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Identification of serotonin 5-HT_3 recognition sites by radioligand binding in NG108-15 neuroblastoma-glioma cells

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The 5-HT (5-hydroxytryptamine, serotonin) receptors have been subdivided into 3 main classes termed 5-HT_1, 5-HT_2 and 5-HT_3 (Bradley et al., 1986). Although well characterized in various isolated tissue models, 5-HT_3 receptors, in contrast to 5-HT_1 and 5-HT_2 receptors (Hoyer et al., 1985), have not yet been identified by means of radioligand binding techniques.

We now report on the identification of 5-HT_3 recognition sites in NG 108-15 neuroblastoma-glioma cells done with [^{3}H]ICS 205-930, a potent and selective 5-HT_3 receptor antagonist (Richardson et al., 1985).

NG 108-15 cells were grown in Dulbecco's modified Eagle medium (DMEM) with Hepes (7.6 mM) and sodium bicarbonate (30 mM). The antibiotics penicillin (100 IU/ml) and streptomycin (100 μg/ml) were added, as well as 7.5% fetal calf serum (Gibco) and the following amino acids (in mM concentrations): cysteine-HCl (30), l-alanine (40), asparagine (45), l-aspartic acid (40), l-proline (40) and l-glutamic acid (40). The cells were cultured at 37° C, in closed tissue culture roller bottles (Falcon 850 ml) at a density of 10^8 cells/bottle and harvested by vigourous shaking. A crude membrane fraction was prepared after centrifugation at 4° C at 900 × *g* for 5 min and resuspension in Tris buffer (20 mM, pH 7.5) containing 154 mM NaCl followed by homogenisation with a Brinkmann polytron (position 9, 2 × 15 s). Membranes diluted to about 2×10^6 cells/ml in Tris NaCl buffer (150 μl) were added to 50 μl [^{3}H]ICS 205-930 (33 Ci/mmol, final concentration 2-4 nM) and 50 μl buffer or drug in polystyrene tubes and were incubated at 37° C for 60 min. The incubation was stopped by rapid filtration and washing over Whatman GF/B glass fiber filters as described earlier (Hoyer et al., 1985). Non-specific binding was defined in the presence of 10 μM MDL 72222 (Merrel-Dow, Strasbourg). Competition and saturation experiments were performed and analysed as described earlier (Hoyer et al., 1985).

[^{3}H]ICS 205-930 labelled with high affinity a single population of recognition sites on membranes of NG 108-15 cells: $B_{max} = 60.4 \pm 3.9$ fmol/mg protein, pK_D ($-\log$ M) $= 8.91 \pm 0.08$, $n = 7$. Binding was rapid, reversible and stable for at least 90 min (data not shown). The sites labelled by [^{3}H]ICS 205-930 displayed intermediate to high affinity for the 5-HT_3 receptor antagonists metoclopramide, quipazine, MDL 72222 and ICS 205-930 (table 1). The 5-HT_3 receptor agonists, 2-methyl-5-HT (Richardson et al., 1985) and phenylbiguanide (Ireland and Tyers, 1987) were only slightly less potent than 5-HT to inhibit [^{3}H]ICS 205-930 binding (table 1). In contrast, other 5-HT_1 and 5-HT_2 receptor antagonists (ketanserin, spiperone, pindolol) did not significantly affect

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TABLE 1

Affinity values of various drugs for [^{3}H]ICS 205-930 recognition sites on NG 108-15 cell membranes. The results are expressed as pK_D values (−log M, means ± S.E.M. of 3 individual experiments).

Drug	pK_D
ICS 205-930	8.98 ± 0.06
MDL 72222	7.89 ± 0.08
Quipazine	8.47 ± 0.11
Metoclopramide	6.58 ± 0.02
5-HT	6.05 ± 0.06
2-Methyl-5-HT	6.02 ± 0.10
Phenylbiguanide	5.87 ± 0.08

[^{3}H]ICS 205-930 binding at 10 μM (data not shown). All competition curves were steep, monophasic and showed best fit for a one-site model.

The rank order of affinity of both agonists and antagonists for [^{3}H]ICS 205-930 recognition sites was in good agreement with their rank order of potency determined in various functional models, e.g. 5-HT induced depolarisation in rabbit vagal afferents and sympathetic ganglia (Round and Wallis, 1987) or rat vagus nerve (Ireland and Tyers, 1987).

In conclusion, the present results demonstrate that [^{3}H]ICS 205-930 labels with high affinity a single population of recognition sites which display the pharmacological characteristics of a 5-HT_3 receptor. [^{3}H]ICS 205-930 will be a useful tool for study of the pharmacology and distribution of 5-HT_3 recognition sites in mammalian tissues by radioligand binding and autoradiography.

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