Enhancement of Serotonin 2C Receptor mRNA Expression by Antidepressants Possessing the Receptor-Blocking Activity in the Rat Brain

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ABSTRACT—The effects of repeated oral administration of antidepressants on the serotonin 2C receptor subtype (5-HT2CR) mRNA level in the rat brain were examined. Imipramine (20 mg/kg, p.o.) enhanced the hybridization signal in a time (days)-dependent manner, reaching a maximum at day 4 and maintaining a high level until day 14. Desipramine and mianserin, which have 5-HT2CR antagonistic activity, also stimulated the mRNA expression to about same extents as imipramine, but nomifensine, which has no effect on 5-HT2CR, was ineffective. These results suggest that long-term treatment with antidepressants, which act as 5-HT2CR antagonists, stimulates 5-HT2CR mRNA expression.

Keywords: Serotonin 2C receptor, Antidepressant, mRNA expression

It has long been recognized that many antidepressants bind to and inhibit in serotonin transporters and thereby achieve their therapeutic effects. In fact, a single amino acid-binding site for tricyclic antidepressants has been identified in the human serotonin transporter (1). However, long-lasting, repeated treatment with antidepressants for several weeks is necessary to determine their therapeutic effects. This fact suggests that some physiological mechanisms are changed during long-lasting exposure to antidepressants. We previously reported that repeated oral administration of imipramine elevated the level of serotonin 2C receptor subtype (5-HT2CR) mRNA in rat brain (2). Many antidepressants also bind to 5-HT2CR (3) and inhibit its function (4, 5). In the present report, we describe the effect of some antidepressants on 5-HT2CR mRNA expression in rat brain, discuss the relationship between the mRNA-inducing activity and the 5-HT2CR antagonistic effect, which we reported previously (4), and speculate on the physiological significance.

The 5-HT2CR inserted in the EcoRI site of the plasmid, PMV7ST1C, was a kind gifted from Dr. D. Julius (6). The inserted vector of 5-HT2CR cDNA was firstly changed to pBluescript KS(−) II by ourselves. The drugs used were imipramine HCl (Nacalai Tesque, Kyoto), desipramine HCl, mianserine HCl (Sigma, St. Louis, MO, USA) and nomifensin malate (RBI, Natick, MA, USA). The materials for digoxigenin labelings were purchased from Boehringer (Ingelheim, Germany). Enzymes and other materials for gene technology were purchased from Takara (Kusatsu), Gibco (Gaithersburg, MD, USA), NEN (Boston, MA, USA), Stratagene (La Jolla, CA, USA) or Fermentas (Vilnius, Lithuania). To obtain the digoxigenin-labeled RNA probe corresponding to the sequence of the third intracellular loop domain, which is unique to each G protein-coupled receptor, the pBluescript II KS(−) vector containing rat 5-HT2CR cDNA was rearranged using restriction enzymes, DNA polymerase and ligase (2). The resultant 275 bp 5-HT2CR cDNA fragment (nucleotides 1355–1630) was inserted between nucleotides 657 and 753 of the pBluescript vector. The reconstituted vector, which was used to make a probe, was cut by Hinc II at nucleotide 1368 of the 5-HT2CR cDNA for the T3 RNA polymerase reaction to make antisense RNA or by Kpn I at nucleotide 759 of the vector for the T7 RNA polymerase reaction to make sense RNA. The reactions to make these probes were performed essentially as described previously (2). Statistical analysis was carried out by Student’s t-test.

Imipramine or vehicle (water) was administered orally at 3:00 p.m. everyday to male Wistar rats (8-week-old, 220–250 g; Japan SLC, Inc., Hamamatsu). One hour after the last administration, the rats were killed by decapi-
tion. Frozen brain sections (16 μm) were made from these rats. In situ hybridization was carried out and quantitative analysis of stained sections was performed by using an NIH Image analysis system as described previously (2).

The digoxigenin-labeled antisense probe showed strong hybridization with the choroid plexus of the lateral and third ventricles (Fig. 1) and also but weakly hybridized to the hippocampus, medial and lateral habenular nucleus, amygdala and piriform cortex (data not shown). These findings are in good agreement with previous results obtained using a radiolabeled probe (7). The repeated treatment with imipramine dose-dependently enhanced the hybridization signal, and this enhancement increased with increasing days of treatment: although a single administration of imipramine caused only a slight, but not significant, increase in the mRNA level (Image Density: 201.7 ± 11.6% in the choroid plexus compared with the water-administered control (181.0 ± 10.4%, N = 4)), the expression intensity reached a peak at day 4 (236.0 ± 3.6%) and remained at a high level at least until day 14 (228.3 ± 7.7%). The effects of 4 days of oral administration of other antidepressants: desipramine, mianserin and nomifensine, at 20 mg/kg, on 5-HT2CR mRNA expression were also examined (Figs. 1 and 2). Desipramine and mianserin stimulated the mRNA expression to the same extent as imipramine with about the same potency, although nomifensine had no effect.

Previously, we showed that some antidepressants, including imipramine, desipramine and mianserin, but not

Fig. 2. Effects of repeated treatment with antidepressants on 5-HT2CR mRNA expression in the rat brain. Each antidepressant (20 mg/kg) or vehicle was administered once a day for 4 days. The staining densities were quantified using an NIH Image scanner. Values represent the mean ± S.E.M. percent of the blank (the area not labeled by 5-HT2CR probe) of 4 individual experiments. Differences were analyzed for significance by Student's t-test. *P < 0.05, compared with vehicle treatment.

Fig. 1. Effects of repeated treatment with antidepressants on 5-HT2CR mRNA expression in the rat brain. Each antidepressant (20 mg/kg) or vehicle (water) was administered once a day for 4 days. Each panel shows the choroid plexus of the lateral ventricles. A: vehicle, B: imipramine, C: desipramine, D: mianserin and E: nomifensine.
nomifensine, act as 5-HT2CR antagonists, using Xenopus oocytes injected with rat brain mRNA (4). The IC<sub>50</sub> values of imipramine, desipramine and mianserin are 20 nM, 20 μM and 60 nM, respectively. We also found that these three antidepressants, but not nomifensine, also inhibited [³H]mesulergine binding in rat brain synaptic membrane with IC<sub>50</sub> values of 1–10 μM. Palvimäki et al. (3) also reported the K<sub>i</sub> value for inhibition of [³H]mesulergine in the cloned 5-HT2C receptor: the values of imipramine, desipramine, mianserin and nomifensine are 94 nM, 244 nM, 0.4 nM and >1000 nM, respectively. The present results show similar abilities among these antidepressants at 20 mg/kg, which is about fivefold higher than the dose in humans, to induce 5-HT2C mRNA expression, suggesting that long-term occupation of 5-HT2CR by antidepressants possessing the receptor-blocking activity may stimulate 5-HT2CR gene expression in order to compensate for the receptor function. Laakso et al. (8) found that chronic treatment (for 14 days) with fluoxetine and citalopram, both of which are antidepressants with high affinity to the 5-HT uptake site, cause a marked up-regulation of 5-HT2CR in the rat choroid plexus. Blockage of 5-HT2CR by fluoxetine was shown by using Xenopus oocyte system (9). Citalopram also has 5-HT2CR-binding activity (3). These previous and the present results suggest that 5-HT2CR functions are altered by antidepressants. The role of monoamine uptake inhibition in antidepressants’ mechanisms of action has been recognized. The amine uptake inhibition, however, is an acute effect. Days or weeks of treatment are necessary to show the clinical effects of antidepressants and 5-HT2CR mRNA expression. Concerning this point, studying the increase of 5-HT2CR mRNA level may offer some advantages for clarifying the mechanisms of action of antidepressants.

Four days of treatment was enough to maximally increase the expression of 5-HT2CR mRNA. This seems to be considerably more rapid than the expression of therapeutical effects. This may be due to the additional time required to make protein from mRNA, or alternatively, other factors may need to be expressed after 5-HT2CR. Jullius et al. (6) showed that 5-HT2CR has transforming activity. In fact, our preliminary experiments showed that expression of some genes is induced by 14 days of imipramine treatment (10). The increased 5-HT2CR may stimulate expression of other genes such as those encoding anti-stress proteins. Further studies should reveal the roles of gene expression changes in the mechanisms of action of antidepressants.

REFERENCES


