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Identification of 5-HT_3 recognition sites in human brain tissue using [^{3}H]zacopride

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The present classification of 5-hydroxytryptamine (5-HT) receptors outlines three main sub-types denoted 5-$HT_{1\text{-like}}$, 5-HT_2 and 5-HT_3 (Bradley et al 1986). To date only the first two sub-types have been identified in human brain tissue. Recently, using a variety of different ligands, 5-HT_3 recognition sites have been demonstrated in rodent neuronal tissues (reviewed by Watling 1988). We now report the first direct evidence for the existence of 5-HT_3 recognition sites in human brain tissue using the tritiated derivative of the potent and selective 5-HT_3 receptor antagonist zacopride (U.S. Patent Number 4657911 assigned to Delalande; Smith et al 1988), a ligand that we have used to demonstrate 5-HT_3 recognition sites in rat and ferret brain tissue (Barnes et al 1988a,b).

Tissue from the amygdala and hippocampus was obtained at autopsy from patients who had died within 48 h from a non-neurological disorder and was frozen on dry-ice until required for assay. To prepare the brain homogenate, tissue was thawed and homogenized (Polytron, setting 7 for 10 s) in 20 volumes of HEPES buffer (50 mM) containing all the constituents of Krebs (NaCl 118·0, KCl 4·75, KH_2PO_4 1·2, $MgSO_4$ 1·2, $CaCl_2$ 2·5, $NaHCO_3$ 25·0 glucose 11·0 mM) with a final pH of 7·4. The homogenate was centrifuged at 48000 *g* for 10 min at 4°C and the pellet was then resuspended and again centrifuged. This pellet was finally resuspended in the HEPES/Krebs buffer at a concentration of 0·6–0·8 mg protein mL^{-1}. Protein estimation was performed using the Bio-Rad Coomassie Blue method using bovine serum albumin as the standard.

For binding studies assay tubes, used in triplicate, contained 100 μL [^{3}H]zacopride (54·9 $Cimmol^{-1}$), in a range of at least 9 concentration (0·1–16·0 nM) for saturation studies and at 0·5 nM for competition and kinetic studies, and 650 μL of competing drug or its vehicle. 250 μL of homogenate was added to initiate binding and the tubes were incubated at 37°C for 30 min before rapid filtration under vacuum through pre-wet Whatman GF/B filters followed by a rapid wash with 7 mL of ice-cold HEPES/Kreb's buffer using a Brandel cell harvester. Bound radioactivity was assayed by liquid scintillation spectroscopy at an efficiency of 47%.

Using the 5-HT_3 receptor antagonist BRL 43694 (10·0 μM) to define specific binding, saturation studies interpreted by Scatchard analysis revealed a single, saturable site of high affinity in both the amygdala and hippocampus (amygdala; $K_d = 3{\cdot}55$ nM, $B_{max} = 57$ fmol (mg protein)$^{-1}$, hippocampus; $K_d = 3{\cdot}36$ nM, $B_{max} = 54$ fmol (mg protein)$^{-1}$ mean, n = 2). As shown in Table 1, total [^{3}H]zacopride binding was inhibited in both areas by 5-HT_3 receptor agonists and antagonists (by approximately 40 and 30% in the amygdala and hippocampus, respectively) whilst methysergide, ritanserin, fluphenazine, sulpiride, SCH 23390, ranitidine, mepyramine, idazoxan, prazosin, propranolol, atropine, histamine, dopamine and noradrenaline (10·0 μM) all failed to inhibit binding. Specific binding was linear with protein content for both areas (0·04–0·22 and 0·04–0·24 mg protein from the amygdala and hippocampus, respectively). Using hippocampal tissue association was complete within 9 min ($K_{+1} = 7{\cdot}65 \times 10^5$ M^{-1} s^{-1}) and after full association BRL 43694 (10·0 μM) displaced specific binding within 10 min ($K_{-1} = 3{\cdot}45 \times 10^{-3}$ s^{-1}). The dissociation constant calculated from the association and dissociation rate constants gave a value of 4·51 nM which is in good agreement with that obtained from saturation studies.

Table 1. pK_1 ($-\log_{10}$ molar K_1) values for various compounds for the recognition site labelled by [^{3}H]zacopride in human amygdalold or hippocampal tissue. K_1 values (M) were determined using the equation: $K_1 = IC50/1 + (L/K_d)$, where IC50 is the molar concentration of competing compound to reduce specific binding by 50%, L is the molar concentration of [^{3}H]zacopride and K_d is the molar dissociation constant determined from saturation experiments. Data for each area are from a single brain employing 12 concentrations (10^{-10}–10^{-4} M), each in triplicate, of displacing compound

	pK_1	
Compound	Amygdala	Hippocampus
BRL 43694	8·64	8·85
GR 38032F	8·21	8·15
ICS 205-930	7·94	8·19
Metoclopramide	6·12	6·31
Cocaine	5·12	5·15
5-HT	6·12	5·68
2-Methyl-5-HT	6·25	5·46

In conclusion, our data provides the first direct evidence for the existence of 5-HT_3 recognition sites in human brain tissue.

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References

Barnes, N. M., Costall, B., Naylor, R. J. (1988a) [^{3}H]Zacopride: A ligand for the identification of 5-HT_3 recognition sites. J. Pharm. Pharmacol., 40: 548–551

Barnes, N. M., Costall, B., Naylor, R. J., Tattersall, F. D. (1988b) Identification of 5-HT_3 recognition sites in the ferret area postrema. Ibid. 40: 586–588

Bradley, P. B., Engel, G., Feniuk, W., Fozard, J. R., Humphrey, P. P. A., Middlemiss, D. N., Mylecharane, E. J., Richardson, B. P., Saxena, P. R. (1986) Proposals for the classification and nomenclature of functional receptors for 5-hydroxytryptamine. Neuropharmacology 25: 563–576

Smith, W. L., Sancillo, L. F., Owere-Atepo, J. B., Naylor, R. J., Lambert L. (1988) Zacopride: a potent 5-HT_3 antagonist. J. Pharm. Pharmacol. 40: 301–302

Watling, K. J. (1988) Radioligand binding studies identify 5-HT_3 recognition sites in neuroblastoma cell lines and mammalian CNS. T. Pharmacol. Sci., 9: 227–229

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