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The $5HT_{1A}$ receptor ligand, S15535, antagonises G-protein activation: a [^{35}S]GTPγS and [^{3}H]S15535 autoradiography study

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Abstract

4-(Benzodioxan-5-yl)1-(indan-2-yl)piperazine (S15535) is a highly selective ligand at $5\text{-}HT_{1A}$ receptors. The present study compared its autoradiographic labelling of rat brain sections with its functional actions, visualised by guanylyl-5′-[γ-thio]-triphosphate ([^{35}S]GTPγS) autoradiography, which affords a measure of G-protein activation.

[^{3}H]S15535 binding was highest in hippocampus, frontal cortex, entorhinal cortex, lateral septum, interpeduncular nucleus and dorsal raphe, consistent with specific labelling of $5\text{-}HT_{1A}$ receptors. In functional studies, S15535 (10 μM) did not markedly stimulate G-protein activation in any brain region, but abolished the activation induced by the selective $5\text{-}HT_{1A}$ agonist, (+)-8-hydroxy-dipropyl-aminotetralin ((+)-8-OH-DPAT, 1 μM), in structures enriched in [^{3}H]S15535 labelling. S15535 did not block 5-HT-stimulated activation in caudate nucleus or substantia nigra, regions where (+)-8-OH-DPAT was ineffective and [^{3}H]S15535 binding was absent. Interestingly, S15535 attenuated (+)-8-OH-DPAT and 5-HT-stimulated G-protein activation in dorsal raphe, a region in which S15535 is known to exhibit agonist properties in vivo [Lejeune, F., Millan, M.J., 1998. Induction of burst firing in ventral tegmental area dopaminergic neurons by activation of serotonin $(5\text{-}HT)_{1A}$ receptors: WAY100,635-reversible actions of the highly selective ligands, flesinoxan and S15535. Synapse 30, 172–180.].

The present data show that (i) [^{3}H]S15535 labels pre- and post-synaptic populations of $5\text{-}HT_{1A}$ sites in rat brain sections, (ii) S15535 exhibits antagonist properties at post-synaptic $5\text{-}HT_{1A}$ receptors in corticolimbic regions, and (iii) S15535 also attenuates agonist-stimulated G-protein activation at raphe-localised $5\text{-}HT_{1A}$ receptors. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: S15535; (+)-8-Hydroxy-dipropyl-aminotetralin; [^{35}S]GTPγS; Autoradiography; $5\text{-}HT_{1A}$ receptor; G-protein

1. Introduction

$5\text{-}HT_{1A}$ receptors play a major role in the control of mood and cognition, both pre- and/or post-synaptic populations of $5\text{-}HT_{1A}$ receptors being targets for the treatment of anxiety, depression and other affective disorders (see Coplan et al., 1992; De Vry, 1995; Steckler and Sahgal, 1995). The characterisation of $5\text{-}HT_{1A}$ sites has progressed in parallel with the discovery of novel and potent ligands (see Boess and Martin, 1994 for review). However, while 8-hydroxy-dipropyl-aminotetralin (8-OH-DPAT) remains the prototypical and selective $5\text{-}HT_{1A}$ receptor agonist, comparatively few potent and highly selective antagonists have been proposed. These include *N*-{2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl}-*N*-(2-pyridinyl)-cyclohexane-carboxamide (WAY100,635), 4-(2′-methoxyphenyl)-1-[2′-(2″-pyridinyl)-*p*-iodobenzamido]-ethyl]-piperazine (*p*-MPPI), robalzotan (NAD299) and 4-(benzodioxan-5-yl)1-(indan-2-yl)piperazine (S15535) (Millan et al., 1994; Kung et al., 1995; Fletcher et al., 1996; Jerning et al., 1998). S15535 is of particular interest since it is active in preclinical tests of anxiolytic activity (Millan et al., 1997) and is one of the most potent (K_i = 0.6 nM) and selective $5\text{-}HT_{1A}$ receptor ligands identified to date (Newman-Tancredi et al., 1998). In tests of receptor-mediated G-protein activation, determined by [^{35}S]GTPγS binding, S15535 antagonises the actions of $5\text{-}HT_{1A}$ receptor agonists at recombinant human $5\text{-}HT_{1A}$ receptors in Chinese hamster ovary (CHO) cells, while itself exhibiting weak partial

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agonist activity (~30% efficacy relative to 5-HT; Newman-Tancredi et al., 1996). In vivo, S15535 inhibits flat body posture and forepaw treading induced in rats by 8-OH-DPAT (Millan et al., 1994), suggesting that it acts as an antagonist at post-synaptic 5-HT_{1A} receptors. In contrast, in electrophysiological and turnover studies, S15535 completely inhibited the firing of raphe neurons and diminished extracellular 5-hydroxytryptophan (5-HTP) levels, in the same manner as 8-OH-DPAT (Gobert et al., 1995; Lejeune et al., 1997). S15535 displays, therefore, a mixed post-synaptic antagonist, presynaptic agonist profile of activity in in vivo models of 5-HT_{1A} receptor activation. However, the efficacy of S15535 at native 5-HT_{1A} receptors and, in particular, the functional activity of S15535 at post-synaptic 5-HT_{1A} receptors in corticolimbic brain regions, have not yet been investigated at the molecular level.

The present study investigated these issues by a dual approach. First, the localisation of S15535 binding sites in rat brain was investigated using [^{3}H]S15535. The utility of [^{3}H]S15535 has been shown for selective labelling of 5-HT_{1A} receptors in membranes prepared from rat hippocampus and CHO-h5-HT_{1A} cells (Newman-Tancredi et al., 1998). Second, the influence of S15535 on G-protein activation in different postsynaptic (cortical, hippocampal and septal) and presynaptic (dorsal raphe) structures was investigated by [^{35}S]GTPγS autoradiography. The latter technique has been successfully used to show the differential activation of G-proteins in rat brain by opioid and cannabinoid receptors (Sim et al., 1995, 1996). The activation of G-proteins by guinea pig and rat 5-HT_{1A} and 5-HT_{1B} receptors can also be investigated by this method (Sim et al., 1997; Waeber and Moskowitz, 1997; Pauwels et al., 1998). The present study sought to define the actions of S15535, in comparison with two agonists, (+)-8-OH-DPAT and 5-HT, on [^{35}S]GTPγS binding in different rat brain regions. The availability of both [^{3}H]S15535 and [^{35}S]GTPγS autoradiography enabled the parallel mapping of both the receptor labelling and functional activity of S15535 at 5-HT_{1A} receptors.

2. Methods

2.1. Preparation of slide-mounted sections

Male Wistar rats (200–240 g) were sacrificed by decapitation, their brains rapidly removed and frozen by immersion into isopentane (−40°C on dry ice). Brains were stored at −80°C until their use. Serial coronal sections (20 μm thickness, three to four per slide) were cut using a cryostat (Leitz Kryostat 1720 Digital) with the chamber temperature set at −12°C to −18°C. Sections were thaw-mounted onto poly-L-lysine-coated microscope slides (Sigma, S. Quentin Fallavier, France) and stored sealed at −20°C.

2.2. [^{3}H]S15535 autoradiography

Labelling of 5-HT_{1A} receptors in vitro with [^{3}H]S15535 (50 Ci/mmol; Newman-Tancredi et al., 1998) was performed as follows: slides were removed from the freezer and allowed to reach room temperature (1 1/2 h). Slides were preincubated at 22°C for 30 min in Tris–HCl (50 mM) pH 7.5, with 2 mM $CaCl_2$, 10 μM pargyline, 0.01% ascorbic acid. Sections were then incubated for 2 h in the same buffer with [^{3}H]S15535 (2.0 nM). Non-specific binding was defined in adjacent sections in the presence of WAY100,635 (10 μM). Following incubation, sections were washed two times for 5 min with ice-cold buffer and then dipped once into ice-cold deionized distilled water. The slides were dessicated under air flow and placed in X-ray cassettes apposed to tritium-sensitive film Hyperfilm-^{3}H (Amersham, Les Ulis, France). The films were exposed for 8 to 9 weeks at −80°C. Following this exposure period, the cassettes were allowed to reach room temperature and developed with Kodak LX24 solution, development was stopped with in 1% glacial acetic acid and the film fixed with Kodak AL4 solution. Calibration of binding densities was carried out using [^{3}H] microscales (Amersham).

2.3. [^{35}S]GTPγS autoradiography

Labelling of rat brain sections with [^{35}S]GTPγS was carried out essentially as described by Sim et al. (1995) with minor modifications. Slides were removed from the freezer and allowed to reach room temperature (2 h). In preliminary experiments (not shown), thorough prewashing of brain sections was found to reduce the [^{35}S]GTPγS binding observed in the absence of added ligands, presumably by removing endogenous agonist(s) and reducing receptor pre-coupling. The procedure was as follows: slides were prewashed three times for 15 min at room temperature: (i) in buffer A (HEPES 50 mM, pH 7.5, NaCl 150 mM, ethylene glycol-bis(β-aminoethyl ether) (EGTA), 0.2 mM, dithiothreitol, 0.2 mM) plus GTP 2.5 mM, (ii) in buffer B (= buffer A plus GDP 2.5 mM), and (iii) in buffer C (= buffer A plus GDP 2.5 mM and $MgCl_2$ 10 mM). The slides were then incubated for 60 min at 37°C in buffer C plus [^{35}S]GTPγS (0.05 nM) and agonist/antagonist ligands. Following incubation, sections were washed two times for 2 min with ice-cold buffer C and then dipped once into ice-cold deionized distilled water. The slides were dessicated under airflow and placed in X-ray cassettes apposed to [^{35}S]sensitive film (Hyperfilm β-max, Amersham). Calibration of binding densities was carried out using [^{14}C] standard microscales (Amersham). The films were exposed for 4 1/2 days at −20°C. Following exposure, the cassettes were allowed to reach room temperature and films developed as described above.

2.4. Autoradiographic quantification

All autoradiograms were analysed by computerised densitometry using a Leica Q600 image analyser. Images of specific binding were produced by subtraction of two aligned images from adjacent (total and non-specific binding) brain sections. Anatomical nomenclature used in describing the distribution of binding sites and structures was by reference to the atlas of Paxinos and Watson (1998). Results are expressed as means ± s.e.m. of average measurements from three different rats. For [^{3}H]S15535 autoradiography, data are expressed as femtomoles of [^{3}H] per milligram of tissue equivalent, according to Amersham calibration scales. For [^{35}S]GTPγS autoradiography, data are expressed as the percentage increase in [^{35}S]GTPγS binding induced by drug treatment relative to that observed under basal (non-drug-treated) conditions.

2.5. Materials

Drugs: (+)-8-OH-DPAT hydrobromide was obtained from RBI (Natick, MA); 5-HT creatinine sulphate was obtained from Sigma; S15535 base and WAY100,635 fumarate were synthesised by J.L. Peglion, Servier. The drug concentrations tested were: 1 μM for (+)-8-OH-DPAT and 10 μM for 5-HT and S15535.

3. Results

3.1. [^{3}H]S15535 autoradiography

[^{3}H]S15535 (2 nM) bound to rat brain sections at the densities shown in Table 1. Specific binding to 5-HT$_{1A}$

Table 1
Autoradiographic labelling of rat brain sections with [^{3}H]S15535
Coronal rat brain sections were incubated with [^{3}H]S15535. Non-specific binding was defined with WAY100,635 (10 μM). Sections were exposed to film for 9 weeks and autoradiographic images analysed by computerised densitometry. Binding calibration was carried out using [^{3}H] standard microscales (Amersham). The mean amount of [^{3}H]S15535 binding observed, expressed as fmol per mg of tissue equivalent, was used to calculate an estimated B_{max} (maximal binding capacity) value, based on a correction for receptor occupancy (see Section 2). Values are means ± s.e.m. of three independent experiments carried out on brains from three different animals. Percentage specific binding is shown in brackets.

Brain region	Specific binding (fmol mg^{-1})	Estimated B_{max} (fmol mg^{-1})	Specific binding (% of total)
Hippocampus			
CA1	111.9 ± 9.5	246	81.0 ± 1.4
CA2	42.6 ± 2.6	94	59.0 ± 1.5
CA3	60.8 ± 2.1	134	63.9 ± 4.0
Dentate gyrus	136.7 ± 17.8	300	80.4 ± 3.4
Cortex			
Entorhinal	82.3 ± 17.8	181	75.6 ± 8.4
Cingulate	50.3 ± 11.5	110	63.1 ± 4.8
Piriform	44.1 ± 5.4	97	58.1 ± 2.9
Frontal	33.4 ± 9.0	73	51.6 ± 10.2
Occipital	30.8 ± 5.5	68	55.5 ± 6.8
Temporal	28.4 ± 2.2	62	56.9 ± 4.3
Parietal	19.9 ± 1.1	44	40.5 ± 2.0
Insular	17.5 ± 3.0	38	40.3 ± 8.9
Midbrain			
Interpeduncular nucleus	79.0 ± 15.1	173	73.9 ± 7.6
Dorsal raphe	61.1 ± 28.0	134	57.6 ± 8.3
Superior colliculus[a]	25.7 ± 4.3	56	47.7 ± 6.0
Central Grey	16.1 ± 6.8	35	26.0 ± 7.6
Substantia nigra	7.1 ± 2.1	16	21.8 ± 7.6
Other			
Lateral septum	77.7 ± 8.7	171	72.4 ± 4.4
Tenia tecta	71.3 ± 7.8	157	75.6 ± 6.0
Ventromedial hypothalamus	47.0 ± 6.0	103	58.8 ± 3.6
Amygdala	42.5 ± 3.6	93	58.2 ± 1.3
Claustrum	35.3 ± 2.3	78	53.1 ± 1.0
Zona incerta	32.1 ± 5.5	71	48.7 ± 7.3
Endopiriform nucleus	30.2 ± 3.5	66	49.9 ± 4.1
Nucleus accumbens	3.2 ± 1.3	7	13.5 ± 7.5
Anterior commissure	3.1 ± 1.0	7	15.0 ± 2.0
Corpus callosum	1.7 ± 2.2	4	9.3 ± 11.3
Caudate nucleus	−0.4 ± 1.8	−1	−0.5 ± 7.4

[a] Superficial grey layer.

receptors, defined with the selective 5-HT_{1A} receptor antagonist, WAY100,635, was highest in hippocampus (particularly in the dentate gyrus), lateral septum, interpeduncular nucleus, entorhinal cortex and dorsal raphe (Figs. 1–3). Marked [^{3}H]S15535 binding was also observed in cingulate cortex, piriform cortex and amygdala. Specific

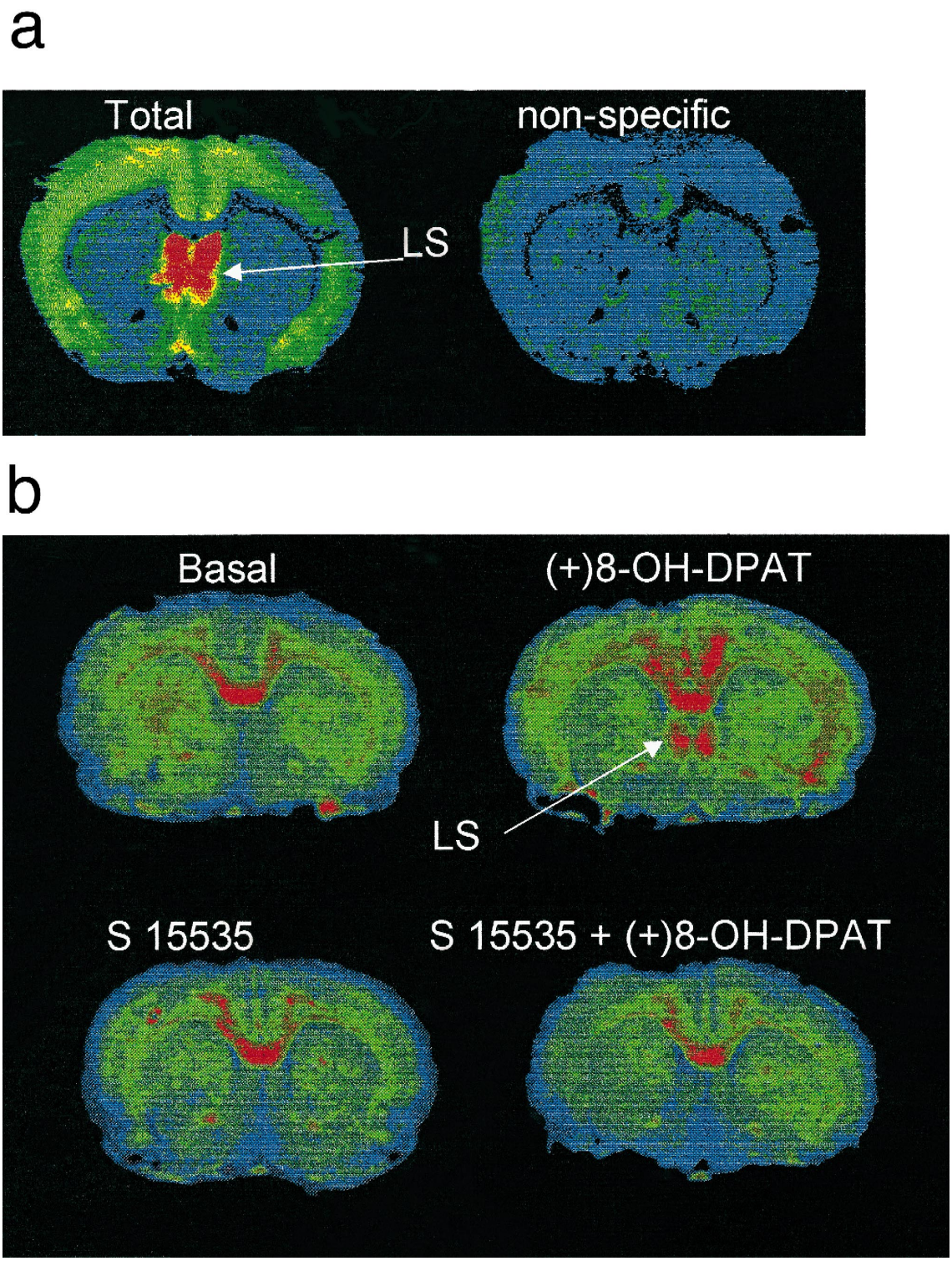

Fig. 1. (a) Pseudocolour autoradiographic localisation of specific [^{3}H]S15535 (2 nM) binding sites in septal regions of rat brain coronal sections. Non-specific binding was defined with WAY100,635 (10 μM). (b) Pseudocolour autoradiographic localisation of [^{35}S]GTPγS binding sites in septal regions of rat brain coronal sections under basal conditions (no drug treatment) or incubated with S15535 (10 μM), (+)-8-OH-DPAT (1 μM) or S15535 (10 μM) and (+)-8-OH-DPAT (1 μM) together. LS: lateral septum.

binding was low or absent in the caudate nucleus, substantia nigra and in the corpus callosum. An estimate of the maximal binding capacity of [^{3}H]S15535 to different tissues was derived by correcting for receptor occupancy

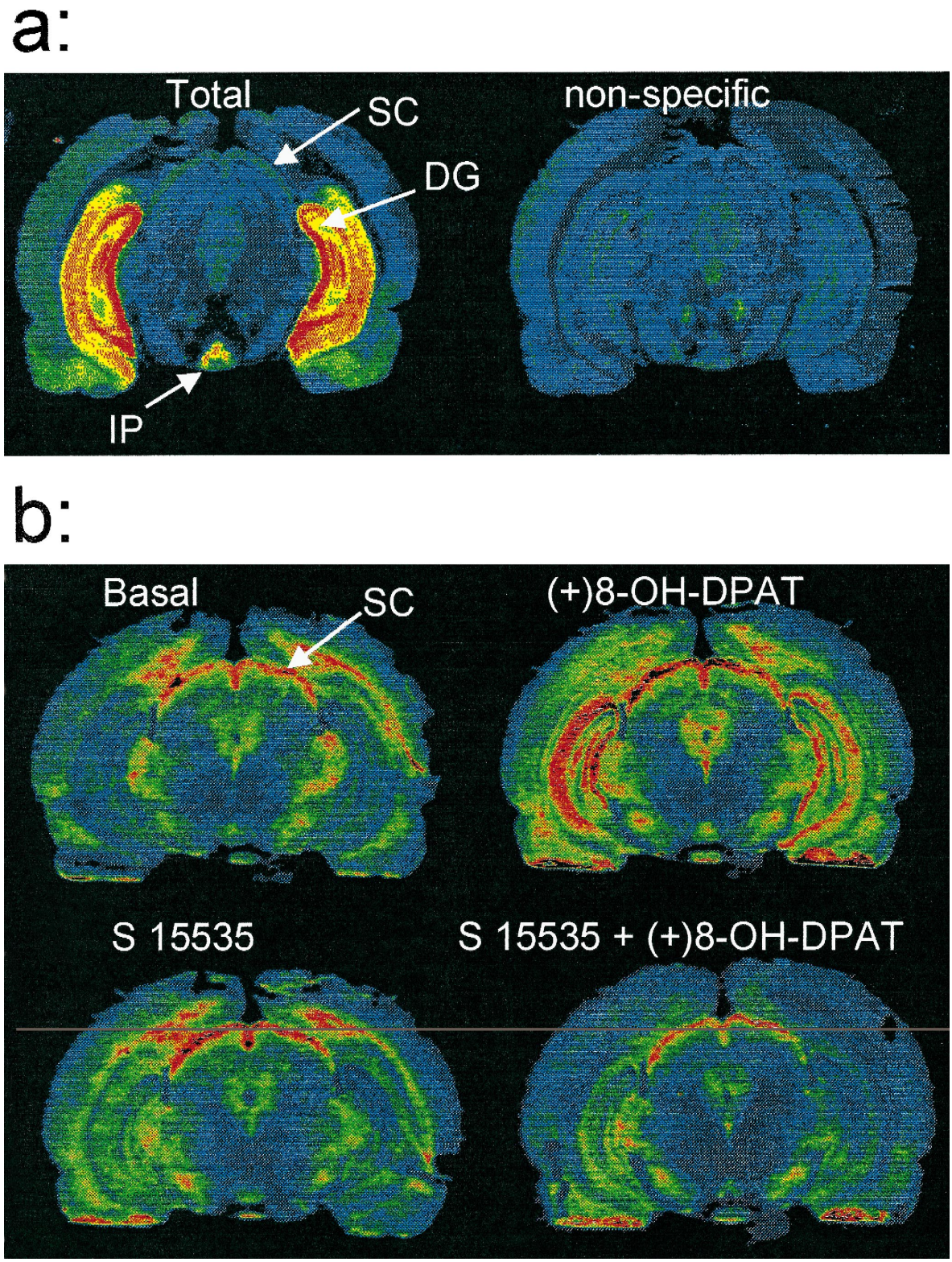

Fig. 2. (a) Pseudocolour autoradiographic localisation of specific [^{3}H]S15535 (2 nM) binding sites in hippocampal regions of rat brain coronal sections. Non-specific binding was defined with WAY100,635 (10 μM). (b) Pseudocolour autoradiographic localisation of [^{35}S]GTPγS binding sites in hippocampal regions of rat brain sections under basal conditions (no drug treatment) or incubated with S15535 (10 μM), (+)-8-OH-DPAT (1 μM) or S15535 (10 μM) and (+)-8-OH-DPAT (1 μM) together. DG: dentate gyrus; IP: interpeduncular nucleus; SC: superior colliculus.

$O = L/(L + K_D)$; where L is the concentration of [^{3}H]S15535 (2 nM) and K_D is the dissociation constant of [^{3}H]S15535 at rat brain 5-HT$_{1A}$ receptors (2.39 nM; Newman-Tancredi et al., 1998). The resulting B_{max} values bore

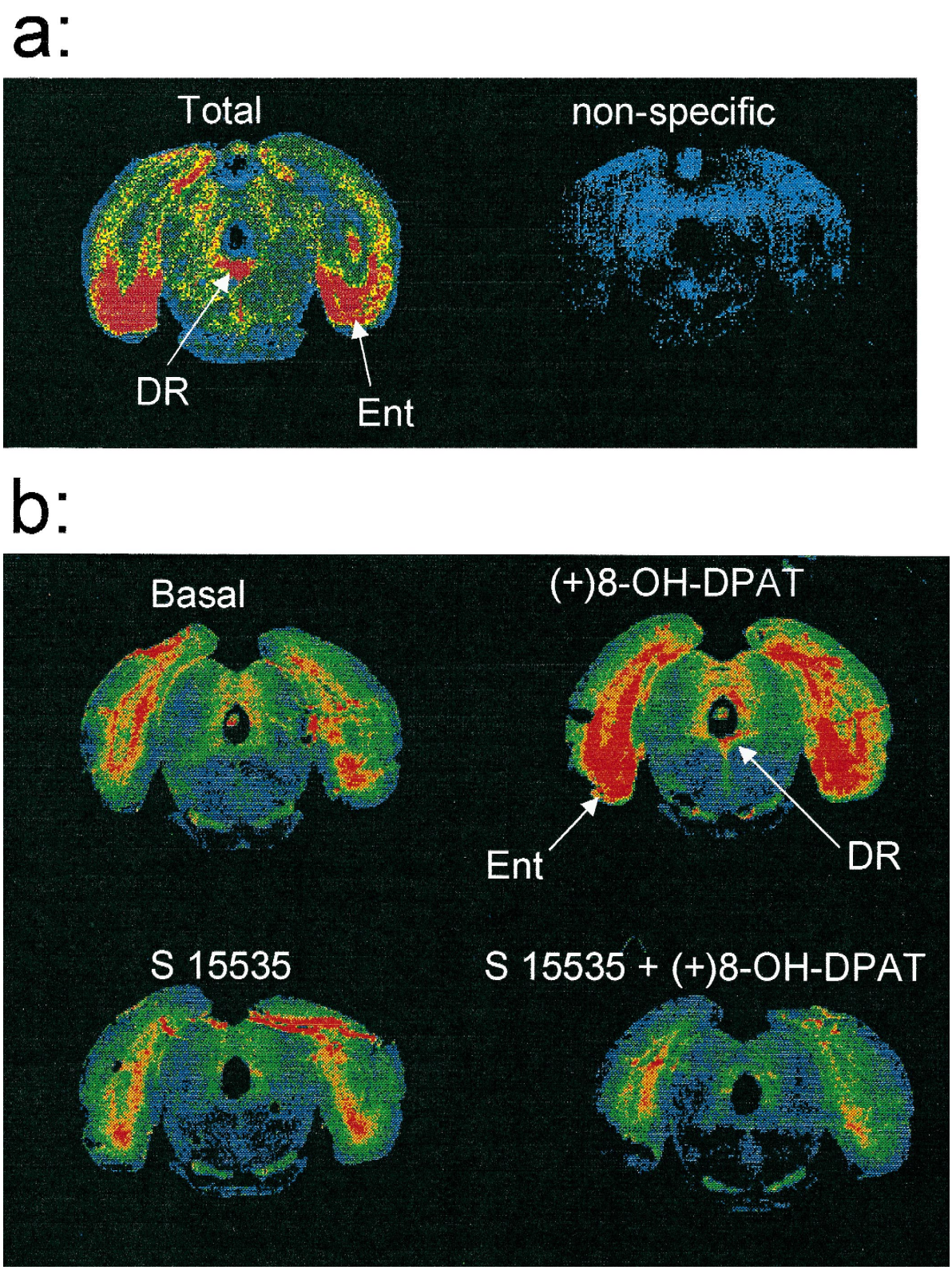

Fig. 3. (a) Pseudocolour autoradiographic localisation of specific [^{3}H]S15535 (2 nM) binding sites in midbrain rat brain coronal sections. Non-specific binding was defined with WAY100,635 (10 μM). (b) Pseudocolour autoradiographic localisation of [^{35}S]GTPγS binding sites in midbrain rat brain coronal sections under basal conditions (no drug treatment) or incubated with S15535 (10 μM), (+)-8-OH-DPAT (1 μM) or S15535 (10 μM) and (+)-8-OH-DPAT (1 μM) together. DR: dorsal raphe; Ent: entorhinal cortex.

Table 2
Binding of [^{35}S]GTPγS to rat brain sections in the presence of serotonergic ligands
Rat brain coronal sections (20 μm) were incubated with [^{35}S]GTPγS and (+)-8-OH-DPAT, 5-HT and/or S15535. Sections were exposed to film and autoradiographic images analysed by densitometry. Calibration was carried out using [^{14}C] standard microscales. Drug concentrations were as follows: S15535 10 μM, 5-HT 10 μM, (+)-8-OH-DPAT 1 μM. Values shown are means ± s.e.m. of (*n*) independent determinations and are expressed as % stimulation above basal values (= 0%).

	S15535 (%) (*n*)	(+)-8-OH-DPAT (%) (*n*)	(+)-8-OH-DPAT + S15535 (%) (*n*)	5-HT (%) (*n*)	5-HT + S15535 (%) (*n*)
Hippocampus					
CA1	9.4 ± 7.1 (9)	63.6 ± 17.0 (4)	−7.4 ± 9.9 (4)	96.5 ± 13.6 (5)	27.8 ± 9.3 (5)
CA2	14.0 ± 6.3 (7)	99.5 ± 35.1 (4)	0.5 ± 16.7 (3)	90.9 ± 11.9 (4)	24.1 ± 5.4 (4)
CA3	18.3 ± 6.2 (7)	55.4 ± 8.6 (3)	5.1 ± 12.6 (3)	90.6 ± 16.0 (4)	29.7 ± 7.3 (4)
Dentate gyrus	18.3 ± 5.2 (12)	70.2 ± 9.1 (6)	4.3 ± 5.7 (6)	124 ± 12.3 (7)	44.2 ± 7.3 (6)
Cortex					
Entorhinal	0.0 ± 6.6 (8)	35.8 ± 17.1 (4)	−11.0 ± 9.3 (4)	82.2 ± 26.1 (4)	30.4 ± 17.6 (3)
Frontal	11.6 ± 6.3 (9)	30.7 ± 6.8 (3)	0.2 ± 6.4 (4)	65.4 ± 14.1 (6)	11.6 ± 8.8 (3)
Occipital	4.0 ± 4.6 (11)	21.4 ± 3.6 (5)	−5.7 ± 4.8 (5)	77.1 ± 17.5 (7)	28.2 ± 7.2 (6)
Temporal	3.4 ± 5.5 (10)	21.2 ± 6.8 (5)	−8.7 ± 5.2 (5)	51.2 ± 7.9 (6)	26.3 ± 8.3 (5)
Parietal	−2.7 ± 4.0 (11)	17.2 ± 1.2 (4)	0.4 ± 3.4 (5)	40.6 ± 7.1 (7)	7.8 ± 4.5 (5)
Midbrain					
Dorsal raphe	0.0 ± 5.5 (8)	35.6 ± 6.0 (4)	−2.0 ± 9.1 (4)	68.6 ± 11.2 (6)	12.4 ± 6.4 (5)
Central grey	−3.4 ± 6.9 (8)	4.2 ± 4.9 (4)	−9.2 ± 6.0 (4)	30.5 ± 14.8 (6)	18.3 ± 5.8 (3)
Substantia nigra	6.4 ± 9.0 (9)	5.9 ± 7.3 (4)	−8.7 ± 6.8 (4)	90.6 ± 23.4 (6)	74.4 ± 18.1 (3)
Other					
Lateral septum	−5.3 ± 3.5 (9)	42.2 ± 7.9 (4)	−2.5 ± 5.3 (4)	95.6 ± 21.8 (5)	16.4 ± 5.2 (4)
Corpus callosum	14.4 ± 4.8 (9)	13.5 ± 6.8 (4)	−4.8 ± 9.7 (4)	24.1 ± 7.6 (4)	29.7 ± 12.5 (5)
Caudate nucleus	−2.2 ± 2.8 (9)	1.8 ± 5.4 (4)	−0.2 ± 3.8 (4)	27.9 ± 4.0 (6)	20.0 ± 5.3 (3)

marked similarity, and were significantly correlated ($P < 0.01$, $r = 0.77$, slope = 0.99, $n = 14$), with those reported by Khawaja (1995) using the selective 5-HT$_{1A}$ receptor antagonist [^{3}H]WAY100,635.

3.2. [^{35}S]GTPγS autoradiography

[^{35}S]GTPγS bound to rat brain sections in a heterogeneous manner (Fig. 1–3). Typical basal binding in hippocampus was 250 to 280 nCi g^{-1} of tissue equivalent (as calibrated with Amersham [^{14}C] standards). Typical basal binding in cortical regions was 150 to 200 nCi g^{-1} whereas in dorsal raphe it was 300 to 350 nCi g^{-1}. Marked basal [^{35}S]GTPγS binding was also observed in substantia nigra and caudate nucleus (200 to 250 nCi g^{-1}). Results are shown in Table 2 for the percentage stimulation of [^{35}S]GTPγS binding by different drug treatments, relative to basal values determined in parallel in the same experiment. (+)-8-OH-DPAT (1 μM) increased [^{35}S]GTPγS binding in hippocampus, lateral septum, cortex and dorsal raphe. 5-HT (10 μM) also increased [^{35}S]GTPγS binding but some marked differences were apparent, compared with (+)-8-OH-DPAT. First, the stimulation of [^{35}S]GTPγS binding induced by 5-HT in hippocampal and cortical regions was consistently higher than that induced by (+)-8-OH-DPAT. Second, 5-HT stimulated [^{35}S]GTPγS binding in caudate nucleus and substantia nigra, areas in which (+)-8-OH-DPAT did not stimulate [^{35}S]GTPγS binding.

S15535 did not markedly modify [^{35}S]GTPγS binding in any brain region, although small increases were noted in dentate gyrus and the CA3 region of hippocampus. However, S15535 reversed (+)-8-OH-DPAT-induced stimulation in a statistically significant manner ($P < 0.05$, two-tailed *t*-test) in hippocampus, cortex, septum and dorsal raphe (see Fig. 4). S15535 also attenuated (but did not abolish) 5-HT-induced stimulation in these regions ($p < 0.05$, two-tailed *t*-test). In contrast, S15535 did not antagonise 5-HT-induced [^{35}S]GTPγS binding in substantia nigra or caudate nucleus (Fig. 4; $p > 0.05$, two-tailed *t*-test), where [^{3}H]S15535 labelling is low or absent (Table 1).

4. Discussion

4.1. Distribution of [^{3}H]S15535 binding sites in rat brain sections

As noted in the Introduction, S15535, is one of the most potent and selective 5-HT$_{1A}$ receptor ligands described to date (Newman-Tancredi et al., 1998) and the present study investigated its binding and functional distribution in rat brain tissue. The tissue density of [^{3}H]S15535 labelling was highest in the CA1 and dentate gyrus regions of hippocampus, intermediate in the CA2 and CA3 regions of hippocampus, lateral septum, interpendicular nucleus, dorsal raphe and entorhinal cortex, and lower in temporal and

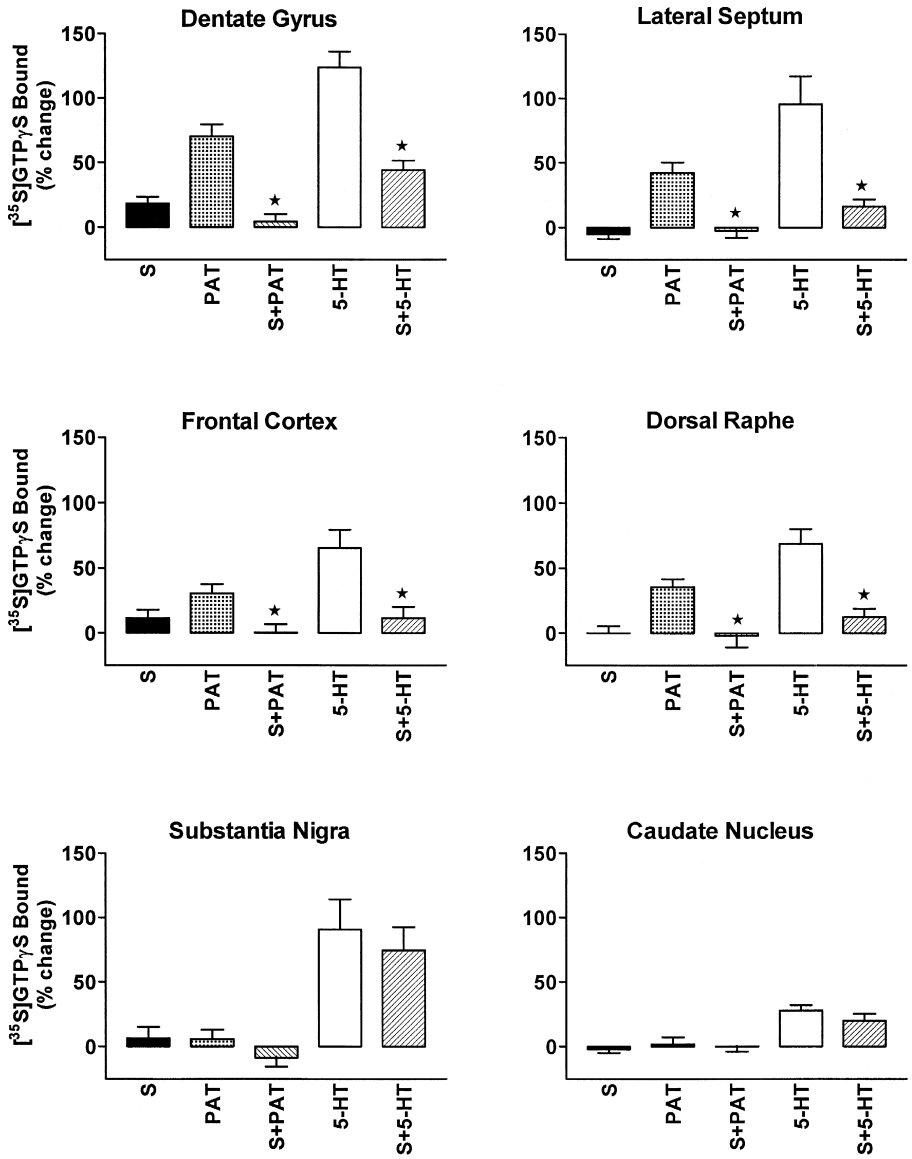

Fig. 4. The effects of the serotonergic ligands (+)-8-OH-DPAT (PAT; 1 μM), 5-HT (10 μM) and S15535 (S; 10 μM) on [^{35}S]GTPγS binding to rat brain sections are shown in different brain regions. Bars represent the mean percentage increase over basal [^{35}S]GTPγS binding induced by the different drug treatments. S15535 significantly antagonised the stimulation induced by (+)-8-OH-DPAT or 5-HT in dentate gyrus, frontal cortex, lateral septum and dorsal raphe (★$P < 0.05$, two-tailed Student's *t*-test). S15535 did not significantly affect 5-HT-induced [^{35}S]GTPγS binding in substantia nigra or caudate nucleus.

parietal cortex, central grey and substantia nigra (Table 1). This localisation of labelling is consistent with that previously reported in rat and human brain using other 5-HT$_{1A}$ receptor radioligands (Marcinkiewicz et al., 1984; Pazos et al., 1987; Sijbesma et al., 1991; Burnet et al., 1995; Khawaja, 1995; Kung et al., 1995). Indeed, there is general agreement on the relative expression levels of 5-HT$_{1A}$ receptors in different brain regions, although antagonists such as [^{3}H]WAY100,635 detect a higher proportion of 5-HT$_{1A}$ sites than the agonist, [^{3}H]8-OH-DPAT (Khawaja, 1995; Burnet et al., 1997). It is interesting to note that the number of sites detected by [^{3}H]S15535 in the present study closely resembles that detected in rat brain with [^{3}H]WAY100,635 (see Table 1 and Khawaja, 1995) suggesting that S15535 may exhibit predominantly antagonist properties. The localisation of [^{3}H]S15535 binding was also similar to that detected using anti-5-HT$_{1A}$ receptor antibodies (Hamon et al., 1991; Kia et al., 1996) and in situ hybridisation studies of 5-HT$_{1A}$ mRNA (Chalmers and Watson, 1991; Miquel et al., 1992; Pompeiano et al., 1992; Burnet et al., 1995). It appears, therefore, that [^{3}H]S15535 labels the same population of rat brain 5-HT$_{1A}$ receptors as

previously characterised. The use of [^{35}S]GTPγS autoradiography, in parallel with [^{3}H]S15535 autoradiography, allowed us to address the issue of the functional efficacy of S15535 and to relate this to the localisation of its binding sites.

4.2. Actions of S15535 on G-protein activation in corticolimbic brain regions

When S15535 was tested alone, no marked stimulation of [^{35}S]GTPγS binding was observed in any brain region, although very slight increases were noted in hippocampus (Table 2; Fig. 4), possibly reflecting weak partial agonist properties in this brain region. These observations are consistent with the essentially antagonist properties of S15535 at post-synaptic 5-HT$_{1A}$ receptors in limbic regions predicted on the basis of results from a recombinant cell line expressing human 5-HT$_{1A}$ receptors (efficacy: ~30% relative to 5-HT; Newman-Tancredi et al., 1996) and with the antagonist properties of S15535 at post-synaptic 5-HT$_{1A}$ receptors in vivo (see Section 1). Indeed, although S15535 did not markedly stimulate [^{35}S]GTPγS binding to brain sections, it antagonised the stimulation induced by both (+)-8-OH-DPAT and 5-HT in those brain regions which exhibited high [^{3}H]S15535 labelling, such as hippocampus and lateral septum, whereas it showed no activity in regions where [^{3}H]S15535 labelling was low or absent, such as substantia nigra and caudate nucleus. However, whereas S15535 abolished stimulation induced by (+)-8-OH-DPAT, it only partially antagonised the stimulation of [^{35}S]GTPγS binding induced by 5-HT in hippocampus and cortex. Further, in substantia nigra and caudate nucleus, 5-HT induced a pronounced stimulation of [^{35}S]GTPγS binding in the absence of [^{3}H]S15535 labelling, indicating that the stimulation is due to stimulation of a 5-HT receptor subtype other than 5-HT$_{1A}$ (Waeber and Moskowitz, 1997; Dupuis et al., 1998). Indeed, we have recently shown that 5-HT-induced stimulation in substantia nigra is entirely blocked by SB229,284 (Millan et al., 1999), a selective 5-HT$_{1B}$ receptor antagonist (Roberts et al., 1997).

4.3. Actions of S15535 on G-protein activation in the raphe

In the dorsal raphe, (+)-8-OH-DPAT stimulated [^{35}S]GTPγS binding, consistent with its agonist activity at presynaptic 5-HT$_{1A}$ receptors (Lejeune et al., 1997). In contrast, S15535 did not stimulate [^{35}S]GTPγS binding in the dorsal raphe. Instead, it antagonised G-protein activation therein, inhibiting the stimulation of [^{35}S]GTPγS binding by (+)-8-OH-DPAT and 5-HT as in hippocampus, cortex and lateral septum (Fig. 4). Thus, the present study found no qualitative difference in the actions of S15535 at pre- or postsynaptic 5-HT$_{1A}$ receptors. These results, which indicate antagonist actions of S15535 on G-protein activation in the raphe, appear at variance with electrophysiological and neurochemical studies which demonstrated agonist properties of S15535 on serotonergic neurones (see Section 1 and Millan et al., 1994). There are several possible explanations. First, the populations of G-proteins involved in mediating the agonist actions of S15535 at presynaptic receptors (inhibition of raphe neuron firing) may not be the same as those labelled by [^{35}S]GTPγS in the autoradiographic experiments. Various coupling mechanisms of 5-HT$_{1A}$ receptors have been reported, including inhibition of adenylyl cyclase activity, stimulation of inositol phosphate synthesis, activation of Na^+/H^+ exchange, elevation of cytosolic Ca^{2+} and modulation of arachidonic production (De Vivo and Maayani, 1986; Sprouse and Aghajanian, 1987; Harrington et al., 1988; Fargin et al., 1991; Raymond et al., 1992; Boess and Martin, 1994; Garnovskaya et al., 1997). All of these responses are mediated via Gi/o G-proteins ($G_{i\alpha 2}$ and $G_{i\alpha 3}$ being particularly important; Harrington et al., 1988; Bertin et al., 1992; Raymond et al., 1993) but it is unclear what signal transduction mechanisms are involved at 5-HT$_{1A}$ autoreceptors (Innis and Aghajanian, 1987; Kelly et al., 1991; Penington et al., 1993; Katayama et al., 1997). Indeed, no coupling of 5-HT$_{1A}$ receptors to adenylyl cyclase inhibition is detectable in raphe nucleus (Clarke et al., 1996; Johnson et al., 1997), but the *inhibition* of phosphoinositide hydrolysis has been reported therein (Johnson et al., 1997). Hence, marked differences in the activation cascades of somatodendritic versus limbic 5-HT$_{1A}$ receptors are likely, possibly related to coupling to different subtypes of Gi proteins and/or discrimination between Gi and Go proteins. A second possibility which may explain the absence of agonist activity by S15535 in dorsal raphe nucleus is that S15535 does, in fact, exert weak agonist actions (too slight to be reliably detected by the present methodology) and that these are amplified, downstream of G-protein activation, to yield a 'full efficacy' response which results in total suppression of raphe neuron firing. Indeed, the presence of receptor reserve at somatodendritic, but not at post-synaptic hippocampal, 5-HT$_{1A}$ receptors has been documented (Meller et al., 1990; Yocca et al., 1992). Consistent with this hypothesis, it is noteworthy that, despite their similar affinity at h5-HT$_{1A}$ receptors (K_i = 0.6 nM; Newman-Tancredi et al., 1998), the ID_{50} values for inhibition of raphe neuron firing by S15535 and 8-OH-DPAT show a 14-fold difference (7 μg/kg and 0.5 μg/kg i.v., respectively; Millan et al., 1994), possibly reflecting the lower efficacy of S15535 for activation of 5-HT$_{1A}$ receptors. Third, the possibility cannot be excluded that other, as yet unidentified, receptor subtypes may be present in the raphe nucleus, via which S15535 could exert its presynaptic agonist properties, perhaps through G-protein subtypes other than Gi/o. However, this hypothesis remains speculative in view of the high level of selectivity of

S15535 for 5-HT_{1A} receptors (see Section 1 and Newman-Tancredi et al., 1998).

4.4. Other considerations

Two further points should be made concerning the present data. First, as in the case of previous studies of [^{35}S]GTPγS autoradiography, we examined the effects of concentrations of ligands in the micromolar range. Indeed, (+)-8-OH-DPAT attains its maximal stimulation of [^{35}S]GTPγS binding at ≥ 1 μM (Sim et al., 1997; Waeber and Moskowitz, 1997; Dupuis et al., 1998, 1999). The necessity for such high concentrations may be related to the high concentration of GDP (2.5 mM), which is necessary to reduce basal [^{35}S]GTPγS binding (Sim et al., 1995; Waeber and Moskowitz, 1997). However, GDP also reduces agonist affinity at G-protein coupled receptors, shifting agonist concentration-response curves to the right. Future studies will address the issue of the concentration-response relationships of S15535 on G-protein activation in brain sections. Second, although the localisation of (+)-8-OH-DPAT-induced [^{35}S]GTPγS binding corresponded well with the distribution of 5-HT_{1A} receptors as determined by [^{3}H]S15535 autoradiography (Scott and Bruinvels, 1997; Waeber and Moskowitz, 1997), the data suggested possible mismatches between 5-HT_{1A} receptor expression levels and stimulation of [^{35}S]GTPγS binding. In frontal cortex, for example, the density of [^{3}H]S15535 binding sites was only half of that observed in entorhinal cortex, but the stimulation of [^{35}S]GTPγS binding was similar (30.7% and 35.8%, respectively). Further, although 5-HT_{1A} receptor expression in dentate gyrus is $>$ 2-fold higher than in the CA2 and CA3 regions of hippocampus, (+)-8-OH-DPAT induces a comparable stimulation of [^{35}S]GTPγS binding (Tables 1 and 2). The functional responses to 5-HT_{1A} receptor agonists may, therefore, be not only related to 5-HT_{1A} receptor density but also to region-specific variations in coupling efficiency (Sim et al., 1995, 1996).

4.5. Conclusions

The present study characterised both the autoradiographic receptor localisation and the functionality of S15535 in rat brain sections. The results indicate that [^{3}H]S15535 displays a distribution of binding which is consistent with selective labelling of 5-HT_{1A} receptors. S15535 behaved essentially as an antagonist in limbic brain regions, inhibiting agonist-induced G-protein activation. Interestingly, S15535 also antagonises G-protein activation at 5-HT_{1A} receptors in dorsal raphe, a region in which it has been previously shown to inhibit neuronal firing (Lejeune and Millan, 1998). The relationship between the molecular and in vivo actions of S15535, particularly at the presynaptic level, require further investigation.

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