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Myocardial and pulmonary uptake of *S*-1′-[^{18}F]fluorocarazolol in intact rats reflects radioligand binding to β-adrenoceptors

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Abstract

The biodistribution of *S*-(−)-4-(2-hydroxy-3-(1′-[^{18}F]fluoroisopropyl)-aminopropoxy)carbazole ([^{18}F]*S*-fluorocarazolol, a non-selective β-adrenoceptor antagonist) was studied in rats (60 min after ^{18}F injection when specific binding in peripheral organs was maximal). ^{18}F uptake in brain, erythrocytes, heart and lung appeared to be linked to β-adrenoceptors. CGP-20712A and ICI-89,406 inhibited ^{18}F uptake in heart (predominantly β_1-adrenoceptors) more potently than in lungs (predominantly β_2-adrenoceptors). In contrast, ICI-118,551 and procaterol were more potent in the lungs than in the heart. ICI-118,551 inhibited ^{18}F uptake in cerebellum (predominantly β_2-adrenoceptors) more potently than in cerebral cortex (predominantly β_1-adrenoceptors). Stereoselectivity of the in vivo binding was demonstrated since *S*-(−)-propranolol inhibited uptake in target tissues more effectively than *R*-(+)-propranolol. Myocardial and cerebral imaging may be hampered by poor heart-to-lung contrast and low signal-to-noise ratios, but [^{18}F]*S*-fluorocarazolol seems suitable for positron emission tomography (PET) of pulmonary β-adrenoceptors.

Keywords: PET (positron emission tomography); Receptor imaging; β-Adrenoceptor; Radioligand

1. Introduction

Carazolol is a very potent ($K_d \leq 2 \times 10^{-10}$ M), lipophilic β-adrenoceptor antagonist (Cohen et al., 1980; Costin et al., 1983; Innis et al., 1979; Manalan et al., 1981; Lemoine and Kaumann, 1978; Morris and Kaumann, 1979; Morris et al., 1978; Porzig et al., 1982). The compound has been claimed to be selective for the β_2 subtype (Lemoine and Kaumann, 1978), but in later studies these results were not confirmed and a β_2 selectivity of at best 5- to 10-fold was observed (Cohen et al., 1980; Costin et al., 1983; Innis et al., 1979; Manalan et al., 1981). Preliminary studies with tritiated carazolol have indicated the potential usefulness of carazolol as a receptor-binding radiotracer (Eckelman et al., 1980).

An ^{18}F analogue of carazolol has been prepared by reaction of [^{18}F]fluoroacetone or [^{18}F]fluoroisopropyl-tosylate with a desisopropyl precursor (Zheng, 1992; Zheng and Berridge, 1992; Elsinga et al., 1994). Introduction of fluorine in the isopropyl group does not significantly reduce the binding affinity of carazolol to β-adrenoceptors (Zheng, 1992; Zheng and Berridge, 1992). The use of [^{18}F]fluorocarazolol and positron emission tomography (PET) may therefore allow the estimation of β-adrenoceptor density (i.e. both β_1- and β_2-adrenoceptors) in intact experimental animals or in humans.

Previously, we have shown that [^{18}F]*S*-fluorocarazolol meets some of the criteria for quantitative receptor imaging. After intravenous injection in rats, the radioligand is rapidly cleared from blood and avidly taken up by brain, heart, lung, erythrocytes, spleen, submandibular gland and trachea (Elsinga et al., 1994). A significant fraction (45–95%) of the uptake in these organs

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can be blocked by prior treatment of the animals with the β-adrenoceptor antagonist, *RS*-propranolol (Elsinga et al., 1994). Labelled metabolites are formed in the liver and they appear rapidly in the circulation (< 2 min), but are not significantly taken up by the lung and heart (Elsinga et al., 1994).

However, lowered radioligand uptake in the presence of propranolol is not definitive proof that the ligand is bound to β-adrenoceptors. Lipophilic β-adrenoceptor antagonists such as propranolol (Geddes et al., 1979; Pang et al., 1982; Dollery and Junod, 1976; Schneck et al., 1977; Hayes and Cooper, 1971), dihydroalprenolol (Altière et al., 1982) and oxprenolol (Hemsworth and Street, 1981) are known to accumulate in the lung. First-pass uptake in humans can be as high as 75–81% (Geddes et al., 1979; Dargent et al., 1985; Morel et al., 1985). The mechanism underlying lung uptake is not only binding to β-adrenoceptors on the cell surface; there is also a facilitated transport to intracellular compartment(s) by carrier protein(s) (Dollery and Junod, 1976; Hemsworth and Street, 1981). Endothelial cells and alveolar macrophages appear to be involved in pulmonary uptake and retention of propranolol and other basic amines (Vestal et al., 1980; Kornhauser et al., 1980). Uptake of β-blockers via the amine carrier is inhibited by drugs like chlorpromazine, imipramine, desmethylimipramine, nortryptiline, amphetamine and methadone which compete for the same transport mechanism (Hemsworth and Street, 1981; Dollery and Junod, 1976; Vestal et al., 1980; Kornhauser et al., 1980) but not by the β-adrenoceptor agonist isoprenaline (Bryan-Lluka and O'Donnell, 1992). In isolated membranes prepared from bovine lung parenchyma and rat brainstem, dihydroalprenolol has been shown to bind to at least two different 'receptors': β-adrenoceptors and non-β-adrenergic binding sites. Isoprenaline displaces dihydroalprenolol from a single site which is probably the β_2-adrenoceptor. Propranolol displaces dihydroalprenolol from an additional second site which displays no stereoselectivity (Nahorski and Richardson, 1979; Stone and U'Prichard, 1981). This second site may be a protein which transports basic amines in intact cells (Vestal et al., 1980).

For this reason, we have examined the tissue distribution of [^{18}F]*S*-fluorocarazolol in rats that had been treated with various β-adrenoceptor agonists and antagonists, and with chlorpromazine. It was our intention to test whether ligand uptake in the target organs specifically reflects binding to β-adrenoceptors.

2. Materials and methods

2.1. Materials

S-Desisopropyl-carazolol (enantiomeric excess > 98%) was prepared as reported previously (Elsinga et al., 1994). [^{18}F]Fluorocarazolol (specific activity 48 ± 11 TBq [1300 ± 300 Ci]/mmol) was synthesized from [^{18}F]fluoroacetone and *S*-desisopropylcarazolol according to published procedures (Zheng, 1992; Zheng and Berridge, 1992; Elsinga et al., 1994). (±)-CGP-12177 (hydrochloride) and CGP-20712A (sulphate) were kindly donated by Dr. K. Scheibli (Ciba-Geigy, Basel, Switzerland). *RS*-, *R*-(+)- and *S*-(−)-propranolol hydrochloride, (±)-isoprenaline hydrochloride, procaterol hydrochloride and chlorpromazine (hydrochloride) were from Sigma (St. Louis, MO). ICI-89,406 (free base) and ICI-118,551 (hydrochloride) were gifts of Imperial Chemical Industries (Macclesfield, UK).

2.2. Animal handling

The animal experiments were carried out in compliance with the Law on Animal Experimentation of The Netherlands, as described previously (Van Waarde et al., 1992a,b). Rats (219 ± 28 g body weight, all males, Wistar strain) were anesthetized with sodium pentobarbital (60 mg/kg i.p.). A syringe needle was placed into a tail vein and 0.5 ml of saline (0.9% NaCl) was injected, which contained various amounts of receptor-blocking drugs (see Tables 1, 2, 3). Five minutes after the first injection, the radioligand (1 MBq [^{18}F]fluorocarazolol in 0.3 ml saline) was administered via the same route. Body temperature was maintained throughout the experiment with an electric heating pad and was checked with a rectal thermometer. After 60 min, a blood sample was drawn by cardiac puncture and the animals were killed by removal of the heart. A piece of tissue (about 200 mg) was taken from the left ventricular wall near the cardiac apex. A sample of pulmonary parenchyma at the apex of the right lung was also taken, and the entire right kidney was taken out. Other tissues (skeletal muscle (vastus medialis), subcutaneous fat, cerebral cortex, cerebellum, small intestine, pancreas, urinary bladder, spleen, stomach, submandibular gland, testis and trachea) were likewise removed and sampled. Plasma and an erythrocyte pellet were obtained from blood by centrifugation (5 min, 2000 × *g*). The tissue samples were then weighed and radioactivity was determined with a calibrated gamma counter (LKB-Wallac CompuGamma 1282 CS). Uptake of radioactivity by the tissues was expressed as a differential adsorption ratio: (cpm recovered/g tissue)/(cpm injected/g body weight). Differences between the various treatments were analyzed using one-way analysis of variance (ANOVA); a probability < 0.05 was considered statistically significant. Non-specific binding was defined as residual tissue uptake in the presence of 2.5 mg/kg *RS*-(±)-propranolol or 0.15 mg/kg *S*-(−)-propranolol.

3. Results

3.1. Time course of pulmonary ^{18}F uptake

The kinetics of ^{18}F uptake in the right lung of untreated and propranolol-treated rats are shown in Fig. 1. A ratio of total/non-specific binding could be calculated on the assumption that uptake in the presence of propranolol represents non-specific binding. The value of this parameter increases during the first 45 min and reaches a plateau from 50 to 90 min (Fig. 1). The kinetics of ^{18}F uptake in animals treated with ICI-118,551 were similar to those in propranolol-treated rats although the residual pulmonary radioactivity observed at intervals > 45 min post-injection is higher.

Because of these observations, a post-injection interval of 60 min was selected for the biodistribution studies reported in this paper.

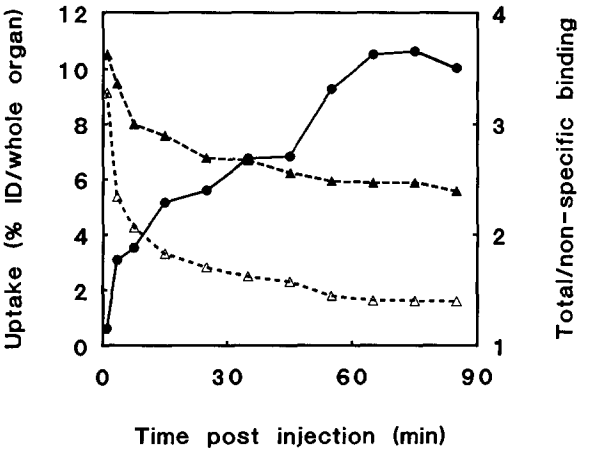

Fig. 1. Uptake of [^{18}F]*S*-fluorocarazolol in the right lung of rats after prior injection of saline (closed triangles) or saline containing propranolol (2.5 mg/kg body weight, open triangles). Uptake was measured with a Siemens ECAT 951/31 PET camera. The ratio of total/non-specific binding (closed circles) is also indicated.

3.2. Blocking of ^{18}F uptake by non-subtype-selective β-adrenoceptor-binding drugs

The results of the experiments in which rats were pretreated with saturating dosages of non-subtype-selective β-adrenoceptor-binding drugs are presented in Table 1.

Treatment of the animals with propranolol (a lipophilic β-adrenoceptor antagonist) caused a strong reduction of ^{18}F uptake in brain (cerebral cortex and cerebellum), heart, lung, erythrocytes, spleen, submandibular gland and trachea, whereas the levels of ^{18}F in plasma and liver increased. CGP-12177 (a hydrophilic β-adrenoceptor antagonist) or isoprenaline (a hydrophilic β-adrenoceptor agonist) had effects similar to those of propranolol, but the brain uptake of ^{18}F in animals treated with these drugs was not significantly

Table 1
Biodistribution of ^{18}F in rats pretreated with non-subtype-selective β-adrenoceptor-binding drugs, 60 min after injection of [^{18}F]*S*-fluorocarazolol

Tissue	Controls ($n = 8$) (saline only)	DL-Propranolol ($n = 7$) (2.5 mg/kg)	CGP-12177 ($n = 5$) (2.5 mg/kg)	Isoprenaline ($n = 7$) (15 mg/kg)
Bladder	1.27 ± 0.35	1.27 ± 0.75	0.97 ± 0.25	0.39 ± 0.10 [f]
Cerebellum	0.85 ± 0.06	0.44 ± 0.12 [f]	0.90 ± 0.17	0.84 ± 0.26
Cortex	1.08 ± 0.15	0.47 ± 0.08 [f]	1.16 ± 0.08	0.96 ± 0.36
Fat	0.24 ± 0.07	0.26 ± 0.08	0.30 ± 0.10	0.22 ± 0.07
Heart	2.68 ± 0.16	0.62 ± 0.11 [f]	0.80 ± 0.30 [f]	0.78 ± 0.14 [f]
Intestine	1.56 ± 0.58	1.74 ± 1.20	0.98 ± 0.33	1.29 ± 0.77
Kidney	1.46 ± 0.24	1.24 ± 0.22	1.13 ± 0.18 [a]	1.44 ± 0.38
Liver	1.90 ± 0.19	2.74 ± 0.41 [f]	2.73 ± 0.12 [f]	2.55 ± 0.77 [a]
Lung	15.86 ± 2.74	1.15 ± 0.13 [f]	2.62 ± 0.17 [f]	4.91 ± 1.60 [f]
Muscle	0.36 ± 0.07	0.38 ± 0.05	0.31 ± 0.06	0.31 ± 0.07
Pancreas	0.61 ± 0.06	0.60 ± 0.16	0.51 ± 0.17	0.46 ± 0.10 [c]
Plasma	0.29 ± 0.03	0.61 ± 0.07 [f]	0.50 ± 0.07 [f]	0.31 ± 0.09
Red blood cells	1.28 ± 0.29	0.45 ± 0.03 [f]	0.40 ± 0.06 [e]	0.39 ± 0.07 [f]
Spleen	2.96 ± 0.48	0.67 ± 0.06 [f]	0.66 ± 0.14 [f]	1.87 ± 0.49 [c]
Stomach	0.61 ± 0.22	0.63 ± 0.25	0.88 ± 0.24	0.89 ± 0.22
Submandibularis	1.80 ± 0.35	0.86 ± 0.14 [f]	1.02 ± 0.27 [c]	1.01 ± 0.28 [c]
Testes	0.35 ± 0.06	0.58 ± 0.15 [c]	0.42 ± 0.11	0.34 ± 0.14
Trachea	0.74 ± 0.10	0.46 ± 0.08 [f]	0.43 ± 0.08 [f]	0.54 ± 0.15 [b]
Bone	0.49 ± 0.10	0.74 ± 0.13 [d]	0.52 ± 0.11	0.48 ± 0.18

Tissue uptake is expressed as a differential adsorption ratio (D.A.R., see Methods) and presented as a mean ± S.D. of n independent observations. Significant differences between control and experimental groups are indicated by the following symbols: [a] $P < 0.05$, [b] $P \leq 0.01$, [c] $P \leq 0.005$, [d] $P \leq 0.001$, [e] $P \leq 0.0005$, [f] $P \leq 0.0001$.

different from the control value. The uptake of radioactivity in adipose tissue, intestine, kidney, muscle, pancreas, stomach, testes and bone was not consistently affected by β-adrenoceptor binding drugs.

In heart, erythrocytes, submandibular gland and trachea, propranolol, CGP-12177 and isoprenaline were equally effective to block ^{18}F uptake. However, in lungs and spleen isoprenaline caused a smaller reduc-

Table 2
Biodistribution of ^{18}F in rats pretreated with propranolol enantiomers or with chlorpromazine, 60 min after injection of [^{18}F]*S*-fluorocarazolol

	S-(−)-Propranolol (0.15 mg/kg) ($n = 4$)	*R*-(+)-Propranolol (0.15 mg/kg) ($n = 4$)	Stereo-selectivity *P*	Chlorpromazine (10 mg/kg) ($n = 5$)
Bladder	1.43 ± 0.48	1.16 ± 0.28		1.07 ± 0.32
Cerebellum	0.45 ± 0.10 [f]	0.81 ± 0.09	< 0.005	1.06 ± 0.05 [f]
Cortex	0.40 ± 0.06 [f]	0.93 ± 0.14	0.001	1.11 ± 0.22
Fat	0.19 ± 0.05	0.19 ± 0.05		0.21 ± 0.04
Heart	0.82 ± 0.24 [f]	2.11 ± 0.20 [e]	< 0.0005	2.14 ± 0.19 [e]
Ileum	0.80 ± 0.31 [a]	0.79 ± 0.25 [a]		0.89 ± 0.29 [a]
Kidney	1.07 ± 0.21 [a]	0.99 ± 0.21 [b]		0.92 ± 0.15 [d]
Liver	3.10 ± 0.51 [e]	1.81 ± 0.28	< 0.01	1.09 ± 0.14 [f]
Lung	2.23 ± 0.42 [f]	8.97 ± 2.49 [c]	< 0.005	12.60 ± 4.64
Muscle	0.30 ± 0.04	0.32 ± 0.05		0.61 ± 0.10 [e]
Pancreas	0.57 ± 0.21	0.48 ± 0.08 [b]		0.73 ± 0.05 [c]
Plasma	0.47 ± 0.10 [d]	0.28 ± 0.03	< 0.05	0.26 ± 0.03 [a]
Red blood cells	0.37 ± 0.12 [e]	0.84 ± 0.17 [a]	< 0.005	1.39 ± 0.23
Spleen	0.71 ± 0.05 [f]	2.04 ± 0.21	< 0.0001	2.77 ± 0.20
Stomach	0.53 ± 0.13	0.39 ± 0.09		1.25 ± 0.49 [b]
Submandibularis	1.45 ± 0.36	1.54 ± 0.24		1.77 ± 0.28
Testes	0.43 ± 0.12	0.31 ± 0.06		0.33 ± 0.06
Trachea	0.45 ± 0.12 [c]	0.80 ± 0.14	< 0.05	0.74 ± 0.04
Bone	0.45 ± 0.04	0.45 ± 0.11		0.75 ± 0.11 [c]

Tissue uptake is expressed as a differential adsorption ratio (D.A.R., see Methods) and presented as a mean ± S.D. of n independent observations. Significant differences between control and experimental groups are indicated by the following symbols: [a] $P \leq 0.05$, [b] $P \leq 0.01$, [c] $P \leq 0.005$, [d] $P \leq 0.001$, [e] $P \leq 0.0005$, [f] $P \leq 0.0001$ (see Table 1 for control values).

Table 3
Biodistribution of ^{18}F in rats pretreated with subtype-selective β-adrenoceptor-binding drugs, 60 min after injection of [^{18}F]*S*-fluorocarazolol

Tissue	CGP20712A ($n = 5$) (2.5 mg/kg)	ICI 89,406 ($n = 5$) (0.15 mg/kg)	ICI 118,551 ($n = 5$) (0.15 mg/kg)	Procaterol ($n = 5$) (0.75 mg/kg)
Bladder	1.12 ± 0.35	0.74 ± 0.34 [a]	1.24 ± 0.56	0.48 ± 0.10 [e]
Cerebellum	0.92 ± 0.30	0.76 ± 0.04 [a]	0.51 ± 0.06 [f]	1.13 ± 0.19 [c]
Cortex	0.99 ± 0.13	0.88 ± 0.07 [a]	0.90 ± 0.28	1.11 ± 0.39
Fat	0.17 ± 0.07	0.18 ± 0.05	0.21 ± 0.04	0.31 ± 0.09
Heart	1.24 ± 0.15 [f]	0.94 ± 0.14 [f]	1.94 ± 0.20 [f]	2.35 ± 0.32
Intestine	0.80 ± 0.24 [a]	0.80 ± 0.34 [a]	0.96 ± 0.23 [a]	1.75 ± 0.64
Kidney	0.82 ± 0.16 [e]	0.77 ± 0.23 [d]	1.28 ± 0.10	1.96 ± 0.34 [d]
Liver	2.00 ± 0.70	2.27 ± 0.31	2.00 ± 0.70	2.03 ± 0.59
Lung	13.30 ± 2.88	7.88 ± 0.48 [e]	3.82 ± 0.34 [f]	10.27 ± 1.82 [c]
Muscle	0.40 ± 0.09	0.38 ± 0.10	0.31 ± 0.05	0.36 ± 0.07
Pancreas	0.62 ± 0.20	0.57 ± 0.19	0.57 ± 0.14	0.75 ± 0.30
Plasma	0.26 ± 0.03	0.26 ± 0.03	0.41 ± 0.03 [e]	0.22 ± 0.03 [d]
Red blood cells	1.25 ± 0.23	1.07 ± 0.20	0.43 ± 0.04 [e]	0.47 ± 0.06 [f]
Spleen	2.71 ± 0.61	1.66 ± 0.25 [e]	1.53 ± 0.18 [f]	2.88 ± 0.68
Stomach	0.51 ± 0.24	0.40 ± 0.11	0.54 ± 0.24	1.27 ± 0.51 [b]
Submandibularis	1.41 ± 0.24 [a]	1.04 ± 0.19 [c]	1.86 ± 0.42	2.12 ± 0.29
Testes	0.32 ± 0.09	0.29 ± 0.02	0.46 ± 0.05 [a]	0.27 ± 0.08
Trachea	0.67 ± 0.18	0.46 ± 0.08 [e]	0.54 ± 0.02 [c]	1.15 ± 0.23
Bone	0.41 ± 0.10	0.37 ± 0.07 [a]	0.54 ± 0.11	0.49 ± 0.16

Tissue uptake is expressed as a differential adsorption ratio (D.A.R., see Methods) and presented as a mean ± S.D. of n independent observations. Significant differences between the control and experimental groups are indicated by the following symbols: [a] $P < 0.05$, [b] $P \leq 0.01$, [c] $P \leq 0.005$, [d] $P \leq 0.001$, [e] $P \leq 0.0005$, [f] $P \leq 0.0001$ (see Table 1 for control values).

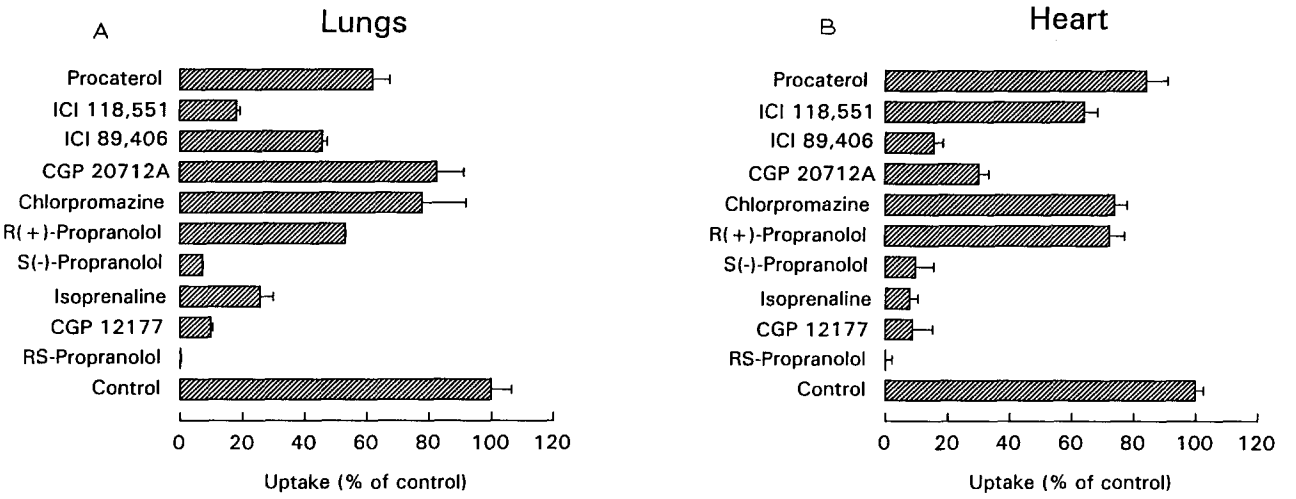

Fig. 2. Cardiac and pulmonary uptake of [^{18}F]*S*-fluorocarazolol, 60 min after injection. The animals had been treated with the listed drugs 5 min before administration of the radioligand. Tissue uptake is expressed as a percentage of the control (after subtraction of the non-specific binding, which was 7% of the total radioactivity in the lungs and 23% in the heart). The data are means ± S.E. of four to eight experiments.

tion of ^{18}F uptake than CGP-12177 ($P = 0.02$ and $P = 0.001$, respectively) or propranolol ($P < 0.0001$ and $P = 0.0001$, respectively).

3.3. Blocking of ^{18}F uptake by chlorpromazine and by the enantiomers of propranolol

The results of the experiments in which rats were pretreated with the enantiomers of propranolol or with chlorpromazine are presented in Table 2. In brain, heart, lung, erythrocytes, spleen and trachea but not in submandibular gland, *S*-(−)-propranolol (0.15 mg/kg) was almost as effective as *RS*-(±)-propranolol (2.5 mg/kg) to inhibit [^{18}F]*S*-fluoro-carazolol uptake. The *R*-(+)-isomer of propranolol (0.15 mg/kg) was a less effective blocker of [^{18}F]*S*-fluorocarazolol binding in these tissues than the *S*-(−)-isomer. Chlorpromazine did not lower ^{18}F uptake in brain, lung, red blood cells, spleen, submandibular gland and trachea but the radioactivity in ileum, kidney and liver was strongly reduced after treatment with this drug. A slight (< 20%) reduction was also observed in the heart.

3.4. Blocking of ^{18}F uptake by subtype-selective β-adrenoceptor-binding drugs

The results of the experiments in which rats were pretreated with subtype-selective β-adrenoceptor-blocking drugs are presented in Table 3. Treatment of the animals with CGP-20712A (a hydrophilic β_1-adrenoceptor antagonist) caused a reduction of ^{18}F uptake in heart and submandibular gland, but not in lung, erythrocytes, spleen, trachea and brain. Administration of ICI-89,406 (a lipophilic β_1-adrenoceptor antagonist) before the radioligand lowered ^{18}F uptake in brain, heart, lung, erythrocytes, spleen, submandibular gland and trachea. Treatment of rats with ICI-118,551 (a lipophilic β_2-adrenoceptor antagonist) reduced ^{18}F up-

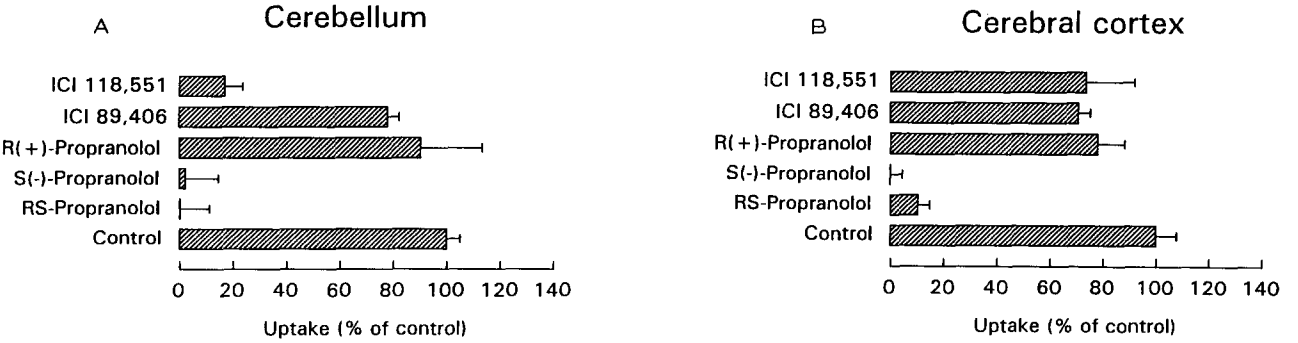

Fig. 3. Cerebral and cerebellar uptake of [^{18}F]*S*-fluorocarazolol, 60 min after injection. The animals had been treated with the listed drugs 5 min before administration of the radioligand. Tissue uptake is expressed as a percentage of the control (after subtraction of the non-specific binding, which was 37% of the total radioactivity in the cerebral cortex and 52% in the cerebellum). The data are means ± S.E. of four to eight experiments.

take in brain, heart, lung, erythrocytes, spleen and trachea, while the plasma levels of ^{18}F were increased. Injection of procaterol (a hydrophilic β_2-adrenoceptor agonist) before the radioligand suppressed ^{18}F uptake in lung and erythrocytes, but not in brain, heart, spleen, submandibular gland or trachea (Table 3).

Cerebellar binding of ^{18}F was reduced more by ICI-118,551 than by ICI-89,406 ($P < 0.0005$, Fig. 3). Pulmonary ^{18}F uptake was more potently blocked by ICI-118,551 than by ICI-89,406 ($P < 0.0001$) or by CGP-20712A ($P = 0.0001$, Fig. 2). Erythrocyte binding of ^{18}F-carazolol was strongly reduced by ICI-118,551, lowered less in the presence of ICI-89,406 ($P < 0.05$) and not at all affected by CGP-20712A ($P = 0.0002$). Myocardial uptake of ^{18}F was higher after treatment with ICI-118,551 than after injection of ICI-89,406 ($P = 0.0002$) or CGP-20712A ($P = 0.0005$, Fig. 2). More ^{18}F was found in the submandibular gland upon administration of ICI-118,551 than after injection of ICI-89,406 ($P < 0.005$).

4. Discussion

4.1. Blocking of ^{18}F uptake with non-subtype-selective β-adrenoceptor-binding drugs

After treatment of rats with propranolol (2.5 mg/kg), the [^{18}F]*S*-fluorocarazolol uptake in brain, heart, lung, erythrocytes, spleen, submandibular gland and trachea was reduced (Table 1). The ^{18}F uptake in these tissues may therefore involve association of fluorocarazolol to β-adrenoceptors. The hydrophilic antagonist, CGP-12177, and the hydrophilic agonist, isoprenaline, reduced [^{18}F]*S*-fluorocarazolol uptake in heart, lung, erythrocytes, spleen, submandibular gland and trachea but not in brain. Hydrophilic drugs probably have little effect on cerebral [^{18}F]*S*-fluorocarazolol uptake because these compounds do not cross the blood-brain barrier.

Isoprenaline is a less effective blocker of ^{18}F uptake in lung and spleen than either CGP-12177 or propranolol. Moreover, CGP-12177 is a less effective blocker of pulmonary ^{18}F uptake than propranolol (Table 1). These data on [^{18}F]*S*-fluorocarazolol uptake contrast with previous findings in our laboratory which showed that isoprenaline, unlabeled CGP-12177 and propranolol cause a similar reduction of the in vivo uptake of *S*-[^{3}H]CGP-12177 (Van Waarde et al., 1992b).

The different effects of isoprenaline, CGP-12177 and propranolol on pulmonary fluorocarazolol uptake and the identical effects of these compounds on pulmonary CGP-12177 uptake may be explained by the different lipophilicity of the radioligands and by agonist-induced receptor internalization.

Hydrophilic [^{3}H]CGP-12177 binds only to β-adrenoceptors on the cell surface (Hertel et al., 1983a; Hertel et al., 1983b; Staehelin and Hertel, 1983; Staehelin et al., 1983; Staehelin and Simons, 1982) whereas lipophilic ligands like [^{125}I]iodopindolol (Hertel et al., 1983b), [^{3}H]dihydroalprenolol (Hertel et al., 1983a; Staehelin and Hertel, 1983; Staehelin et al., 1983) and [^{3}H]carazolol (Staehelin et al., 1983) interact with surface and internalized receptors. Rapid redistribution of pulmonary β_2-adrenoceptors from the cell surface to an intracellular compartment (onset within 5 min, maximum after 10 min) occurs upon administration of isoprenaline (Strasser et al., 1984). Since [^{3}H]CGP-12177 binds only to receptors on the cell surface and since isoprenaline, unlabeled CGP-12177 and propranolol bind to these receptors, the three compounds have similar effects on pulmonary [^{3}H]CGP-12177 uptake. However, [^{18}F]fluorocarazolol will bind both to surface and to internalized receptors as its lipophilicity is comparable to that of carazolol. Propranolol treatment results in blocking of β-adrenoceptors in all subcellular compartments, but treatment with CGP-12177 only blocks the surface receptors. The residual uptake of ^{18}F in lung after injection of [^{18}F]fluorocarazolol was thus higher in rats treated with CGP-12177 than in animals treated with propranolol (Table 1). Isoprenaline, like CGP-12177, binds only to surface receptors. In addition, isoprenaline may induce receptor internalization because it is an agonist. The fraction of internalized receptors will thus increase after isoprenaline administration. Therefore, the residual uptake of ^{18}F in the lung of isoprenaline-treated animals is even greater than in lungs of animals treated with CGP-12177 (Table 1, Fig. 2).

Isoprenaline and CGP-12177 have different effects on [^{18}F]*S*-fluorocarazolol uptake in lung and spleen, but not in heart and red blood cells (Table 1). This may indicate a different rate of receptor internalization in different tissues upon agonist infusion. In Chinese hamster fibroblasts transfected with cDNA encoding either β-adrenoceptor subtype, agonist-induced redistribution of β_2-adrenoceptors is much faster than redistribution of β_1-adrenoceptors (Suzuki et al., 1992).

A higher ^{18}F uptake in the lung of animals treated with isoprenaline (or procaterol) than of animals treated with propranolol (or ICI 118,551) may also be due to transport of fluorocarazolol by an amine carrier. Carrier-mediated uptake can thus be estimated to contribute maximally 16% to the pulmonary signal at 60 min post-injection.

4.2. Blocking of ^{18}F uptake with the enantiomers of propranolol and with chlorpromazine

The *S*-(−)enantiomer of propranolol has an about 100-fold higher affinity to β_1- and β_2-adrenoceptors than the *R*-(+)enantiomer (Tsuchihashi et al., 1990).

Blocking experiments with both enantiomers indicate stereoselectivity of [^{18}F]*S*-fluorocarazolol binding in brain (both cortex and cerebellum), heart, lung, erythrocytes, spleen and trachea, but not in submandibular gland (Table 2). When the effect of *S*-(−)-propranolol (0.15 mg/kg, Table 2) on [^{18}F]fluorocarazolol uptake is compared to that of *RS*-(±)-propranolol (2.5 mg/kg, Table 1) it is clear that significant blocking of [^{18}F]fluorocarazolol uptake in the submandibular gland occurs only at high dosages of propranolol. In the gland, fluorocarazolol may thus bind to non-β-adrenergic binding sites, which have a low affinity for propranolol and which do not display stereoselectivity.

Chlorpromazine inhibits active transport and retention of propranolol and other basic amines in the lung (Hemsworth and Street, 1981; Dollery and Junod, 1976; Vestal et al., 1980; Kornhauser et al., 1980). We have examined the influence of this drug (10 mg/kg) on the biodistribution of [^{18}F]*S*-fluorocarazolol (see Table 2). The ^{18}F uptake in ileum, kidney and liver was strongly reduced, consistent with inhibition of active transport systems (Barber et al., 1980). No inhibition occurred in brain, erythrocytes, spleen, submandibular gland and trachea, which suggests that the amine carrier does not significantly contribute to ^{18}F uptake in these tissues. However, chlorpromazine caused a slight (i.e. 20%) reduction of ^{18}F uptake in the lungs and heart. Due to inter-individual variability, only the change in the heart was statistically significant (Table 2).

4.3. Blocking of ^{18}F uptake with subtype-selective β-adrenoceptor-binding drugs

If a radioligand is associated with β-adrenoceptors in vivo, its tissue uptake should be inhibited by β-adrenoceptor agonists and antagonists which bind to the proper subtype but not by other drugs. In the present study, we have used the following β-adrenoceptor antagonists: (i) ICI-89,406 which is lipophilic and moderately (75-fold) β_1-selective (Janssen and Daniel, 1990); (ii) CGP-20712A which is hydrophilic and highly (1000–10000-fold) β_1-selective (Dooley et al., 1986); (iii) ICI-118,551, which is lipophilic and has 100–300 times higher affinity for β_2- than for β_1-adrenoceptors (Bilski et al., 1983; Lemoine et al., 1985). We also used (iv) procaterol which is a moderately (22-fold) β_2-selective, hydrophilic agonist (Rugg et al., 1978).

Literature data indicate that cerebral cortex, heart and submandibular gland of rats contain predominantly β_1-adrenoceptors, whereas in cerebellum, lung, erythrocytes and spleen β_2-adrenoceptors are more abundant (see Table 4).

The results of our blocking experiments are consistent with the hypothesis that [^{18}F]*S*-fluorocarazolol uptake in the target organs represents binding to β-adrenoceptors. β_2-Selective antagonists (ICI-118,551) and agonists (procaterol) have a more pronounced effect on ^{18}F uptake in tissues containing mainly β_2-adrenoceptors (lung, erythrocytes) than in tissues containing predominantly β_1-adrenoceptors (heart, see Table 3, Fig. 2). In contrast, β_1-selective antagonists (ICI-89,406 and CGP-20712A) have a greater influence on ^{18}F uptake in the heart than on radioactivity in lung or erythrocytes (see Table 3, Fig. 2).

In theory, the contribution of β-adrenoceptor subpopulations to tissue B_{max} can be estimated by determining tissue uptake after three injections of the radioligand: (1) in untreated animals (estimation of total binding); (2) in animals pretreated with a non-selective β-adrenoceptor antagonist like propranolol (estimation of non-specific binding); (3) in animals pretreated with a subtype-selective β-adrenoceptor antagonist (estimation of binding to the subtype in question). The contri-

Table 4
β-Adrenoceptor subpopulations in tissues of the rat

Tissue	Estimated from reduction of the in vivo binding of [^{18}F]fluorocarazolol by propranolol (β_1 plus β_2), CGP-20712A (β_1) and ICI-118,551 (β_2)		Literature values based on in vitro binding assays [a] β_1:β_2 % (Range)
	β_1 %	β_2 %	
Cerebral cortex	n.d.	30	63:37–80:20
Cerebellum	n.d.	83	9:91–20:80
Heart	70	36	59:41–83:17
Lung	17	82	14:86–34:66
Red blood cells	< 4	100	0:100
Submandibularis	46	0	90:10
Spleen	11	62	33:67

The fraction of total β-adrenoceptors belonging to a particular subtype is indicated. n.d. = not determined since CGP-20712A does not cross the blood-brain barrier. [a] Data from: Abrahamsson et al. (1988); Barnett et al. (1978); Beer et al. (1988); Bojanic and Nahorski (1983); Dickinson and Nahorski (1981); Dickinson et al. (1981); Ek and Lundgren (1986); Erdtsieck-Ernste et al. (1991); Joyce et al. (1992); Juberg et al. (1985); Kramer et al. (1986); Kuriyama et al. (1981); Minneman et al. (1979); Nahorski et al. (1979); Rainbow et al. (1984); Rothwell et al. (1985); Rugg et al. (1978); Schneyer and Humphreys Beher (1987); Tiong and Richardson (1989); Vago et al. (1984); Vanscheeuwijck et al. (1989); Winter et al. (1986).

bution of the subpopulation is then: [([Tissue uptake in condition 1] − [Tissue uptake in condition 3])/([Tissue uptake in condition 1] − [Tissue uptake in condition 2]) · 100%.

CGP-20712A and ICI-118,551 have the highest subtype selectivity of all compounds which were used in this study. When the β_1 and β_2 subpopulations are estimated from the [^{18}F]*S*-fluorocarazolol uptake in the presence or absence of these drugs and of propranolol as outlined above, interesting results are obtained which are presented in Table 4. The β_1-subpopulation in brain cannot be estimated from the tissue uptake in the presence of CGP-20712A since this antagonist does not cross the blood-brain barrier (Van Waarde et al., 1992a).

With the exception of spleen (where the β_1-adrenoceptor subpopulation is underestimated after blocking with CGP-20712A for reasons which are not clear) and submandibular gland (where the ^{18}F-fluorocarazolol uptake cannot be stereoselectively blocked and propranolol blocks a greater fraction of ^{18}F uptake than a β_1- and a β_2-adrenoceptor antagonist combined) all the in vivo data are within the range of in vitro determinations of the ratio of the β_1 and β_2 subtypes (Table 4). Thus, the results of the blocking experiments with subtype-selective drugs support the view that [^{18}F]*S*-fluorocarazolol uptake in brain, erythrocytes, heart and lung represents binding to β-adrenoceptors.

4.4. Conclusion

The conclusions of this paper can be summarized in the following way:

(i) Propranolol blocks the uptake of [^{18}F]*S*-fluorocarazolol in cerebellum, cerebral cortex, heart, lung, erythrocytes, spleen and trachea, i.e. in tissues with relatively high densities of β-adrenoceptors.

(ii) Fluorocarazolol uptake can be stereoselectively inhibited in cerebellum, cerebral cortex, heart, lung, red blood cells, spleen and trachea but not in submandibular gland. Inhibition of ^{18}F uptake in submandibular gland occurs at high dosages of propranolol only. Glandular radioactivity may thus represent association of [^{18}F]*S*-fluorocarazolol to non-β-adrenergic binding sites.

(iii) Attempts to block radioligand uptake in the target organs with subtype-selective agonists and antagonists suggest that fluorocarazolol is bound to β-adrenoceptors. The relative sizes of the β_1- and β_2-subpopulations in heart, lung and brain can be estimated from these in vivo data and such estimations are within the range of in vitro assay data for the two subtypes.

(iv) Treatment of animals with hydrophilic and lipophilic β-adrenoceptor-binding drugs causes similar reductions of [^{18}F]*S*-fluorocarazolol uptake in erythrocytes and heart, but not in lung and spleen. Isopreline is a less effective inhibitor than propranolol in the latter tissues. The reason for this discrepancy is not clear. Probably fluorocarazolol and lipophilic antagonists bind to surface and internalized receptors, whereas hydrophilic drugs bind to surface receptors only. It is also possible that an amine carrier contributes to pulmonary ^{18}F uptake; results of blocking experiments with chlorpromazine suggest that its contribution is of relatively minor importance (< 20%).

(v) PET studies of the human heart with fluorocarazolol may prove difficult since PET images from two animal species (rats, sheep) lacked sufficient heart-to-lung contrast (Elsinga et al., 1994). Cerebral PET scans may be hampered by relatively poor signal-to-noise ratios (see Table 1). However, the information in this and the previous (Elsinga et al., 1994) paper suggests that [^{18}F]*S*-fluorocarazolol is suitable for in vivo studies of pulmonary β-adrenoceptors.

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