT$_6$ receptor mRNA expression. Desensitization of AMPA receptors has been shown to limit serotonin release in the striatum, but perhaps desensitization is maximally occurring at synaptic glutamate concentrations. Therefore, there is no additive effect with desensitization facilitated by GYKI52466 treatment. This assumes that the mechanism driving altered striatal 5-HT$_6$ receptor mRNA lev-

els is altered serotonin release, which is speculative. Alternatively, the short half-life of GYKI52466 may preclude sufficient time to cause an effect on 5-HT$_6$ receptor mRNA expression.

Selective AMPA Receptor Competitive Antagonist

NBQX treatment resulted in significant decreases in striatal 5-HT$_6$ receptor mRNA levels of similar magnitude to aniracetam. However, NBQX would actually be expected to have the opposite effect of aniracetam, if its mechanism of action was on raphe AMPA receptors. One explanation is that NBQX is blocking AMPA receptors on GABAergic interneurons in the raphe, resulting in increased serotonin release in the striatum. This mechanism is similar to that invoked for the effects of MK-801 on striatal serotonin release and, again, presumes that altered serotonin release is related to changes in 5-HT$_6$ expression (Figure 4).

dorsal raphe

striatum
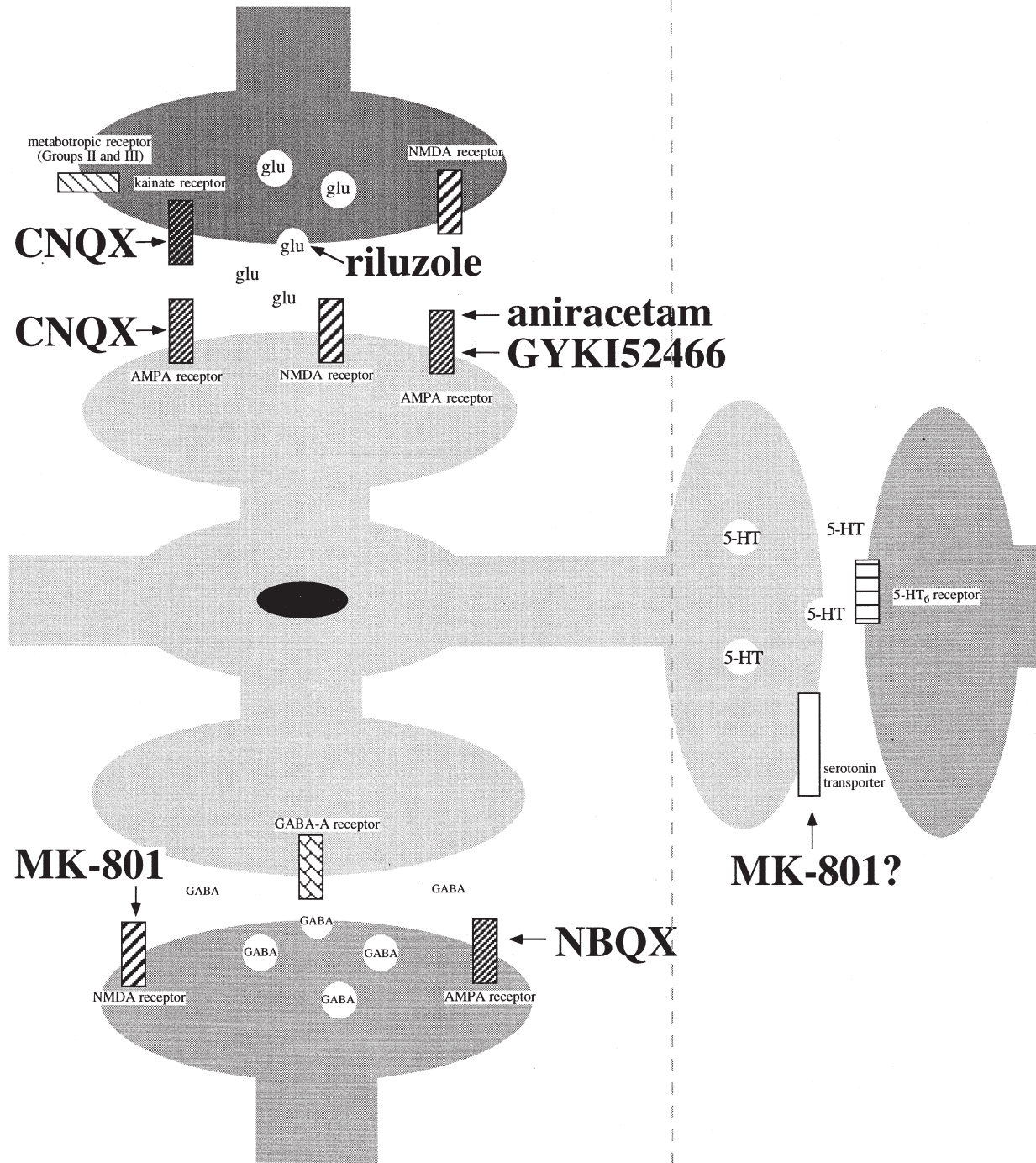
Glutamate Release Inhibitor

The lack of effect of riluzole treatment, a glutamate release inhibitor, is surprising, because there seems to be tonic glutamatergic input to the raphe. Altering this tonic input would be expected to change striatal serotonin release, and, thus, 5-HT$_6$ receptor mRNA levels, if this were the mechanism of action. Perhaps the lack of effect of riluzole is similar to the lack of effect of GYKI52466, in that AMPA receptor-mediated activity is already maximally limited. AMPA receptors are invoked in the mechanism of riluzole, because these receptors mediate fast glutamatergic activity and, presumably, would be affected before NMDA receptors (Hollmann and Heinemann 1994). Alternatively, the lack of effect of riluzole suggests that altered 5-HT$_6$ receptor mRNA expression may be attributable to a direct effect on striatal cells expressing this receptor rather than to altered glutamatergic input to the raphe or striatum.

Nonselective AMPA/Kainate Receptor Competitive Antagonist

CNQX also did not alter 5-HT$_6$ receptor mRNA expression in the striatum. Both AMPA and kainate receptors have significant affinity for CNQX. Kainate receptors have the highest affinity for this drug, as compared to the other drugs used in this study; disrupting kainate receptor-mediated glutamatergic activity may yield a similar effect to riluzole treatment on glutamate release, attenuating the direct effect of AMPA receptor blockade by CNQX (Figure 4). CNQX might be expected to have similar effects to NBQX, if CNQX blocked AMPA receptors on GABAergic interneurons. However, at the doses used, there may be selectivity of NBQX for GABAergic interneuron AMPA receptors while CNQX caused a more general effect because of its promiscuity for glutamate receptors.

Hippocampal Formation

5-HT$_6$ receptor mRNA expression in the hippocampus was minimally affected by the treatments examined in our study. In fact, only GYKI52466 treatment resulted in significant changes. This increase was seen in four of the five hippocampal formation subfields, which is more extensive than a previous study demonstrating an increase in 5-HT$_6$ receptor mRNA expression restricted to the CA1 subfield resulting from metyrapone treatment (Yau et al. 1997). The increase in our study may be consistent with the effect described above of GYKI52466 on AMPA receptor desensitization. Glutamate receptor-mediated hippocampal serotonin release is limited by AMPA receptor desensitization, and the facilitation of desensitization may lead to depressed serotonin levels. The compensatory increase in 5-HT$_6$ receptor mRNA expression is consistent with a putative decrease in serotonin availability. However, it is incongruous that NBQX would not have a similar effect.

As stated previously, 5-HT$_7$ mRNA has been shown to be sensitive to regulation by decreased corticosterone or antipsychotic treatment (Fredrick and Meador-Woodruff in press; LeCorre et al. 1997; Yau et al. 1997). However, none of the treatments in this experiment resulted in significant changes in 5-HT$_7$ receptor mRNA, similar to our MK-801 experiment.

CONCLUSIONS

These data are consistent with our hypothesis that glutamatergic modulators of serotonin release lead to changes in novel serotonin receptor expression, but these changes were specific to glutamate receptor modulator, serotonin receptor subtype, and region. To our knowledge, this is the first demonstration of the regulation of striatal 5-HT$_6$ receptor mRNA expression, and provides neurochemical anatomical evidence for the in-

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Figure 4. Summary figure of the putative effects of ionotropic glutamate receptor modulators used in Experiments 1 and 2 on raphe and striatum. Excitatory glutamatergic input to serotonergic raphe neurons (top) is modulated by these drugs as shown. Postsynaptic AMPA receptors are affected by aniracetam and GYKI52466, such that aniracetam may result in increased striatal serotonin release (right), but GYKI52466 may not. This is the mechanism proposed to result in decreased striatal 5-HT$_6$ expression. In addition, both AMPA and kainate receptors are blocked by CNQX (top), which does not lead to alterations in striatal 5-HT$_6$ expression. The lack of effect may be attributable to CNQX's ability to affect pre- and postsynaptic processes. Changes in glutamate release induced by riluzole (top) also do not affect striatal 5-HT$_6$ expression. In addition, glutamate receptor antagonists are purported to affect GABAergic inhibitory input to the raphe (bottom). NBQX may block AMPA receptors on GABAergic neurons, putatively leading changes in GABA release (bottom). Striatal serotonin release is thus increased, leading to decreased striatal 5-HT$_6$ expression. Similarly, MK-801 blocks NMDA receptors on GABAergic neurons, eventually producing altered striatal 5-HT$_6$ expression (bottom). In addition to the effects on GABA, MK-801 may also exert its effect via the serotonin transporter (right). As described in the Discussion section, the dose-dependent effects of MK-801 may be attributable to decreased serotonin reuptake, resulting in the reduction of striatal 5-HT$_6$ expression.

teraction of serotonergic and glutamatergic systems. Modulators of both NMDA and AMPA receptors decreased 5-HT$_6$ receptor mRNA levels, suggesting that multiple glutamate receptor subtypes participate in this interaction. Although these two neurotransmitter systems are separately implicated in schizophrenia, alterations in serotonin receptor expression in schizophrenia may result, in part, from altered glutamatergic activity.

ACKNOWLEDGMENTS

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