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Evaluation in rat of RS-79948-197 as a potential PET ligand for central α_2-adrenoceptors

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Abstract

Tritium-labelled RS-79948-197 {(8a*R*,12a*S*,13a*S*)-5,8,8a,9,10,11,12,12a,13,13a-decahydro-3-methoxy-12-(ethylsulphonyl)-6*H*-isoquino[2,1-g][1,6]naphthyridine} was evaluated in rat brain as an in vivo ligand for central α_2-adrenoceptors, as a preliminary step in the development of a radioligand for positron-emission tomography (PET) studies. The maximal receptor-specific signal was achieved within 90–120 min after i.v. injection of [ethyl-^{3}H]RS-79948-197 and was selective for the α_2- compared with the α_1-adrenoceptor, with no detectable binding to the imidazoline-I_2 site. Estimates for binding potential (approximating to B_{max}/K_d) ranged between 3.4 in entorhinal cortex and 0.5 in medulla oblongata. The results, which indicate a similarly localised but 2-fold increase in specific binding compared with that previously demonstrated using [^{3}H]RX 821002 (2-methoxy-idazoxan), are sufficiently encouraging as to support further investment in the development of ^{11}C-labelled RS-79948-197, or a close structural analogue, as a ligand for clinical PET.

Keywords: RS-79948-197; (Rat); Brain; α_2-Adrenoceptor; Radioligand; In vivo; PET (positron-emission tomography)

1. Introduction

Positron-emission tomography (PET) enables the visualisation and quantification of receptors in man in vivo. In addition to its value in clinical studies of synaptic pathology or dysfunction, PET now attracts the attention of pharmaceutical companies, particularly in the determination of in vivo receptor occupancy using pharmacologically active doses of drugs still in development (see Comar, 1995). This pre-supposes the availability of potent and selective ligands which can feasibly be labelled with a positron emitter and given to man. In fact, the lack of such compounds is often the limiting factor in the advancement of such studies.

If the compound is available in its tritiated form, then its temporal biodistribution (and thus the estimated size, location and time course of any specific radioactive signal) can be assessed in the laboratory. The present study comprises this initial and rapid phase in the development of a PET ligand for central α_2-adrenoceptors, describing the α_2-specific distribution of [ethyl-^{3}H]RS-79948-197 (Fig. 1), (8a*R*,12a*S*,13a*S*)-5,8,8a,9,10,11,12,12a,13,13a-decahydro-3-methoxy-12-(ethylsulphonyl)-6*H*-isoquino-[2,1-g][1,6]naphthyridine, in rat brain as a function of time after i.v. injection. When assessed in vitro, its close structural homologue RS-15385-197 (Fig. 1), (8a*R*,12a*S*,13a*S*)-5,8,8a,9,10,11,12,12a,13,13a-decahydro-3-methoxy-12-(methylsulphonyl)-6*H*-isoquino[2,1-g][1,6]-naphthyridine (Clark et al., 1991), has been classed as an α_2-adrenoceptor antagonist with a > 1000-fold selectivity over α_1-adrenoceptors (MacKinnon et al., 1992), binding to α_{2A}-, α_{2B}- and possibly α_{2D}-adrenoceptor subtypes (MacKinnon et al., 1994).

The extent of the regional specific, compared with non-specific binding of [^{3}H]RS-79948-197 was measured in both brain and body tissues by pre-dosing the rats with non-radioactive compound such that maximal receptor occupancy was achieved. The in vivo selectivity was checked by pre-dosing with the 5-hydroxytryptamine $(5\text{-HT})_{1A}$ receptor antagonist WAY 100635 (Fletcher et al., 1993), the

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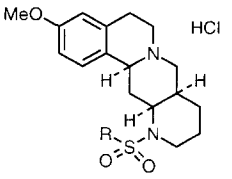

R = Me, RS-15385-197
R = Et, RS-79948-197

Fig. 1. The structures of RS-79948-197 and its related homologue RS-15385-197.

α_1-adrenoceptor antagonist prazosin (Latifpour and Bylund, 1983; MacKinnon et al., 1994), the α_2-adrenoceptor antagonist RX 821002 (2-methoxy-idazoxan) (Stillings et al., 1985) and the imidazoline-I_2 receptor antagonists RS-45041-190 (MacKinnon et al., 1995) and 2-BFI [(2-benzo-furanyl)2-imidazoline] (Lione et al., 1996).

2. Materials and methods

2.1. Materials

[Ethyl-^{3}H]RS-79948-197 (3.0 TBq/mmol in ethanol; 99.3% radiochemical purity by high-performance liquid chromatography) was purchased from Amersham International. RX 821002 was from Research Biochem (Natick, MA, USA) and prazosin from Sigma (Poole, UK). RS-79948-197 and RS-45041-190 were synthesised by Roche Bioscience. WAY 100635 was kindly donated by Wyeth Research (UK) and 2-BFI by Dr. Alan Hudson (Psychopharmacology Unit, University of Bristol, Bristol, UK).

2.2. Biological studies

All studies used adult male Sprague-Dawley rats (260–320 g; Harlan Olac, Bicester, UK) and were carried out by licensed investigators in accordance with the British Home Office's Guidance on the Operation of the Animals (Scientific Procedures) Act 1986 (HMSO, February 1990). During the biodistribution studies, the rats were awake but under light restraint. The experimental procedures were similar to those which have been reported elsewhere (Hume et al., 1991, 1992a).

Radioactivity was given as a bolus injection (approximately 0.3 MBq in 0.2 ml saline with 10% ethanol), via a tail vein catheter. Blood samples were taken at designated times via a tail artery catheter and the rats killed by i.v. injection of euthatal (Rhône Mérieux) at graded times between 1 and 150 min after radioligand injection. A selection of body and brain tissues were rapidly sampled and, after overnight treatment with a tissue solubiliser, radioactivity measured using a Beckman LS 6800 scintillation counter with automatic quench correction. The brain tissues sampled were: olfactory tubercle, frontopolar cortex, septum, anterior cingulate cortex, striatum, frontal + parietal cortex, hypothalamus, thalamus, amygdala + piriform cortex, occipital cortex, inferior colliculi, hippocampus, superior colliculi, entorhinal cortex, medulla, cerebellar vermis and cerebellar lobules, all dissected according to the guide compiled by Palkovits and Brownstein (1988). The body tissues and fluids sampled were: heart (auricle and ventricle), lung, liver, kidney (primarily cortex), fat, muscle and urine. Radioactivity content in blood (Bq/ml) or tissue (Bq/g) was expressed as a percentage of that injected, giving units of [(Bq/g)/Bq] × 100.

For the blocking studies, groups of rats (3 per group) were given an i.v. injection of either RS-79948-197, RX 821002, prazosin, WAY-100635, RS-45041-190 or 2-BFI, each at a dose of 2 mg/kg body weight, 5 min prior to injection of [ethyl-^{3}H]RS-79948-197. Statistical significance in the difference between treatment vs. control was assessed using Student's t-test with Bonferroni correction.

2.3. Time-radioactivity curve analysis

Assuming negligible specific binding to cerebellum, brain tissue time-radioactivity curves were fitted to a reference-tissue compartmental model, with the cerebellum data set (fitted to a double exponential plus constant) as an indirect input function (Hume et al., 1992b; Lammertsma et al., 1996). The kinetic parameter obtained was the binding potential (k_3/k_4), where k_3 and k_4 represent the rate constants for movement to and from the specific binding compartment, respectively. For the high specific radioactivity injections used in the present studies (approximately 0.3 nmol/kg body weight), minimal receptor occupancy is assumed and binding potential approximates to B_{max}/K_d, where B_{max} represents receptor number and K_d represents the dissociation constant.

3. Results

3.1. Blood and plasma

Fig. 2 illustrates the rapid clearance of radioactivity from plasma following a single i.v. injection of [ethyl-^{3}H]RS-79948-197. At 1 min after ligand injection, counts/ml of plasma were approximately 1% of that injected and 0.2% at 10 min. The inset illustrates the similar fall in whole blood counts. The ratio of whole blood/plasma counts was approximately 0.6 throughout the time of the study which, assuming an haematocrit of approximately 40%, indicates negligible binding to the cellular component of blood.

3.2. Brain tissues

Biodistribution of radioactivity within brain was heterogeneous, showing a temporal redistribution after an 'ini-

tial' (1 min), relatively homogeneous uptake of the order of 0.6%/g of that injected. Fig. 3 illustrates time-radioactivity curves for three of the tissues sampled, viz. entorhinal cortex, thalamus and striatum showing high, intermediate and low retentions of radioactivity, respectively. Also illustrated are the corresponding cerebellum data from the same rats (vermis and lobules gave essentially similar data and have therefore been combined).

The cerebellum curve was initially assumed to reflect uptake and subsequent clearance of radioactivity from the free and/or non-specifically bound tissue compartments, allowing the regional data to be fitted to a reference tissue model, with cerebellum data as the indirect input function (Hume et al., 1992b). The solid lines in Fig. 3 are the fitted curves through each of the brain tissues shown and the values for the binding potential (approximating to B_{max}/K_d) are listed in Table 1, together with the values for all the brain tissues sampled. The numbering system relates to subsequent figures. High specific binding was observed in entorhinal cortex, septum and amygdala, with low binding in striata and medulla oblongata. Tissue/cerebellum radioactivity ratios derived from the time-activity curves, and illustrated in Fig. 4 for the three representative tissues, demonstrate that 90–120 min was sufficient for the development of secular equilibrium. The 120-min ratios are listed in Table 1; the linear regression between this ratio and binding potential gave a correlation coefficient of 0.965 ($P < 0.001$).

The results of the blocking studies are presented in Table 2. Pre-treating the rats with non-radioactive RS-79948-197 at a dose of 2 mg/kg, 5 min before injection of [ethyl-^{3}H]RS-79948-197, reduced the 120-min radioactivity content in all regions, eliminating the specific signal. In addition to the reduction in counts in regions of interest, however, the radioactivity content of cerebellum itself was

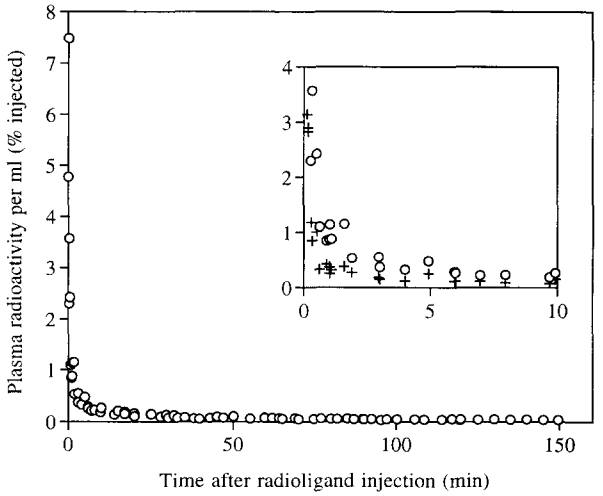

Fig. 2. Radioactivity content/ml plasma (o) and blood (inset (+)) as a function of time after i.v. injection of [ethyl-^{3}H]RS-79948-197, expressed as a percentage of radioactivity injected per rat. The curve is a composite from several rats (6 data points per rat).

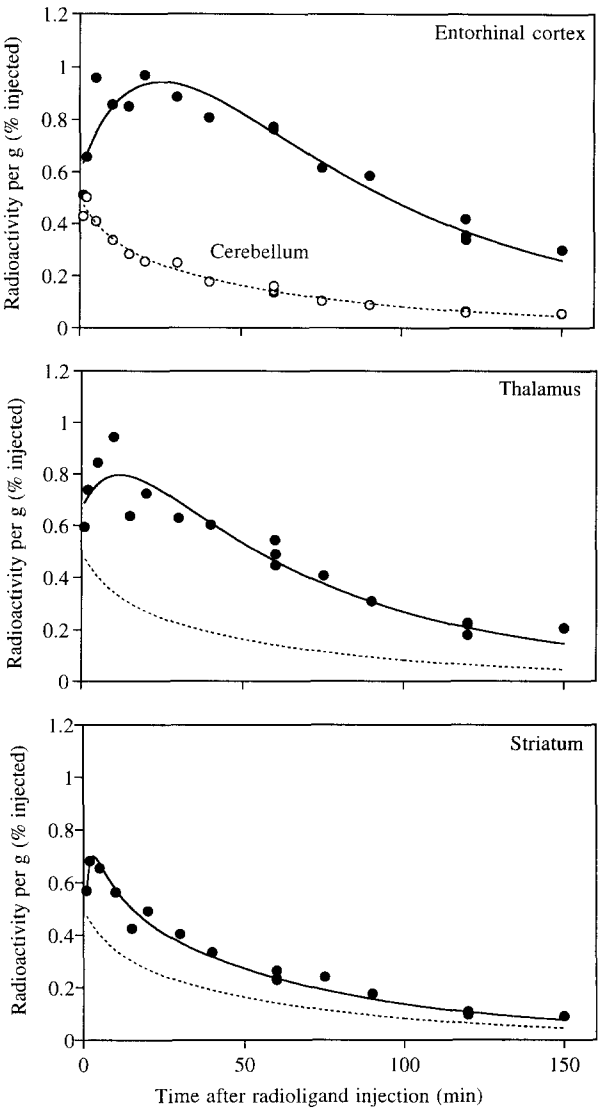

Fig. 3. Radioactivity per g of the 3 tissues indicated (●), as a function of time after i.v. injection of [ethyl-^{3}H]RS-79948-197. Data points are from individual rats, shown paired with the cerebellum data (o) in the uppermost plot. The dashed line is a multi-exponential fit through the cerebellum data. The solid lines are the best fits through the regional data sets, using the cerebellum curve as the input function in the reference tissue compartmental model (Hume et al., 1992b).

reduced (0.046 ± 0.004 compared with a control value/g of tissue of 0.067 ± 0.008% of that injected). Thus, the binding potential values based on cerebellum as an input function slightly under-estimate the expected specific signal, and the tissue/cerebellum ratios at equilibrium are consistently lower than those obtained when control tissue counts are expressed relative to regional counts obtained in RS-79948 pre-dosed rats. The latter ratios are listed in the final column in Table 1 and are linearly related to the individual control tissue/cerebellum ratios ($r = 0.986$). The increase in standard deviation is due to inter-rat variation, which is avoided in the tissue/cerebellum estimates.

Table 1
Binding potential values in control rats (with S.E. from the fit), estimated from time-radioactivity curves using a reference tissue compartmental model

Tissue	Binding potential (k_3/k_4)	Tissue/cerebellum ratio	Tissue/RS pre-dosed ratio
1. Olfactory tubercle	1.89 ± 0.13	3.4 ± 0.6	4.2 ± 1.0
2. Frontopolar cortex	1.58 ± 0.08	3.2 ± 0.1	3.7 ± 0.5
3. Septum	2.76 ± 0.26	5.4 ± 0.5	6.1 ± 1.0
4. Anterior cingulate cortex	1.47 ± 0.12	2.6 ± 0.1	3.6 ± 0.5
5. Striatum	0.65 ± 0.04	1.7 ± 0.1	2.3 ± 0.3
6. Frontal + parietal cortex	1.57 ± 0.09	2.7 ± 0.5	3.4 ± 0.7
7. Hypothalamus	2.26 ± 0.15	3.9 ± 0.5	3.9 ± 0.6
8. Thalamus	1.94 ± 0.13	3.3 ± 0.3	3.7 ± 0.4
9. Amygdala + piriform cortex	2.78 ± 0.25	5.7 ± 1.0	6.6 ± 1.0
10. Occipital cortex	1.40 ± 0.11	2.6 ± 0.1	2.9 ± 0.5
11. Inferior colliculi	1.68 ± 0.08	2.4 ± 0.1	2.9 ± 0.3
12. Hippocampus	0.97 ± 0.10	2.5 ± 0.2	3.1 ± 0.5
13. Superior colliculi	0.79 ± 0.04	1.9 ± 0.1	2.6 ± 0.3
14. Entorhinal cortex	3.44 ± 0.16	6.1 ± 0.4	6.7 ± 1.0
15. Medulla	0.50 ± 0.02	1.6 ± 0.2	2.1 ± 0.5
16. Cerebellum	–	–	1.5 ± 0.2

Tissue/cerebellum ratios (individual rats) and ratios for control/RS-79948-197 pre-dosed tissue (individual rats compared with group mean) were assayed 120 min after [ethyl-^{3}H]RS-79948-197 injection. Values are means with S.D. from 3 rats per group.

Of the other pre-dosing compounds tested, only RX 821002 caused a significant reduction in radioactive content. Again, the specific (now selective), signal was eliminated. For all of the regions sampled, the effects of RS-79948-197 and RX 821002, given at the same high dose, were not significantly different. Although caution in any statistical analysis is advised due to the small sample size, neither prazosin, RS-45041-190 nor 2-BFI had any significant effect in any of the tissues sampled. Pre-dosing with WAY 100635 appeared to cause an increase in binding of [ethyl-^{3}H]RS-79948-197 in all regions including cerebellum, reaching the 1% significance level in thalamus.

3.3. Body tissues

Of the tissues sampled, only liver, kidney and body fat showed an accumulation of radioactivity with time. Much of the radioactivity was excreted in the urine. Table 3 lists the radioactivity contents of the body tissues sampled at 120 min after injection of [ethyl-^{3}H]RS-79948-197 in control rats and those pre-dosed as indicated. Only the kidney

Table 2
Radioactivity content (mean ± S.D. from 3 rats) of brain tissues sampled at 120 min after injection of radioligand, in control rats or rats pre-dosed with the compounds shown

Tissue	Radioactivity content/g (% injected)						
	Control	RS-79948	RX 821002	Prazosin	RS-45041	2-BFI	WAY 100635
Olfactory tubercle	0.23 ± 0.06	0.06 ± 0.02 [c]	0.05 ± 0.01 [c]	0.24 ± 0.06	0.27 ± 0.03	0.23 ± 0.04	0.37 ± 0.01
Frontopolar cortex	0.21 ± 0.03	0.06 ± 0.01 [b]	0.05 ± 0.01 [a]	0.19 ± 0.06	0.22 ± 0.03	0.17 ± 0.01	0.28 ± 0.03
Septum	0.37 ± 0.07	0.06 ± 0.01 [c]	0.05 ± 0.01 [c]	0.35 ± 0.08	0.28 ± 0.06	0.24 ± 0.03	0.50 ± 0.06
Cingulate cortex	0.18 ± 0.02	0.05 ± 0.01 [b]	0.06 ± 0.01 [b]	0.18 ± 0.05	0.19 ± 0.01	0.15 ± 0.02	0.24 ± 0.02
Striatum	0.11 ± 0.02	0.05 ± 0.01 [c]	0.05 ± 0.01 [c]	0.13 ± 0.03	0.14 ± 0.03	0.13 ± 0.02	0.18 ± 0.02
Frontal cortex	0.18 ± 0.04	0.05 ± 0.01 [c]	0.05 ± 0.01 [c]	0.18 ± 0.03	0.18 ± 0.03	0.14 ± 0.02	0.27 ± 0.02
Hypothalamus	0.26 ± 0.04	0.07 ± 0.01 [b]	0.06 ± 0.01 [b]	0.30 ± 0.08	0.29 ± 0.03	0.23 ± 0.01	0.39 ± 0.02 [c]
Thalamus	0.23 ± 0.02	0.06 ± 0.01 [a]	0.06 ± 0.01 [a]	0.25 ± 0.06	0.28 ± 0.06	0.22 ± 0.04	0.34 ± 0.01 [b]
Amygdala	0.39 ± 0.07	0.06 ± 0.01 [c]	0.05 ± 0.01 [c]	0.33 ± 0.02	0.38 ± 0.07	0.31 ± 0.03	0.49 ± 0.06
Occipital cortex	0.17 ± 0.03	0.06 ± 0.01 [c]	0.05 ± 0.01 [c]	0.15 ± 0.04	0.19 ± 0.02	0.15 ± 0.01	0.23 ± 0.01
Inferior colliculi	0.16 ± 0.02	0.06 ± 0.01 [b]	0.06 ± 0.01 [b]	0.19 ± 0.07	0.19 ± 0.01	0.16 ± 0.01	0.24 ± 0.03
Hippocampus	0.17 ± 0.03	0.05 ± 0.01 [c]	0.05 ± 0.01 [c]	0.18 ± 0.03	0.17 ± 0.02	0.13 ± 0.02	0.23 ± 0.02
Superior colliculi	0.13 ± 0.02	0.05 ± 0.01 [c]	0.06 ± 0.01 [c]	0.14 ± 0.04	0.14 ± 0.01	0.12 ± 0.01	0.17 ± 0.01
Entorhinal cortex	0.41 ± 0.06	0.06 ± 0.01 [b]	0.06 ± 0.01 [b]	0.42 ± 0.08	0.39 ± 0.07	0.31 ± 0.03	0.59 ± 0.02
Medulla	0.11 ± 0.03	0.05 ± 0.01	0.05 ± 0.01	0.12 ± 0.02	0.11 ± 0.02	0.11 ± 0.01	0.15 ± 0.01
Cerebellum	0.07 ± 0.01	0.05 ± 0.01	0.05 ± 0.01	0.07 ± 0.01	0.08 ± 0.01	0.08 ± 0.01	0.09 ± 0.01

Values significantly different from control are indicated (Student's t-test with Bonferroni correction: [a] $P < 0.001$; [b] $P < 0.01$; [c] $P < 0.05$).

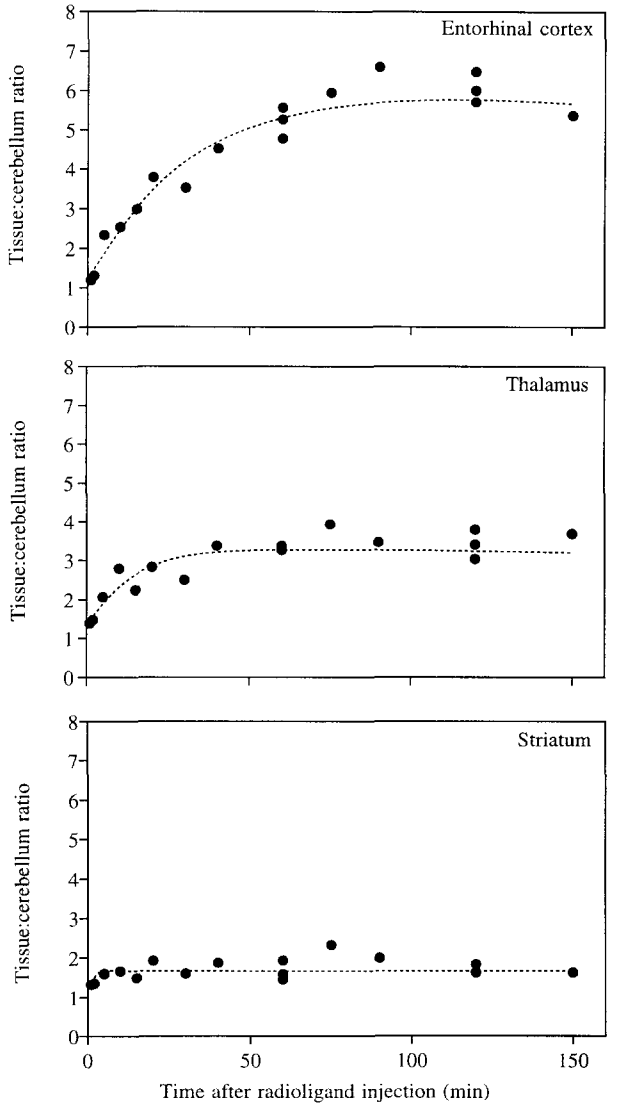

Fig. 4. Ratio of tissue/cerebellum counts for each of the tissues shown as a function of time after injection of [ethyl-^{3}H]RS-79948-197. Data points are from individual rats and the dashed lines were obtained by dividing each pair of the fitted curves shown in Fig. 3.

showed a significantly reduced radioactivity content after any of the pre-dosing regimes, RS-79948-197 itself causing a marked reduction compared with control. Prazosin, RX 821002, RS-45041-190 and 2-BFI appeared to have no significant effect whereas WAY 100635 caused a generalised increase in radioactivity content.

4. Discussion

The results describe the time course of development of the specific signal achieved in rat brain after i.v. injection of [ethyl-^{3}H]RS-79948-197. Within 90–120 min, the localisation of the signal reflected noradrenergic innervation, being qualitatively similar to that defined by in vitro autoradiographic studies using [^{3}H]idazoxan (binding to both α_2-adrenoceptors and the pharmacologically related imidazoline-I_2 sites) (Boyajian and Leslie, 1987; Mallard et al., 1992) and the more selective ligands [^{3}H]bromoxidine (Pascual et al., 1992) and [^{3}H]RX 821002 (Hudson et al., 1992). The close relationship between the present in vivo distribution and the in vitro binding obtained with the latter compound is presented in Fig. 5.

RX 821002 itself has been suggested as a potential PET ligand and a synthetic route for carbon-11-labelling identified (Pike et al., 1993). Using the tritiated compound, its in vivo labelling of central α_2-adrenoceptors was demonstrated in rats, giving a primarily post-synaptic maximal 'signal' (the ratio between regional radioactivity content in control compared with idazoxan pre-dosed rats) of the order of three (Hume et al., 1992a). The consistently greater 'signal' achieved with [ethyl-^{3}H]RS-79948-197 relative to [^{3}H]RX 821002 is illustrated in Fig. 6. The greater ability of radiolabelled RS-79948-197 to resolve α_2-adrenoceptor binding in vivo is probably due to a lower level of non-specific binding, which in turn may be related to its rapid clearance from plasma.

The similarity in pattern of labelling with the two compounds implies an in vivo selectivity of [ethyl-^{3}H]RS-

Table 3
Radioactivity content (mean ± S.D. from 3 rats, except for urine, where the range is given) of various body tissues sampled at 120 min after injection of radioligand, in control rats or rats pre-dosed with the compounds shown

Tissue	Radioactivity content/g or ml (% injected)						
	Control	RS-79948	RX-821002	Prazosin	RS-45041	2-BFI	WAY 100635
Bood	0.03 ± 0.01						
Plasma	0.05 ± 0.01						
Heart (a)	0.06 ± 0.01	0.07 ± 0.01	0.08 ± 0.01	0.06 ± 0.01	0.08 ± 0.01	0.09 ± 0.01	0.07 ± 0.01
Heart (v)	0.07 ± 0.01	0.07 ± 0.01	0.08 ± 0.01	0.06 ± 0.01	0.08 ± 0.01	0.08 ± 0.01	0.08 ± 0.01
Lung	0.08 ± 0.02	0.08 ± 0.01	0.10 ± 0.02	0.07 ± 0.01	0.12 ± 0.01	0.11 ± 0.01	0.09 ± 0.02
Liver	0.45 ± 0.08	0.38 ± 0.06	0.67 ± 0.14	0.38 ± 0.09	0.53 ± 0.06	0.54 ± 0.01	0.63 ± 0.13
Kidney	0.35 ± 0.02	0.14 ± 0.01 [a]	0.35 ± 0.03	0.28 ± 0.04	0.41 ± 0.05	0.42 ± 0.04	0.46 ± 0.08
Fat	0.12 ± 0.02	0.13 ± 0.03	0.18 ± 0.03	0.13 ± 0.06	0.16 ± 0.07	0.12 ± 0.03	0.21 ± 0.03 [c]
Skeletal muscle	0.05 ± 0.01	0.05 ± 0.01	0.06 ± 0.01	0.05 ± 0.01	0.06 ± 0.01	0.07 ± 0.01	0.06 ± 0.01
Urine	2.5–3.7	0.8–3.9	1.8–3.9	1.7–8.6	2.6–5.4	3.0–4.0	2.2–3.9

The heart samples are either auricle (a) or ventricle (v). Values significantly different from control are indicated (Student's t-test with Bonferroni correction: [a] $P < 0.001$; [b] $P < 0.01$; [c] $P < 0.05$).

79948-197 for the α_2-adrenoceptor and this is confirmed by the results of the blocking studies. Thus, RX 821002 completely eliminated the specific signal while prazosin and the imidazoline-I_2 receptor antagonists had no significant effect. It should be noted, however, that imidazoline-I_2 sites are so few in number in rat brain (Mallard et al., 1992) that the gross dissection technique used in the present study may be insufficiently sensitive to enable the detection of any significant binding of [ethyl-^{3}H]RS-79948-197 to the latter site.

Supporting evidence for selective α_2-adrenoceptor binding does, however, come from the peripheral tissue binding studies. The specific binding identified in kidney at the 120-min assay time was unaffected by pre-dosing with either RS-45041-190 or 2-BFI despite the fact that imidazoline-I_2 sites are reportedly 9-fold more prominent than α_2-receptors, at least in guinea-pig kidney (Wikberg et al., 1992). Of the α_2-adrenoceptors in rat kidney, $\approx 80\%$ are of the α_{2B}-subtype (Xia et al., 1993). The finding that these were blocked by pre-dosing with RS-79948-197 but not by RX 821002 (Table 3) is unexplained but may be related to differences in affinity for the α_{2A}- and α_{2B}-subtypes of the adrenoceptor (Devedjian et al., 1994).

Although a reportedly common feature of many α_2-adrenoceptor ligands in vitro, including [^{3}H]RX 821002 (Vauquelin et al., 1990), is some affinity for 5-HT_{1A} receptors (Leysen et al., 1988), the selective 5-HT_{1A} receptor antagonist WAY 100635 caused no reduction in binding of [ethyl-^{3}H]RS-79948-197 in vivo. In fact, pre-dosing with WAY 100635 resulted in a generalised increase in radioactive content assayed at 120 min after radioligand injection. The reason for this remains to be resolved but is probably related to an increase in bioavailablity of tritiated compound. Since the increase was apparent in both periphery (including fat) and brain (including cerebellum), it is probably the non-specific rather than the specific binding which is increased.

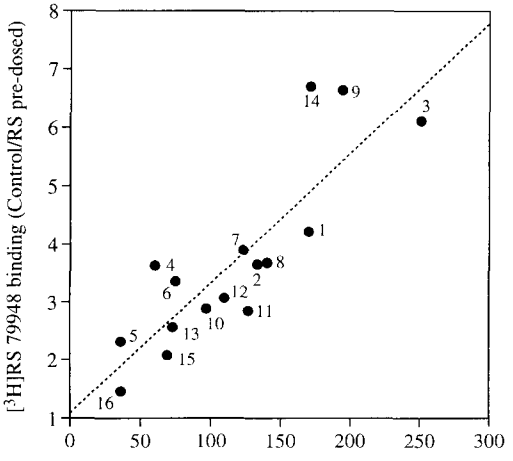

Fig. 5. Correlation between individual region (control tissue/RS pre-dosed tissue) ratios at 120 min after [ethyl-^{3}H]RS-79948-197 injection and the regional distribution of α_2-adrenoceptors in rat brain, reported by Hudson et al. (1992) using conventional in vitro autoradiography of [^{3}H]RX 821002. The in vitro data (from 2 nM, 20 min incubations at 24°C) are averages of the published values for subregions or discrete nuclei within the post-mortem dissected regions. The numbering relates to the individual regions listed in Table 1. The dashed line is the linear regression, giving a correlation coefficient, $r = 0.853$ ($P < 0.001$).

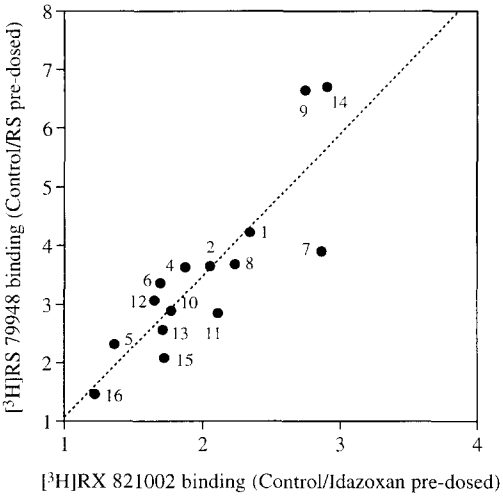

Fig. 6. Correlation between the [control tissue/RS 79948-197 pre-dosed tissue] ratio at 120 min after [ethyl-^{3}H]RS-79948-197 injection and the equivalent equilibrium ratio obtained after injection of [^{3}H]RX 821002, namely (control tissue/Idazoxan pre-dosed tissue) as reported previously (Hume et al., 1992a). The regions were sampled similarly in the two studies and are enumerated as listed in Table 1. The dashed line is the linear regression, giving a correlation coefficient, $r = 0.854$ ($P < 0.001$).

Recently, the α_2-adrenoceptor antagonist WY26703 has been labelled with carbon-11 and tested in PET scans of Rhesus monkeys but failed as an in vivo ligand because of high non-specific binding which was, in fact, predicted by post-mortem dissection experiments using rats (Pleus et al., 1992a). The same group has suggested carbon-11-labelled MK-912 as a better alternative for mapping α_2-adrenoceptor with PET but in rats, at least, they report the specific signal (tissue/cerebellum ratio) to be < 2 (Pleus et al., 1992b). Similarly, a preliminary report on the biodistribution in rats of [^{18}F]fluoroatipamezole, an analogue of the the α_2-adrenoceptor antagonist atipamezole, indicates only a small specific signal (Solin et al., 1996).

In contrast, the specific signal obtained in rat brain after i.v. injection of [ethyl-^{3}H]RS-79948-197 is high (≈ 7), being approximately twice that previously observed with [^{3}H]RX 821002 (Hume et al., 1992a). The binding is selective for α_2-adrenoceptors and is achieved within a time period commensurate with PET scanning using short-lived radioisotopes. Although caution is required in the extrapolation of rat data to man, with respect to both the extent of non-specific binding and rate of metabolism of the ligand in addition to the species differences detected from in vitro post-mortem material (e.g. Pascual et al., 1992), the results support the development of carbon-11-labelled RS-79948-197, or a closely related analogue, as a

PET ligand for central α_2-adrenoceptors. To this end, Enas et al. (1996) have recently reported the successful synthesis of fluorine-18-labelled RS-15385-FP, (8a*R*,12a*S*,13a*S*)-5,8,8a,9,10,11,12,12a,13,13a-decahydro-3-methoxy-12-(3-fluoropropylsulphonyl)-6*H*-isoquino-[2,1-g][1,6]naphthyridine, which appears to show regional selective receptor-mediated binding.

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