Autoradiographic Evidence for the Interaction of SM-3997 with 5-HT₁A Receptors in the Rat Brain

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Abstract—[³H]8-OH-DPAT binding to rat brain sections and inhibition by SM-3997 were investigated. Very high densities of [³H]8-OH-DPAT binding sites were found in the dentate gyrus, entorhinal cortex, dorsal raphe, interpeduncular nucleus and lateral septum. In contrast, their densities were sparse in the substantia nigra, caudate putamen and choroid plexus. In the presence of 1 μM of SM-3997, [³H]8-OH-DPAT binding was strongly inhibited in all the brain structures we examined. These results indicate that SM-3997 binds to 5-HT₁A receptors of rat brain sections.

Benzodiazepines (BZs) are widely used as therapeutic drugs for anxiety, but often produce undesirable side effects such as drowsiness, ataxia and sedation. These agents bind to BZ receptor sites of GABA receptor/Cl⁻ channel complexes and modulate GABAergic functions. This GABA-related mechanism is considered to mediate not only the anxiolytic effect but also other side effects of BZs.

SM-3997 (3αα,4β,7αα-hexahydro-2-(4-(4-(2-pyrimidinyl)-1-piperazinyl)-butyl)-4,7-methano-1H-isooindole-1,3(2H)-dione citrate) is a clinically effective anxiolytic that is devoid of these side effects. We previously reported that SM-3997 did not directly interact with GABA receptor/Cl⁻ channel complexes, but is suggested to have 5-HT₁A agonistic properties by pharmacological, biochemical and electrophysiological experiments (1-5). The present study was conducted to determine the regional distribution of 5-HT₁A receptors and SM-3997 binding to these receptors in the rat brain.

Male Sprague-Dawley rats (weighing about 300 g) were decapitated, and the brains were quickly frozen. Coronal and sagittal sections (20 μm) were thaw-mounted onto gelation-coated slides and stored at -25°C until use. The [³H]8-OH-DPAT binding assay was carried out according to the partially modified procedure of Rainbow et al. (6). Tissues were preincubated at 25°C for 30 min in 0.17 M Tris-HCl buffer, pH 7.4, and then incubated at 25°C for 1 hr in the same buffer containing 4 mM CaCl₂, 0.5 μM chlorimpramine (Ciba-Geigy), a 5-HT uptake inhibitor, and about 2 nM [³H]8-OH-DPAT (Amersham) in the presence or absence of 1 μM SM-3997 (Sumitomo). After incubation, sections were washed in ice cold buffer (2 x 5 min), quickly dipped in distilled water, dried with cold nitrogen gas and exposed to Hyperfilm-³H (Amersham) for 3 weeks. Analyses of autoradiograms were performed with a computer-assisted image analyzer (UHG-1, Unique Medical). The amount of [³H]8-OH-DPAT binding was calculated using [³H]-micro-scales (Amersham) as standards. Non-specific binding was generated by the addition of 1 μM 8-OH-DPAT (Sumitomo).

8-OH-DPAT has been shown to selectively interact with 5-HT₁A receptors (7), so we used this ligand to label this receptor subtype. The autoradiographic localization of specific [³H]-8-OH-DPAT binding sites in the rat brain sections are shown in Fig. 1A and Table 1. Binding sites for [³H]8-OH-DPAT were concentrated in the areas of 5-HT neuronal cell bodies and synaptic terminals. A high degree of binding was observed in the hippocampus.
especially in the dentate gyrus and CA1 field of Ammon's horn. Intense labeling was also present in the dorsal raphe, entorhinal cortex, interpeduncular nucleus and lateral septum. Moderate labeling occurred in the cingulate cortex, ventral hypothalamus, amygdalocortical nucleus, CA3 field of Ammon's horn and frontal cortex. There was only little binding in the caudate putamen, substantia nigra and choroid plexus. These results are in agreement with previous reports by Marcinkiewicz et al. (8) and Verge et al. (9).

In the presence of 1 µM SM-3997, [3H]8-OH-DPAT binding in the dentate gyrus markedly decreased by 90%. Similarly in the dentate gyrus, [3H]8-OH-DPAT binding was intensely inhibited by SM-3997 in all the brain structures we examined (Table 1 and Fig. 1B). These autoradiographic results suggest that SM-3997 binds to 5-HT_{1A} receptors in all regions of the rat brain.

The autoradiographic image in Fig. 1C, which was made by subtracting the image represented in Fig. 1B from that in Fig. 1A, indirectly indicates the regional distribution of SM-3997 binding sites in the rat brain. We previously reported that SM-3997 possessed a high affinity for 5-HT_{1A} receptors, and very low affinity for dopamine_{2} and 5-HT_{2} receptors, but had no affinity for other receptors in rat brain membrane preparations (2, 3). Therefore, the regional distribution of SM-3997 binding sites should be similar to the image represented in Fig. 1C. In the rat brain,
Table 1. Quantitative autoradiographic analysis of [3H]8-OH-DPAT binding and inhibition by SM-3997

| Brain structure       | Specific [3H]8-OH-DPAT binding (fmol/mg tissue) |
|-----------------------|-----------------------------------------------|
|                       | Control                                      | SM-3997 (1 µM)                                      |
| Dentate gyrus         | 148 ± 3                                      | 13.4 ± 0.9                                        |
| Interpeduncular nuc.  | 115 ± 7                                      | 6.3 ± 0.7                                         |
| Entorhinal cortex     | 113 ± 3                                      | 2.1 ± 0.2                                         |
| Lateral septum nuc.   | 108 ± 6                                      | 13.2 ± 0.9                                        |
| Dorsal raphe nuc.     | 98.9 ± 5.6                                   | 6.9 ± 1.2                                         |
| CA1 field             | 74.9 ± 6.4                                   | 6.5 ± 0.6                                         |
| Cingulate cortex      | 65.0 ± 1.3                                   | 7.6 ± 0.4                                         |
| Amygdalocortical nuc. | 54.5 ± 6.3                                   | 2.3 ± 0.6                                         |
| Ventral hypothalamus  | 49.9 ± 2.0                                   | 3.2 ± 1.0                                         |
| CA3 field             | 42.0 ± 3.6                                   | 4.2 ± 0.4                                         |
| Frontal cortex        | 37.5 ± 0.7                                   | 4.6 ± 0.3                                         |
| Medial septum nuc.    | 31.6 ± 1.4                                   | 2.8 ± 0.7                                         |
| Median raphe nuc.     | 24.4 ± 1.3                                   | 1.1 ± 0.7                                         |
| Central amygdaloid nuc.| 21.8 ± 2.3                                 | 2.1 ± 0.5                                         |
| CA2 field             | 18.1 ± 1.3                                   | 1.4 ± 0.3                                         |
| Caudate putamen       | 1.8 ± 0.5                                    | 0.6 ± 0.3                                         |
| Substantia nigra      | N.D.                                         | N.D.                                              |
| Choroid plexus        | N.D.                                         | N.D.                                              |

Coronal and sagittal sections were incubated with 2 nM [3H]8-OH-DPAT in the presence or absence of 1 µM SM-3997. Nonspecific binding determined by incubation of adjacent sections in the presence of 1 µM 8-OH-DPAT was subtracted from all density readings. Results are the mean ± S.E.M. of 4–25 measurements performed in 3–4 independent experiments. N.D.: not detectable.

the highest density of SM-3997 binding sites may exist in the hippocampus. To clarify this hypothesis, the mapping of radiolabeled SM-3997 binding sites in the rat brain is in progress.

Previously we reported that SM-3997 produced marked increases in punished responding of rats in Vogel's conflict test (1). These actions were antagonized by the administration of spiperone (5-HT1A, 5-HT2, D2 mixed antagonist), but not by that of ketanserin (5-HT2 antagonist) or haloperidol (D2 antagonist) (10); and the 5-HT neuronal lesion induced by the intracerebral administration of 5,7-dihydroxytryptamine failed to attenuate the anticonflict action of SM-3997 in rats (11). From these findings, we concluded that central 5-HT1A receptors, which may be located on the post-synaptic membranes of 5-HT neuronal terminals, contributed directly to the anxiolytic or anticonflict actions of SM-3997.

In the present study, preferential localization of 5-HT1A receptors (SM-3997 binding sites) was found in the hippocampus, where the local injection of SM-3997 was reported to produce anticonflict effects in rats (12). Limbic regions, such as the hippocampus and septum, are considered to regulate affecional experiences and emotions. 5-HT1A receptors in the hippocampus may participate in the manifestation of the anxiolytic effects of SM-3997.

References
1 Shimizu, H., Hirose, A., Tatsuno, T., Nakamura, M. and Katsube, J.: Pharmacological properties of SM-3997: A new anxiolselective anxiolytic candidate. Japan. J. Pharmacol. 45, 493–500 (1987)
2 Shimizu, H., Karai, N., Hirose, A., Tatsuno, T., Tanaka, H., Kumasaka, Y. and Nakamura, M.: Interaction of SM-3997 with serotonin receptors in the rat brain. Japan. J. Pharmacol. 46, 311–314 (1988)
3 Shimizu, H., Tatsuno, T., Hirose, A., Tanaka, H., Kumasaka, Y. and Nakamura, M.: Characterization of the putative anxiolytic SM-3997 recognition sites in rat brain. Life Sci. 42, 2419–
Short Communications

Japan. J. Pharmacol. 52, 507 (1990)

4 Hirose, A., Sasa, M., Akaite, A. and Takaori, S.: Inhibition of hippocampal CA1 neurons by 5-hydroxytryptamine, derived from the dorsal raphe nucleus and the 5-hydroxytryptamine_{1A} agonist SM-3997. Neuropharmacology (in press)

5 Hirose, A., Tsuji, R., Shimizu, H., Tatsuno, T., Tanaka, H., Kumasaka, Y. and Nakamura, M.: Inhibition of 8-hydroxy-2-(di-n-propylamino)tetralin and SM-3997, a novel anxiolytic drug, of the hippocampal rhythmical slow activity mediated by 5-hydroxytryptamine_{1A} receptors. Naunyn Schmiedebergs Arch. Pharmacol. (in press)

6 Rainbow, T.C., Bleisch, W.V., Biegon, A. and McEwen, B.S.: Quantitative densitometry of neurotransmitter receptors. J. Neurosci. Methods 5, 127-138 (1982)

7 Middlemiss, D.N. and Fozard, J.R.: 8-Hydroxy-2-(di-n-propylamino)tetralin discriminates between subtypes of the 5-HT_{1A} recognition sites. Eur. J. Pharmacol. 90, 151-153 (1983)

8 Marcinkiewicz, M., Verge, D., Gozlan, H., Pichat, L. and Hamon, M.: Autoradiographic evidence for the heterogeneity of 5-HT_{1A} sites in the rat brain. Brain Res. 291, 159-163 (1984)

9 Verge, D., Daval, G., Marcinkiewicz, M., Patey, A., El Mestikawy, S., Gozlan, H. and Hamon, M.: Quantitative autoradiography of multiple 5-HT_{1A} receptor subtypes in the brain of control or 5,7-dihydroxytryptamine-treated rats. J. Neurosci. 6, 3474-3482 (1986)

10 Tatsuno, T., Shimizu, H., Hirose, A., Kumasaka, Y., Nakamura, M. and Katsube, J.: The mechanism of anti-conflict action of SM-3997—Involvement of the 5-HT_{1A} system. Japan. J. Psychopharmacol. 8, 33-34 (1988) (in Japanese)

11 Shimizu, H., Tatsuno, T., Tanaka, H., Kumasaka, Y. and Nakamura, M.: The role of central serotonergic neuron systems in the mechanism of action of SM-3997. Japan. J. Pharmacol. 46, Supp. 238P (1988)

12 Miyazaki, A., Kataoka, Y., Yamashita, K., Shibata, K. and Ueki, S.: Localization of the brain areas for anticonflict action of non-benzodiazepine anxiolytics in rat. Japan. J. Psychopharmacol. 8, 273-274 (1988) (in Japanese)