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# 5-$HT_{1D}$ binding sites in porcine brain can be sub-divided by GR43175

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We have examined the binding of 5-carboxamidotryptamine (5-CT) and GR43175 (3-(2-dimethylamino)ethyl-N-methyl-1H-indole-5-methane sulphonamide) to 5-$HT_{1D}$ sites labelled with [$^3$H]-5-hydroxytryptamine ([$^3$H]-5-HT) in neonatal porcine caudate membranes. In competition studies, 5-CT produced shallow inhibition curves ($K_i$ 138 nM, slope 0.31), indicating binding site heterogeneity, while GR43175 interacted with a single population of binding sites ($K_i$ 251 nM, slope 0.98), producing a maximum of only 52% inhibition of [$^3$H]-5-HT binding compared to 100% for 5-HT or 5-CT. In the presence of excess GR43175 (10 $\mu$M), 5-CT produced a monophasic inhibition curve with a $K_i$ value of 800 nM for the remaining sites (slope 0.89). These preliminary data suggest that under the conditions employed, GR43175, and to a lesser extent 5-CT, may discriminate between two sub-populations of 5-$HT_{1D}$ binding sites in porcine brain.

**Introduction** The advent of a new generation of ligands for studying 5-hydroxytryptamine (5-HT) receptors has led to a large increase in the number of putative receptors (5-$HT_1$-like, 5-$HT_2$, 5-$HT_3$) and binding sites (5-$HT_{1A}$, $_{1B}$, $_{1C}$, $_{1D}$, 5-$HT_2$, 5-$HT_3$) for 5-HT (Bradley *et al.*, 1986; Fozard, 1987). As part of an ongoing study aimed at characterizing the properties of the novel 5-$HT_1$-like receptor agonist, GR43175 (3-(2-dimethylamino)ethlyl-N-methyl-1H-indole-5-methane sulphonamide) (Humphrey *et al.*, 1988), we have examined the effect of this compound at 5-$HT_{1D}$ binding sites in membranes prepared from piglet brain. We now report that GR43175 appears to differentiate between two sub-populations of 5-$HT_{1D}$ binding sites in porcine caudate nuclei.

**Methods** Male piglets (8–12 days old, weighing 1.5–4.5 kg) of the Large White variety were killed by captive-bolt pistol and exsanguinated. The brains were removed and the caudate nuclei used to prepare a crude membrane fraction as described by Heuring & Peroutka (1987). Binding assays were performed in triplicate by incubation for 30 min at 37°C using 100 $\mu$l of membrane suspension (2 mg protein ml$^{-1}$) in a total volume of 250 $\mu$l in a Tris-HCl buffer (50 mM, pH 7.4) containing L-ascorbic acid (0.1%), calcium chloride (4 mM), pargyline (10 $\mu$M) and [$^3$H]-5-HT (10 nM) (New England Nuclear, sp. act. 29.7 Ci mmol$^{-1}$; purity consistently greater than 95% by t.l.c.) This buffer also contained 8-hydroxy-2-di-n-propylamino tetralin (8-hydroxy-DPAT, 100 nM) and mesulergine (100 nM) to prevent labelling of 5-$HT_{1A}$ and 5-$HT_{1C}$ binding sites, respectively (Heuring & Peroutka, 1987; Waeber *et al.*, 1988). Assays were terminated by rapid vacuum filtration through glass-fibre filters (Whatman, GFB), and the trapped radioactivity counted by liquid scintillation spectroscopy in 10 ml of Picofluor TM30 scintillation fluid (Packard). Specific [$^3$H]-5-HT binding was defined as that inhibited by 10 $\mu$M 5-HT, and represented 40–60% of total [$^3$H]-5-HT binding. Analyses of binding data (disintegrations per min) were performed using the programmes LIGAND (Munson & Rodbard, 1980) and ALLFIT (DeLean *et al.*, 1978). Results are given as mean ± s.e.mean of at least three separate determinations. $K_d$ and $K_i$ are the equilibrium dissociation constants for the radioligand and inhibitor respectively, and $B_{max}$ is the maximum binding capacity of the radioligand.

**Results** Under the conditions employed, [$^3$H]-5-HT appeared to label a single, homogeneous population of binding sites in piglet caudate nucleus, with a $K_d$ of 19 ± 4 nM, $B_{max}$ 0.36 ± 0.08 nM (corresponding to 158 ± 35 fmol mg$^{-1}$ protein) and Hill coefficient (slope) 0.98 ± 0.01. The following compounds displayed little or no affinity ($K_i$ values ⩾ 1000 nM) for these binding sites: 8-hydroxy-DPAT, mesulergine, spiperone, ketanserin, GR38032 and cyanopindolol. Unlabelled 5-HT produced a monophasic inhibition of specific [$^3$H]-5-HT binding (Figure 1), with a $K_i$ value of 16 ± 5 nM and slope 0.95 ± 0.15. In marked contrast, however, 5-carboxamidotryptamine (5-CT) clearly produced shallow inhibition curves (Figure 1), with an apparent $K_i$ of 138 ± 64 nM and slope 0.31 ± 0.24. Using the LIGAND programme, these curves could be fitted, albeit on the basis of limited data points, to a two-site model ($P < 0.01$ versus a one-site model)

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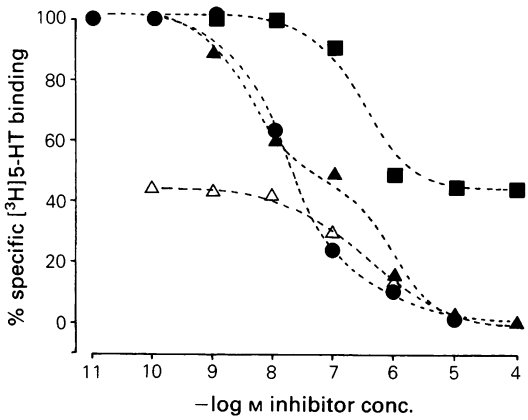


**Figure 1** Inhibition of specific [$^3$H]-5-hydroxytryptamine ([$^3$H]-5-HT) binding to porcine caudate membranes by 5-HT (●), 5-CT (▲), GR43175 (■) and by 5-CT in the presence of 10 $\mu$M GR43175 (△). Data are from at least three independent determinations and the curves were computer-fitted using the LIGAND programme.

described by $K_i$ values of $1.1 \pm 0.4$ nM and $2.5 \pm 1.7$ $\mu$M. The two sites represented $48 \pm 4\%$ and $52 \pm 4\%$ respectively of the total number of specific [$^3$H]-5-HT binding sites. GR43175 produced a monophasic inhibition of specific [$^3$H]-5-HT binding ($K_i$ $251 \pm 103$ nM, slope $0.98 \pm 0.17$), but these curves consistently plateaued at $52 \pm 8\%$ inhibition when compared to 100% for 5-HT or 5-CT (Figure 1). When a maximally effective concentration of GR43175 (10 $\mu$M) was included in the assay, specific [$^3$H]-5-HT binding was reduced by $50 \pm 7\%$. Under these conditions, 5-CT appeared to produce a monophasic inhibition (slope $0.89 \pm 0.20$) with a $K_i$ of $0.80 \pm 0.71$ $\mu$M.

**Discussion** The [$^3$H]-5-HT binding site examined in this preliminary study appeared to be of the 5-HT$_{1D}$ type on the basis of the conditions employed and the lack of affinity displayed by archetypal ligands for each of the other known 5-HT binding sites (Heuring & Peroutka, 1987). However, although 5-HT apparently recognised a single homogeneous population of binding sites, this did not appear to be the case for 5-CT, which produced shallow inhibition curves. Analyses of these curves suggested that 5-CT showed some 2000 fold selectivity for about 50% of the sites ($K_i$ 1.1 nM) over the remainder ($K_i$ 2500 nM). Clearly more extensive studies are needed to confirm these preliminary analyses which are based upon a limited number of data points, but nevertheless it would seem that 5-CT is binding to more than one population of sites. Notably, GR43175 appeared to inhibit the binding of [$^3$H]-5-HT to only one of these sites, with a Hill coefficient not significantly different from unity and maximum inhibition of binding of approximately 50%. Furthermore, in the presence of excess GR43175, the shallow inhibition curves (slope of 0.3) produced by 5-CT were converted to monophasic curves (slope of 0.89), yielding a $K_i$ value for 5-CT of 800 nM. It is therefore tempting, on the basis of these early data, to suggest that neonatal porcine caudate '5-HT$_{1D}$' binding sites are comprised of two subpopulations, namely one that recognises GR43175 and has a high affinity for 5-CT, and another that shows little affinity for either 5-CT or GR43175. It remains to be seen if the same is true in the brains of other mammalian species. If so the 5-HT$_{1D}$ site as first described may actually comprise more than one population of binding sites, an observation that has previously been made (Waeber *et al.*, 1988). One might further speculate that the high affinity site for 5-CT and GR43175 may be equivalent to the 5-HT$_1$-like receptor in the dog saphenous vein (Humphrey *et al.*, 1988) while the site showing a low affinity for 5-CT could correlate with the 5-HT receptor found on vascular endothelial cells (Leff *et al.*, 1987; Sumner & Humphrey, 1988).

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