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RP 62203, a novel naphtosultam derivative with potent and selective $5HT_2$ antagonist properties: I. Binding and second messenger studies

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Serotonin (5HT) is thought to play a role in a wide variety of central nervous system functions, including sleep, appetite, mood and pain perception. Understanding of this role has been hampered by the paucity of selective agonists and antagonists for the different 5HT receptor subtypes. In particular, there are few available $5HT_2$ antagonists devoid of activity at α_1 adrenoceptors. We now report the selective $5HT_2$ antagonist action of the novel naphtosultam derivative, RP 62203.

RP 62203 binds potently to the $5HT_2$ receptor with a Ki value of 0.26 nM in competition experiments against [^{3}H]-ketanserin on rat cortical membranes. Its affinity for 5HT-1A receptors is some threehundred-fold lower (Ki = 68.5 nM, measured with [^{3}H]-8-OH-DPAT on rat hippocampal membranes). RP 62203 displayed no measurable affinity for $5HT_3$ receptors in the rat entorhinal cortex ($Ki > 10^{-6}$ M versus [^{3}H]-GR 65630). Its selectivity for $5HT_2$ over α_1 receptors was 135x (Ki = 35 nM) versus [^{3}H-prazosin), compared to forty times for ritanserin. RP 62203 had negligeable affinity ($Ki > 10^{-6}$ M) for other monoamine receptors (ACH-m, β, D_2, α_2), except for histamine H_1 receptors in the guinea-pig cerebellum, for which a Ki versus [^{3}H]-mepyramine of 25 nM was found, compared to 6 nM for ritanserin. RP 62203 had no detectable affinity for the following receptors: benzodiazepine, CCK-A, CCK-B, neurotensin, neuropeptide Y, vasopressin (V_1), substance P (NK_1), μ, δ- or κ-opiate, PAF or somatostatin. Similarly, RP 62203 was inactive in binding assays for voltage-sensitive sodium and calcium channels.

In vivo, RP 62203 displaced the binding of [^{3}H]-methylspiperone in the rat frontal cortex (a marker for $5HT_2$ receptors) with an ID_{50} of 0.49 mg/kg po, whilst no displacement of the binding of this ligand was observed in the rat striatum (a marker for D_2 receptors) at the highest dose tested (20 mg/kg po). Similar data were obtained in the mouse. After intraperitoneal administration to guinea-pigs, RP 62203 did not displace the binding of [^{3}H]-mepyramine to cerebellar membranes measured ex vivo at the highest dose tested (20 mg/kg po).

In the neonatal rat, serotonin stimulated the formation of [^{3}H]-inositol phosphates from cortical slices prelabelled with [^{3}H]-myo-inositol with an EC_{50} of 8×10^{-6} M. The effect of serotonin was mimicked by the selective $5HT_2$ agonist DOI, but not by the 5HT-1A agonist 8-OH-DPAT. RP 62203 antagonised 5HT-stimulated inositol phophate formation with an IC_{50} of 7.9 nM. This compound also blocked inositol phosphate formation induced by the $5HT_2$ agonist DOI, but not that produced by carbachol. RP 62203 had no effect on basal inositol phosphate formation.

The data presented demonstrate that RP 62203 is one of the most potent and selective $5HT_2$ antagonists described to date, and should prove invaluable in evaluating the role of $5HT_2$ receptors in serotonin-mediated neurotransmission in the central nervous system. Furthermore, the low affinity of RP 62203 for α_1-adrenoceptors should lead to less pronounced cardiovascular effects than those observed for currently available $5HT_2$ antagonists.

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Opioid receptor binding and receptor-effector coupling in intact neurons and their isolated membranes

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A comparison of the receptor-effector properties determined in isolated membranes with those in living cells

frequently reveals qualitative or quantitative differences (e.g., Porzig, 1982). The present study was undertaken to compare the characteristics of the μ opioid receptor in whole cells and in fragmented membranes.

Kinetic constants for ligand binding, stimulation of low-Km GTPase and inhibition of adenylate cyclase were determined in the human neuroblastoma clone SH-5Y5Y. These parameters were measured in both intact cells and isolated membranes (obtained by hypotonic lysis) in physiological buffer using the μ-selective agonist Tyr-D-Ala-Gly(Me)Phe-Gly-ol (DAMGO).

Binding of [^{3}H]DAMGO to cells exhibited two binding components with Kd's of 3 nM and 100 nM. The high affinity sites represented approximately 15% of total binding. In contrast, binding to lysates was monophasic with an affinity similar to that of the high affinity component in cells (Kd: 5 nM). Addition of GTP to the lysates reduced B_{max} by 80% while Just marginally increasing the Kd. Thus, the high affinity sites in cells, representing the form of opioid receptor coupled to G-protein, appear to be identical to those in membranes, and uncoupling of the receptor-G-protein complex by GTP in the cells may account for their lower density.

The coupling of the opioid receptor to its effector, adenylate cyclase, was essentially unperturbed in the isolated membranes as compared to intact cells. The Ki for cyclase inhibition by DAMGO was 11 nM in cells and 26 nM in lysates, whereas the corresponding maximal inhibition was 33% and 27%, respectively. Coupling through stimulation of a low-Km GTPase was also demonstrated in the membranes, with a concentration for half-maximal stimulation (Ks) of 31 nM, and a maximum stimulation of 48%. When cells were homogenized more rigorously than osmotic lysis there was a severe attenuation of inhibition and a 3-fold shift in the Ks. Both cyclase inhibition and GTPase stimulation by DAMGO were blocked by naloxone, a preferentially μ receptor selective antagonist, while the δ receptor antagonist ICI 174864 had no effect.

These results show that in intact SH-SY5Y cells and lysates the characteristics of ligand binding to the μ opioid receptor and receptor coupling to adenylate cyclase are comparable. In both preparations, suspended in physiological medium, [^{3}H]DAMGO bound with similar high affinity. This binding component appears to be that required for coupling since it was the only component apparent in lysates. Signal transduction from the opioid receptor to adenylate cyclase with mediation by G-protein appears to function equally well in cells and lysates, however, this coupling is sensitive to methods of membrane isolation.

Reference

Porzig, H., 1982, Current Rev. Biomed. 2, 25.

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Reconstitution of insulin receptor: influence of lipids on the coupling between binding and activation

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The insulin receptor is a transmembrane heterotetrameric glycoprotein constituted by two extracellular binding units and two transmembrane units which possess a tyrosine kinase activity. We previously demonstrated changes in insulin binding, tyrosine kinase activity and hormonal actions on a hepatoma cell line through modifications of the lipid composition of the culture medium (Bruneau, 1987). In order to assess more precisely the role of membrane lipids in the transmembrane signalling mechanism of insulin, we developped methods for delipidation and reconstitution of purified insulin receptors into lipidic vesicles. The receptors were prepared by detergent solubilisation of placental membranes and affinity chromatography on wheat-germ-agglutinin-agarose (Van Obberghen, 1983). Extensive washing of the column in the absence of detergent resulted in an important loss of phospholipid and cholesterol and a consequent increase of the cholesterol/phospholipid ratio, when compared to control receptor preparations washed with detergent. Control preparations contained about 30 unsaturated fatty acids, while delipidated receptors contained more than so unsaturates. The insulin binding was not altered by this washing procedure, but the tyrosine kinase activity was drastically impaired in the "delipidated" receptors. For reconstitution experiments, natural phospholipid