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SHORT COMMUNICATIONS

[^{3}H]-BRL 43694 (Granisetron), a specific ligand for 5-HT$_3$ binding sites in rat brain cortical membranes

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Functional neuronal 5-hydroxytryptamine$_3$ (5-HT$_3$) receptors have been well characterised in the periphery [1] and recently a number of radioligands have been reported which label 5-HT$_3$ binding sites in central nervous tissue. For instance, [^{3}H]-GR 65630 [2] and [^{3}H]-quipazine [3] label 5-HT$_3$ binding sites in rat cortical membranes, whilst [^{3}H]-ICS 205-930 labels 5-HT$_3$-like binding sites in neuroblastoma–glioma cell cultures [4]. Recently, BRL 43694 (granisetron) has been reported to be a potent and selective 5-HT$_3$ receptor antagonist within the peripheral nervous system, with little or no affinity for 5-HT$_1$-like, 5-HT$_2$, dopamine$_2$, benzodiazepine, α_1, α_2 and β receptors in rat brain tissue [5]. We now report on the characterisation of [^{3}H]-BRL 43694 (see Fig. 1) binding sites in rat cortical membranes.

Materials and methods

For the radioligand binding studies, whole cerebral cortical tissue was obtained from male Hooded Lister rats (150–200 g) and homogenised (Teflon–glass homogeniser) in 10 volumes of ice-cold HEPES buffer (50 mM, pH 7.5). The homogenate was washed 3 times by centrifugation (50,000 *g* for 10 min) and the final pellet suspended in 10 volumes of HEPES buffer and stored on ice until required. Fresh tissue preparations were used throughout for these studies. For the inhibition studies, binding assays consisted of 0.2 ml of [^{3}H]-BRL 43694 (0.5 nM, specific activity 21Ci/mmol; synthesised by Beecham Pharmaceuticals Research Division), 0.2 ml of displacing drug and 0.2 ml of tissue in a total volume of 2ml made up with HEPES buffer. The incubation (at 23° for 30 min) was terminated by filtering rapidly through GF/B filters (presoaked for 30 min in 0.1% polyethyleneimine) using a Millipore filtration system. The filters were rinsed immediately with 3 × 5ml of ice-cold HEPES buffer and radioactivity measured by liquid scintillation spectrometry. Specific binding was defined as the excess over blanks in the presence of unlabelled GR 38032F (1 μM). For saturation studies, 11–12 triplicate concentrations of [^{3}H]-BRL 43694 (0.1–4 nM) were used. The association rate was determined by incubating [^{3}H] BRL 43694 (1 nM) with cortical membranes at 23° in the absence and presence of GR 38032F (1 μM) for various time periods from 0 to 60 min. The results were expressed as specific binding (cpm/20 mg tissue) and plotted against time. In order to calculate the dissociation rate, cortical membranes were incubated with [^{3}H] BRL 43694 (1 nM) at 23° for 30 min. At this time point GR 38032F (1 μM) was added and at various times after this (up to 45 min) specific [^{3}H] BRL 43694 binding determined. The $t_{\frac{1}{2}}$ times and rate constants were estimated from semilogarithmic transformation of the original data.

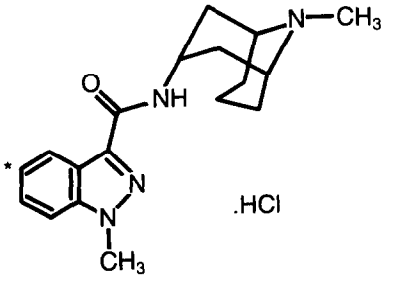

Fig. 1. The structure of BRL 43694 is (*endo-N*-(9-methyl-9-azabicyclo [3.3.1] non-3-yl)-1-methyl-1H-indazole-3-carboxamide hydrochloride). The position of the tritium is denoted by the asterisk.

For the Bezold–Jarisch reflex studies, anaesthetised rats were prepared as described previously [6]. The Bezold-Jarisch reflex was evoked by a rapid, bolus intravenous injection of 5-HT, using the minimum dose which evoked a clear bradycardia, usually 15 μg/kg, and the resultant fall in heart rate measured. 5-HT injections were administered every 12 min and dose–response curves for BRL 43694, or the other drugs tested, were established by injecting increasing doses of drug 5 min before each injection with 5-HT. The ID_{50}'s obtained are the mean of at least three separate experiments.

Results

Scatchard analysis of [^{3}H]-BRL 43694 binding to rat cortex membranes revealed a single saturable binding site of high affinity (K_d = 0.30 nM ± 0.06 nM; B_{max} = 1.29 ± 0.02 fmol/mg wet wt tissue; mean ± SE, N = 3). A typical saturation plot and Scatchard transformation is shown in Fig. 2a and b. At 0.5 nM [^{3}H]-BRL 43694, specific binding (defined in the presence of GR 38032F, 1 μM) represented 66 ± 4% (± SD) of total binding. This specific binding was linear over a range of tissue concentrations, up to 20 mg tissue per assay tube. Kinetic analysis of this binding demonstrated that [^{3}H]-BRL 43694 associates and dissociates rapidly (see Figs 3 and 4). Fifty percent association occurred after 1.4 min ($K_1 = 1.67 \times 10^8$ M^{-1} min^{-1}) and after complete association, GR 38032F (10^{-6} M) displaced 50% of specific binding in 5.5 min (K_2 = 0.045 min^{-1}). The kinetically derived K_d (K_2/K_1) was 0.27 nM.

Drug competition studies (see Table 1) show that known 5-HT$_3$ receptor antagonists, i.e. GR 38032F, ICS 205–930, zacopride, quipazine, MDL 72222, BRL 43694 and BRL 24682 all displaced potently (low nanomolar concentrations) specific [^{3}H]-BRL 43694 binding. Hill analysis of the competition curves for the antagonists revealed slopes close to unity. Metoclopramide (K_i = 160 nM) was a moderate inhibitor of [^{3}H]-BRL 43694 binding. The agonists 5-HT (K_i = 160 nM) and 2-methyl-5-HT (K_i = 620 nM) also inhibited specific binding. Drugs known to interact with other 5-HT receptor sub-types, e.g. ritanserin (K_i = 5300 nM), ICI 169,369 (K_i = 990 nM), spiperone (K_i > 10,000 nM), metergoline (K_i = 7400 nM), methysergide (K_i > 10,000 nM), methiothepin (K_i = 3000 nM) and 8-OH-DPAT (K_i > 10,000 nM) all displaced only weakly. Drugs acting at other neurotransmitter receptors were inactive in the [^{3}H]-BRL 43694 binding system (e.g. prazosin, yohimbine, idazoxan, alprenolol, cyanopindolol, noradrenaline, diazepam, dopamine, sulpiride and GABA) or displaced only weakly (e.g. mepyramine and domperidone).

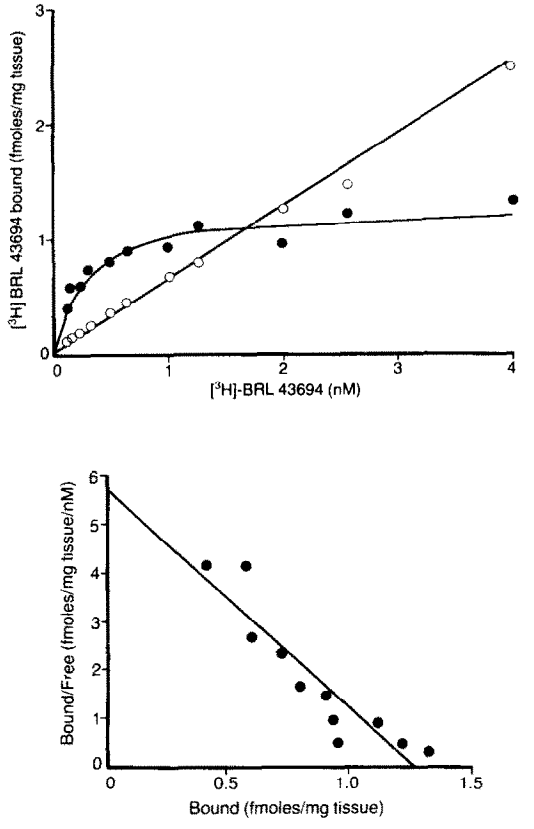

Fig. 2. Plot A shows the saturation curve for the binding of [^{3}H]-BRL 43694 (0.1–4 nM) to rat cortical membranes from one typical experiment. Specific binding (●) was obtained by the difference between total [^{3}H]-BRL 43694 binding and non-specific binding (○), as defined in the presence of GR 38032F, 1 μM. Plot B shows the Scatchard transformation of these data to reveal a K_d value of 0.22 nM and a B_{max} value of 1.26 fmol/mg tissue.

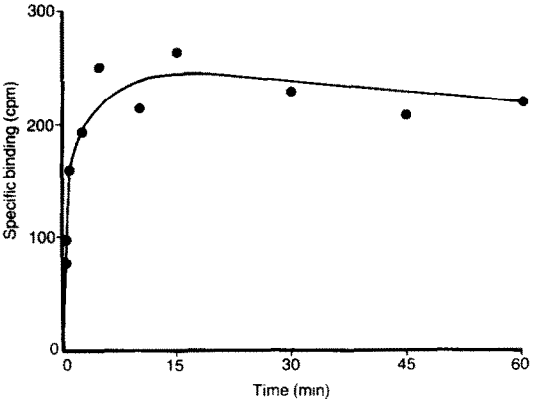

Fig. 3. The time course for the association of [^{3}H]-BRL 43694 (1 nM) to rat cortical membranes at 23°. Specific binding, as defined by GR 38032F (1 μM), is expressed as the cpm/20 mg tissue and is plotted against incubation time (min). The association rate constant (K_1) was estimated to be $1.67 \times 10^8\ M^{-1}\ min^{-1}$.

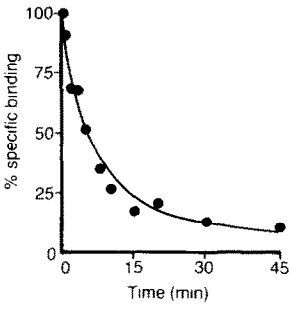

Fig. 4. The time course for the dissociation of [^{3}H]-BRL 43694 binding from rat cortical membranes following incubation with [^{3}H]-BRL 43694 (1 nM) at 23° for 30 min. GR 38032F (1 μM) was added at time zero and aliquots filtered at the times indicated and specific binding determined. The dissociation rate constant was estimated to be 0.045 min^{-1}.

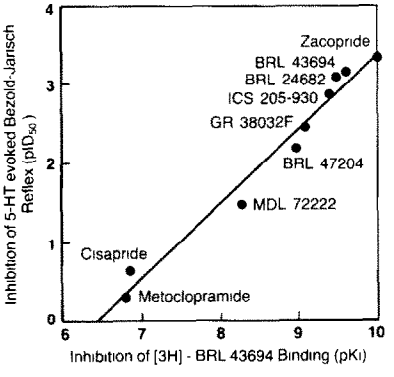

Fig. 5. Correlation of compound affinities for [^{3}H]-BRL 43694 (0.5 nM) binding sites in rat cortex membranes and their ability to inhibit the 5-HT-induced Bezold–Jarisch reflex in the rat. By regression analysis the correlation coefficient is 0.98. pK_i values, the negative log of the inhibitory constant (M), were derived from Table 1 and the inhibition of the 5-HT-induced Bezold–Jarisch reflex in the rat is expressed as the pID_{50}, the negative log of the ID_{50} (mg/kg i.v.).

For those 5-HT_3 receptor antagonists studied, a direct correlation ($r = 0.98$, slope = 0.95; Fig. 5) was observed between the inhibition of [^{3}H]-BRL 43694 binding to rat cortex membranes *in vitro* expressed as the pK_i, (the negative log of the inhibitory constant in molar), and their inhibition of the 5-HT-evoked Bezold–Jarisch reflex expressed as the pID_{50}, (the negative log of the ID_{50} in mg/kg i.v. in the anaesthetised rat).

Discussion

The present results demonstrate that [^{3}H]-BRL 43694 labels potently (K_d 0.30 nM) 5-HT_3 recognition sites in rat cortical membranes but that the number of binding sites is quite low (B_{max} = 1.29 fmol/mg tissue) compared, for example, with central 5-HT_2 binding sites (e.g. B_{max} = 30.9 fmol/mg tissue; [7]). However, the level of binding determined with [^{3}H]-BRL 43694 is similar to that defined with, for example, [^{3}H]-quipazine [3]. Scatchard analysis revealed that [^{3}H]-BRL 43694 binds to a single population of sites which correspond presumably to the high affinity binding sites labelled by [^{3}H]-GR 65630 [2] and [^{3}H]-quipazine [3].

Table 1. The effect of a number of drugs on specific [^{3}H]-BRL 43694 binding to rat cortical membranes

Drug	Mean K_i (nM)
Zacopride	0.10 ± 0.01
BRL 43694	0.26 ± 0.04
BRL 24682	0.33 ± 0.07
ICS 205-930	0.40 ± 0.06
GR 38032F	0.87 ± 0.12
BRL 47204	1.07 ± 0.13
Quipazine	1.23 ± 0.15
MDL 72222	5.3 ± 0.4
Mianserin	5.8 ± 0.9
Cisapride	134 ± 13
5-HT	160 ± 75
Metoclopramide	160 ± 35
2-Methyl-5-HT	620 ± 73
ICI 169, 369	990 ± 170
Methiothepin	3000 ± 810
Ritanserin	5300 ± 1100
Metergoline	7400 ± 1700
8-OH-DPAT	>10,000
Buspirone	>10,000
Methysergide	>10,000
Spiperone	>10,000
Mepyramine	2400 ± 190
Domperidone	3900 ± 1700
Noradrenaline	>10,000
Prazosin	>10,000
Yohimbine	>10,000
Idazoxan	>10,000
Alprenolol	>10,000
Cyanopindolol	>10,000
Dopamine	>10,000
Sulpiride	>10,000
GABA	>10,000
Diazepam	>10,000

Radioligand binding studies were performed as described in the Methods section. IC_{50} values were determined from inhibition curves using five duplicate concentrations. Apparent inhibition constants (K_i's), assuming competitive inhibition, were determined using the Cheng–Prusoff equation ($K_i = IC_{50}/(1 + c/K_d)$) where c = concentration of the radioligand and K_d = affinity constant. Data given are the mean ± SE of three separate experiments except for those K_i values > 10,000 nM which are the mean of two separate experiments. For the structure of BRL 24682 see [5]. BRL 47204, a 5-HT$_3$ antagonist, is 1 - methyl - *N* - (*endo* - 9 - methyl - 9 - azabicyclo - [3,2,1]non - 3 - yl)indol-3-yl carboxylic acid amide.

Inhibition studies have shown that specific [^{3}H]-BRL 43694 binding is displaced by compounds known to interact with 5-HT$_3$ receptors, whilst drugs binding to other 5-HT receptor sub-types, as well as other receptor compound types, only displaced weakly [^{3}H]-BRL 43694 binding. Mianserin (K_i = 5.8 nM) is a potent inhibitor of [^{3}H]-BRL 43694 binding, but this is unlikely to be a consequence of its affinity for 5-HT$_{1C}$ receptors [8] since the potent 5-HT$_{1C}$ antagonist mesulergine inhibited only weakly (K_i > 10,000 nM) [^{3}H]-quipazine binding [3]. Moreover, methysergide, a compound possessing strong affinity for 5-HT$_{1C}$ receptors [9], was very weak at displacing [^{3}H]-BRL 43694 binding (K_i > 10,000 nM). The data indicate, therefore, a strong affinity of mianserin for 5-HT$_3$ recognition sites. It is interesting that Hoyer and Neigt [4] reported mianserin (K_i = 56 nM) to be a moderate inhibitor of [^{3}H]-ICS 205-930 binding in neuroblastoma cells.

Indirect evidence to suggest that [^{3}H]-BRL 43694 binding sites in rat cortex could represent functional receptors is provided by the good correlation (r = 0.98) between the inhibition of [^{3}H]-BRL 43694 binding to central tissue and the inhibition of the 5-HT-evoked Bezold–Jarisch reflex in the rat (a functional assessment of peripheral 5-HT$_3$ receptor antagonism) for those compounds tested so far in both systems.

In conclusion, [^{3}H]-BRL 43694 was shown to bind potently, selectively and reversibly to a single population of recognition sites in rat brain cortical membranes. Drug inhibition studies demonstrated that specific [^{3}H]-BRL 43694 binding was displaced by 5-HT$_3$ receptor antagonists and agonists but was only inhibited weakly by other classes of drugs. A good correlation (r = 0.98) was observed between the inhibition by 5-HT$_3$ antagonists of central [^{3}H]-BRL 43694 radioligand binding and the inhibition of 5-HT-evoked Bezold–Jarisch reflex in the anaesthetised rat. It is concluded that [^{3}H]-BRL 43694 binds to 5-HT$_3$ recognition sites in rat cortex and should provide a valuable aid to biochemical and pharmacological studies designed to characterise further 5-HT$_3$ receptors in the central nervous system.

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