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A novel β-adrenoceptor ligand for positron emission tomography: Evaluation in experimental animals

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Abstract

Myocardial and pulmonary β-adrenoceptors can be imaged and quantified with the antagonist (*S*)-4-[3[(1,1-dimethylethyl)amino]-2-hydroxypropoxy]-1,3-dihydro-2H-benzimidazol-2-[^{11}C]-one (*S*-[^{11}C]CGP-12177). The synthesis of this ligand (based on the reaction of a precursor with [^{11}C]phosgene) is laborious and in many centers the final product has a low and variable specific activity. This prevents widespread use of *S*-[^{11}C]CGP-12177 for studies in patients. We prepared *S*-[^{11}C]CGP-12388, the isopropyl analogue of CGP-12177, by a reliable one-pot procedure and evaluated the radiopharmaceutical for β-adrenoceptor imaging. Blocking experiments with subtype-selective β-adrenergic drugs showed that myocardial and pulmonary uptake of *S*-[^{11}C]CGP-12388 in anesthetized rats reflects ligand binding to β_1- and β_2-adrenoceptors. In this animal model, clearance, metabolism and tissue/plasma ratios of *S*-[^{11}C]CGP-12388 were similar to those of *S*-[^{11}C]CGP-12177. A [^{18}F]fluoroisopropyl analogue of CGP-12177 showed less favorable characteristics. *S*-[^{11}C]CGP-12388 was therefore selected for evaluation in humans and it may become the tracer of choice for clinical studies since it is easily prepared. © 1998 Elsevier Science B.V.

Keywords: β-Adrenoceptor; PET (positron emission tomography); Lung; Heart; CGP-12177; CGP-12388

1. Introduction

The β-adrenoceptor antagonist (*S*)-4-[3[(1,1-dimethylethyl)amino]-2-hydroxypropoxy]-1,3-dihydro-2H-benzimidazol-2-[^{11}C]-one (*S*-[^{11}C]CGP-12177) is suitable for β-adrenoceptor imaging. It binds with sub-nM affinity to β_1- and β_2-adrenoceptors (Affolter et al., 1985; Nanoff et al., 1987). Because of its hydrophilicity (log *P* octanol:buffer, −0.55 at pH 7.4, (Staehelin et al., 1983; Abrahamsson et al., 1988)), it labels only functional receptors at the cell surface (Hertel and Staehelin, 1983; Hertel et al., 1983; Staehelin and Hertel, 1983; Staehelin et al., 1983) and shows little nonspecific binding (Staehelin and Hertel, 1983; Staehelin et al., 1983).

CGP-12177 is labeled with carbon-11 by reaction of a diamine precursor with [^{11}C]phosgene (Boullais et al., 1986; Hammadi and Crouzel, 1991; Aigbirhio et al., 1992a,b). The product, *S*-[^{11}C]CGP-12177, has been successfully employed for quantification of β-adrenoceptors in the myocardium of humans (Lefroy et al., 1993; Merlet et al., 1993; Choudhury et al., 1996) and dogs (Delforge et al., 1991; Delforge, 1994; Valette et al., 1995) and in human lung (Ueki et al., 1993; Qing et al., 1996a,b). Many diseases, such as asthma, chronic obstructive pulmonary disease (COPD), cystic fibrosis, hypertension and heart failure, are associated with altered β-adrenoceptor densities or an altered coupling of β-adrenoceptors to distal parts of the transduction chain (Bristow et al., 1986; Raaijmakers et al., 1987; Sharma and Jeffery, 1990; Lopes et al., 1991; Bai et al., 1992). PET studies of pulmonary and myocardial β-adrenoceptors are therefore of clinical interest.

Unfortunately, we and several other PET centers have experienced unusually great difficulty in the set-up of a reproducible production of *S*-[^{11}C]CGP-12177. Specific activities of the radioligand are variable and usually very low. During the multi-step synthesis of [^{11}C]phosgene from [^{11}C]carbon dioxide, significant amounts of carrier

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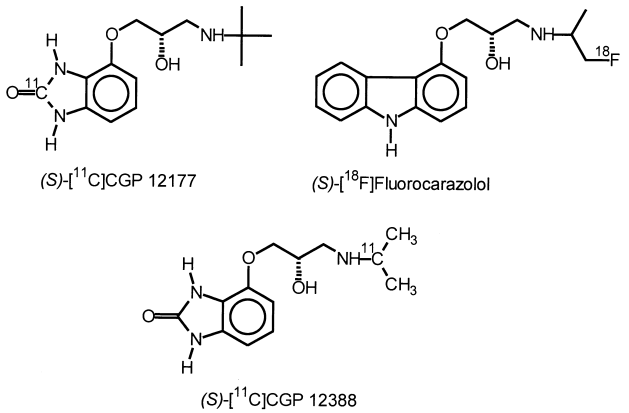

Fig. 1. Chemical structures of antagonist radioligands for β-adrenoceptors.

are introduced. Protracted and systematic attempts to eradicate the source of carrier have not resulted in a reliable synthesis. Yet, reliability is essential for the clinical use of any radiopharmaceutical.

For this reason, we explored possible alternatives to *S*-[^{11}C]CGP-12177. A good candidate for labeling is CGP-12388, the isopropyl analogue of CGP-12177. In functional experiments on isolated guinea pig heart and on the myocardium of the anesthetized cat, CGP-12388 is equally or slightly (<2 fold) less potent than CGP-12177 (Jaeggi et al., unpublished). Moreover, CGP-12388 is as hydrophilic as CGP-12177 (log P, -0.64). In contrast to the laborious synthesis of CGP-12177, *S*-[^{11}C]CGP-12388 can be produced from [^{11}C]acetone by a one-pot procedure (Elsinga et al., 1997).

The present study is an evaluation of this novel ligand in experimental animals. We report the results of biodistribution experiments, PET imaging, subtype-selective β-adrenoceptor blockade, tests on stereoselectivity of the binding, clearance and metabolism. In all in vivo tests, *S*-[^{11}C]CGP-12388 scored comparable to *S*-[^{11}C]CGP-12177 and better than another established β-adrenoceptor ligand, *S*-1′-[^{18}F]fluorocarazolol (Zheng et al., 1994; Elsinga et al., 1996; Visser et al., 1997, see Fig. 1 for chemical structures). Since it can be reliably prepared, *S*-[^{11}C]CGP-12388 may become the radioligand of choice for clinical studies of β-adrenoceptors.

2. Materials and methods

2.1. Materials

S-[^{11}C]CGP-12388 was prepared by reacting a desisopropyl precursor with [^{11}C]acetone (Elsinga et al., 1997). Radiochemical purity of the product was $>99.8\%$ and the specific activity ranged from 22 to 30 TBq (600 to 800 Ci)/mmol. In a few experiments, the fluoroisopropyl analogue of CGP-12388 was produced by reaction of the desisopropyl precursor with [^{18}F]fluoroacetone (Elsinga et al., 1996). The radiochemical purity of this product was also $>99.8\%$ and the average specific activity was 74 TBq (2000 Ci)/mmol. CGP-20712A (sulphate) was kindly donated by Dr. K. Scheibli and CGP-12388 precursor material by Dr. K.A. Jaeggi (both from Novartis, Basle, Switzerland). (±)-Isoprenaline hydrochloride, *RS*-, *R*-(+)- and *S*-(−)-propranolol hydrochloride were from Sigma (St. Louis, MO). ICI-118,551 (hydrochloride) was a gift of Imperial Chemical Industries (Macclesfield, UK).

2.2. Experimental animals

All studies were carried out in compliance with the Law on Animal Experiments of The Netherlands. Rats (235 ± 25 g body weight, males, Wistar strain) were anesthetized by intraperitoneal injection of sodium pentobarbital (60 mg/kg). A needle (0.7×30 mm) was placed in a tail vein and either saline (0.9% NaCl, controls) or saline containing various amounts of β-adrenergic drugs was injected (experimental groups, see Table 1). Immediately (within 1 min) after this injection, the radioligand (0.2 nmol *S*-[^{11}C]CGP-12388, 4.4–6 MBq (120–160 μCi) in saline containing 12% ethanol) was administered via the same route. After 60 min, the thorax and abdomen were opened, a blood sample was drawn and the animals were killed by extirpation of the heart. Plasma and an erythrocyte pellet were obtained from blood by centrifugation (5 min, $2000 \times g$). Samples of the tissues of interest were put into weighed Eppendorf cups. Radioactivity in the samples was determined by gamma counting (LKB/Wallac CompuGamma 1282 CS). Tissue wet weight was assessed by weighing the cups a second time. Tissue uptake was then calculated and expressed as body weight-standardized uptake values or differential absorption ratios: (cpm measured/g tissue)/(cpm injected/g body weight).

In a preliminary PET study with [^{11}C]CGP-12388, we determined the kinetics of [^{11}C]uptake in the lungs of untreated and propranolol-treated rats (Elsinga et al., 1997). A ratio of total/non-specific binding could be calculated on the assumption that uptake in the presence of propranolol represents non-specific binding. This ratio increased during the first 40 min and reached a plateau at about 60 min (Elsinga et al., 1997). Based on these kinetics, a post-injection interval of 60 min was selected for the biodistribution studies reported in this paper.

In experiments which required repeated blood sampling, a canula (PE 50) was placed in the carotid artery of pentobarbital-anesthetized rats. The radioligand (0.2 nmol, 4.4–6 MBq (120–160 μCi) in saline containing 12% ethanol) was administered through a tail vein and arterial blood samples (150–200 μl) were drawn in rapid succession during the first 2 min and subsequently at longer intervals. Plasma was acquired by centrifugation and plasma radioactivity was determined by gamma counting as described above.

Table 1
Biodistribution of [^{11}C]CGP-12388 60 min post injection

Tissue	Untreated animals	Pretreated with *R,S*-propranolol (2.5 mg/kg)		Pretreated with *S*-propranolol (0.15 mg/kg)		Pretreated with *R*-propranolol (0.15 mg/kg)		Pretreated with CGP-20712A (0.15 mg/kg)		Pretreated with ICI 118,551 (0.15 mg/kg)		Pretreated with isoprenaline (15 mg/kg)	
		$n=4$	P	$n=5$	P	$n=4$	P	$n=4$	P	$n=6$	P	$n=4$	P
Bone	0.31±0.06	0.23±0.06	0.05	0.09±0.03	<0.0001	0.23±0.03	<0.05	0.45±0.31	NS	0.07±0.06	<0.0001	0.16±0.10	<0.02
Cerebellum	<0.10	0.26±0.15	0.02	<0.10	NS	<0.10	NS	<0.10	NS	<0.10	NS	<0.10	NS
Cerebral cortex	<0.10	0.25±0.09	<0.005	<0.10	NS	<0.10	NS	<0.10	NS	<0.10	NS	<0.10	NS
Fat	0.28±0.14	0.26±0.06	NS	0.17±0.10	NS	0.14±0.02	NS	0.24±0.02	NS	0.25±0.08	NS	0.20±0.10	NS
Heart	2.71±0.20	0.76±0.23	<0.0001	0.91±0.10	<0.0001	2.54±0.48	NS	1.17±0.11	<0.0001	2.63±0.44	NS	0.96±0.03	<0.0001
Intestine	0.79±0.36	1.14±0.38	NS	1.19±0.44	NS	0.73±0.17	NS	0.91±0.17	NS	1.35±0.82	NS	1.43±0.99	NS
Kidney	1.29±0.36	0.94±0.45	NS	1.06±0.31	NS	1.25±0.64	NS	1.45±0.67	NS	2.07±1.44	NS	12.4±0.9	<0.0001
Liver	0.80±0.12	0.68±0.09	NS	1.16±0.14	<0.002	0.83±0.14	NS	0.91±0.20	NS	0.95±0.12	0.05	1.07±0.22	<0.05
Lung	10.3±1.1	0.88±0.16	<0.0001	1.77±0.24	<0.0001	9.93±2.29	NS	10.4±1.2	NS	5.91±1.40	0.0001	2.02±0.04	<0.0001
Muscle	0.44±0.12	0.36±0.06	NS	0.20±0.02	<0.005	0.37±0.09	NS	0.44±0.07	NS	0.31±0.10	NS	0.41±0.05	NS
Plasma	0.11±0.03	0.40±0.26	<0.05	0.35±0.30	NS	0.13±0.11	NS	0.13±0.03	NS	0.56±0.25	<0.002	0.31±0.06	0.0002
Red blood cells	0.96±0.29	0.41±0.04	<0.02	0.48±0.23	<0.02	0.84±0.63	NS	1.17±0.21	NS	0.64±0.21	0.05	0.52±0.08	<0.05
Spleen	3.11±0.48	0.49±0.05	<0.0001	0.67±0.09	<0.0001	2.82±0.41	NS	2.12±0.71	<0.05	1.52±0.26	<0.0001	0.87±0.12	<0.0001
Trachea	1.25±0.28	0.86±0.19	<0.05	0.45±0.24	<0.001	1.24±0.29	NS	1.21±0.39	NS	0.86±0.28	<0.05	0.67±0.14	<0.01

Mean±standard deviation of n independent observations; data expressed as body-weight-standardized uptake values. Significant differences between control and experimental groups are indicated with probability (P) values (ANOVA).
NS = not significant.

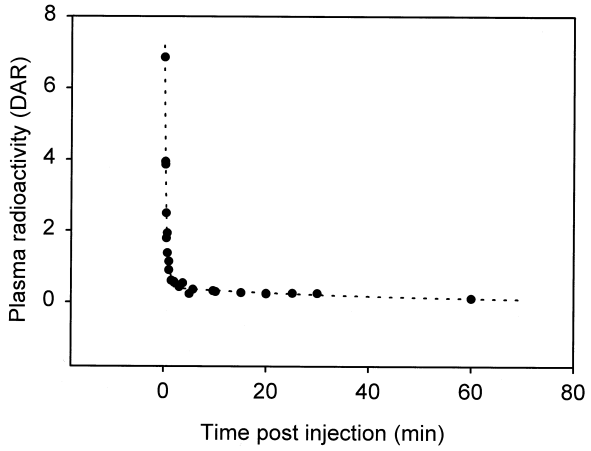

Fig. 2. Clearance of radioactivity from rat plasma after injection of 0.2 nmol *S*-[^{11}C]CGP-12388 (data from 2 rats). The dotted line is a bi-exponential curve fit.

2.3. Analysis of plasma and tissue samples for radioactive metabolites

Plasma samples were deproteinized by addition of 0.1 vol. 70% perchloric acid, vigorous shaking and centrifugation (2 min, $10\,000 \times g$). The supernatant was directly applied to the high-performance liquid chromatography (HPLC) column (Waters Rad-Pak C_{18}, 5 μ provided with a 2 ml sample loop). The mobile phase was 1% triethylamine-acetate pH 4:acetonitrile 85:15 v/v; flow rate 1 ml/min (Van Waarde et al., 1995). Retention time of authentic CGP-12388 was 8 min; time for the dead volume to be voided was 2.5 min. The major metabolite of CGP-12388 eluted at about 6.5 min. Fractions of the eluate (0.5 ml) were collected over a period of 12 min and radioactivity in the fractions was determined with a gamma counter.

Tissues were frozen in liquid nitrogen, powdered with a mortar and homogenized in 6% perchloric acid. Protein was removed by centrifugation, and the acid supernatant was subjected to HPLC as described above.

2.4. PET studies

PET images were acquired with a Siemens ECAT 951/31 positron camera. Data acquisition was performed using a dynamic protocol and in stationary mode. In this mode the in-plane spatial resolution amounts to 6 mm full-width at half-maximum (FWHM). The camera acquires 31 planes simultaneously over a 10.8 cm axial field of view. During image reconstruction a zoom factor of 1.5 was applied and the matrix size was 128×128 pixels. Data analysis was performed using Siemens ECAT software (version 6.3 D) on a Sun workstation.

The animals were anesthetized as described above. The long axis of each rat was positioned parallel to the transaxial plane of the tomograph, to obtain sagittal sections. After a transmission scan, 0.2 nmol *S*-[^{11}C]CGP-12388 (4.4–6 MBq (120–160 μCi) in saline containing 12% ethanol) was administered via a tail vein to untreated animals and animals pretreated with 2.5 mg/kg propranolol (i.v. 5 min before injection of the radioligand). The following frames were defined: 8 of 15 s, 4 of 30 s, 4 of 60 s, 4 of 120 s, 6 of 240 s and 2 of 600 s. The total duration of the study was 60 min. The initial frame rate proved to be too high for acceptable count statistics; thus frames were added and tissue uptake was averaged over 2 min intervals at the beginning of the experiment. Similar experimental protocols were used to study tissue uptake of 0.1–0.2 nmol *S*-[^{18}F]fluoro-CGP 12388, S-[^{11}C]CGP 12177 and *S*-[^{18}F]fluorocarazolol. The pulmonary uptake of these radiotracers was compared to that of *S*-[^{11}C]CGP 12388. Ratios of total/nonspecific binding were calculated at 0–2, 2–4, 4–6, 6–8, 8–10, 10–12, 12–14, 14–16, 16–20, 20–24, 24–28, 28–32, 32–36, 36–40, 40–50 and 50–60 min as (tissue uptake in control animal)/(tissue uptake in propranol-pretreated animal). Three pairs of animals were studied for each tracer.

2.5. Statistical tests

Differences between treatments were examined by 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*-($\pm$)-propranolol. Nonlinear curve fitting was performed on an IBM-compatible PC with commercially available programs (EnzFitter, Elsevier Biosoft; SigmaPlot, Jandel Scientific).

3. Results

3.1. Blocking experiments with non-subtype selective drugs

The results of experiments in which rats were pretreated with non-subtype-selective β-adrenoceptor antagonists are presented in Table 1.

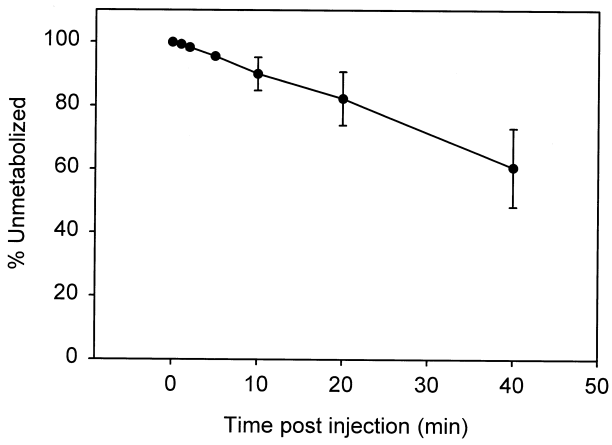

Fig. 3. Fraction of plasma radioactivity representing parent compound after injection of 0.2 nmol *S*-[^{11}C]CGP-12388 (mean $\pm$ S.E.M.).

Table 2
Metabolism of β-adrenoceptor ligands

% Native ligand in:	S-[^{11}C]CGP-12177 (Van Waarde et al., 1995)	S-[^{11}C]CGP-12388 (this paper)	S-1′-[^{18}F]Fluorocarazolol (Elsinga et al., 1996; Van Waarde et al., 1996)
Rat heart (60 min p.i.)	> 90	94 ± 2	83 ± 4
Rat lung (60 min p.i.)	not determined	98 ± 1	92 ± 2
Rat liver (60 min p.i.)	not determined	49	7 ± 3
Rat plasma (60 min p.i.)	67 ± 8	61 ± 9	ca. 2%
Human plasma (60 min p.i.)	> 90	not determined	19 ± 8
Log *P* (octanol:buffer pH 7.4)	−0.49–−0.55	−0.64	+2.2

Treatment of the animals with *RS*-propranolol (a β-adrenoceptor antagonist) reduced the uptake of [^{11}C] in heart, lungs, erythrocytes, spleen and trachea. Plasma radioactivity was increased. Cerebral uptake was minimal and the brain never accumulated radioactivity above plasma levels.

A low dose of the *S*(−)-isomer of propranolol was almost as effective as *RS*(±)-propranolol to inhibit [^{11}C]CGP-12388 uptake. However, the same concentration of the *R*(+)-isomer was completely ineffective and did not inhibit uptake of radioactivity by the target organs (Table 1).

The non-subtype-selective β-adrenoceptor agonist isoprenaline lowered the levels of radioactivity in all target organs and was almost as effective as *RS*-propranolol in inhibiting tissue uptake (Table 1).

3.2. Blocking experiments with subtype-selective drugs

The results of experiments in which rats were pretreated with subtype-selective antagonists are presented in Table 1.

Treatment of the animals with CGP-20712A (a β_1-adrenoceptor antagonist (Dooley et al., 1986)) reduced the uptake of [^{11}C] in the heart and spleen, but not in lungs, erythrocytes and trachea. In contrast, administration of ICI-118,551 (a β_2-adrenoceptor antagonist (Bilski et al., 1983; Lemoine et al., 1985)) inhibited uptake of radioactivity by lungs, erythrocytes, spleen and trachea, but not by the heart. Plasma levels of radioactivity were increased in animals treated with ICI-118,551 but not in rats treated with CGP-20712A.

3.3. Clearance from plasma

Injected S-[^{11}C]CGP-12388 was rapidly cleared from plasma (Fig. 2). A dual exponential function fitted best to these data; 95% of the injected dose (0.2 nmol) disappeared with a half-life of 0.26 min (rapid distribution phase). The remaining 5% was cleared more slowly (elimination phase, half-life 30.9 min).

3.4. Appearance of labeled metabolites and radiolabeled species in target organs

Radiolabeled metabolites of S-[^{11}C]CGP-12388 appeared relatively slowly (Fig. 3). The fraction of plasma radioactivity representing parent compound decreased from 99.9% at time zero to 60.6 ± 8.8% at $t = 40$ min (Fig. 3). Radiochromatograms of tissue extracts made 60 min post injection indicated that heart and lungs contain mainly native [^{11}C]CGP-12388. However, a significant fraction of hepatic radioactivity consists of labeled metabolites (see Table 2).

3.5. PET images

PET images of male Wistar rats clearly showed radioligand binding to pulmonary β-adrenoceptors (Fig. 4). After

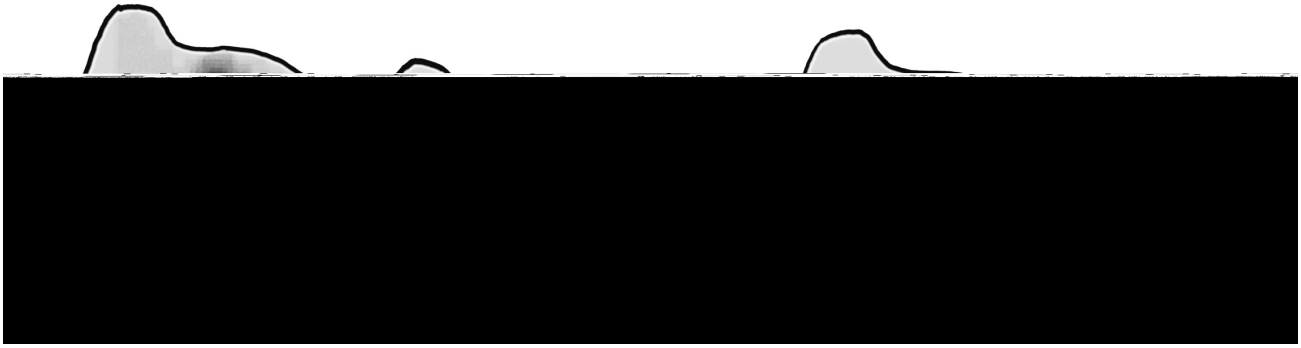

Fig. 4. PET images of male Wistar rats (time frame 50–60 min post injection). Left: untreated control animal, right: propranolol-pretreated animal.

treatment of animals with propranolol, pulmonary uptake was strongly suppressed (see also Table 1) and the lungs were no longer visible (Fig. 4).

4. Discussion

Because the synthesis of *S*-[^{11}C]CGP-12177 from [^{11}C]carbon dioxide via [^{11}C]phosgene was not sufficiently reliable for clinical studies, we prepared the [^{11}C]isopropyl- and [^{18}F]fluoroisopropyl analogs of CGP-12177 ([^{11}C]CGP-12388 and [^{18}F]-fluoro-CGP-12388) and evaluated the suitability of these compounds for β-adrenoceptor imaging. During an early stage of this project, the signal-to-noise ratios of [^{11}C]CGP-12388 were found to be superior to those of [^{18}F]fluoro-CGP-12388. We therefore focused on validation of [^{11}C]CGP-12388 as a receptor-binding radiotracer.

After treatment of rats with *RS*-propranolol (2.5 mg/kg), the uptake of [^{11}C] in heart, lung, erythrocytes, spleen and trachea was reduced (Table 1). Uptake of radioactivity in these target tissues may therefore involve association of CGP-12388 to β-adrenoceptors. As a further proof of receptor binding, we studied the effects of the *R*(+)- and *S*(−)-enantiomers of propranolol. The *S*(−)-enantiomer of this β-adrenoceptor antagonist has about 100-fold higher affinity to β_1- and β_2-adrenoceptors than the *R*(+)-enantiomer (Tsuchihashi et al., 1990). A low dose of the *S*(−)- enantiomer of propranolol (0.15 mg/kg) was about equally effective as a high dose of *RS*-propranolol (2.5 mg/kg) to inhibit uptake in the target organs (Table 1). In contrast, a low dose of the *R*(+)-enantiomer (0.15 mg/kg) was completely ineffective (Table 1). Thus, blocking experiments with the propranolol enantiomers indicated stereoselective competition of propranolol with [^{11}C]CGP-12388 for the same binding sites in tissues which are known to contain β-adrenoceptors.

Additional evidence for receptor binding may be acquired from blocking experiments with subtype-selective β-adrenoceptor antagonists. If a radioligand is associated with β-adrenoceptors, its tissue uptake should be inhibited by compounds which bind to the proper subtype but not by other drugs. Rat heart contains mainly (83%, Minneman et al., 1979) β_1-adrenoceptors. In contrast, lung, erythrocytes and trachea possess mainly β_2-adrenoceptors (respectively 85, 100, and $>$ 85%, see Minneman et al., 1979; Dickinson et al., 1981). Rat spleen contains significant populations of both β-adrenoceptor subtypes (β_1:β_2 35:65, Barnett et al., 1979). In the present study, we have used CGP-20712A which is highly (1000–10 000-fold) β_1-selective (Dooley et al., 1986) and ICI-118,551 which has 100–300 times higher affinity to β_2- than to β_1-adrenoceptors (Bilski et al., 1983; Lemoine et al., 1985). CGP-20712A inhibited the uptake of [^{11}C]CGP-12388 only in heart and spleen. In contrast, ICI-118,551 inhibited radioligand uptake in lung, erythrocytes, spleen and trachea but not in the heart (Table 1). These results are consistent with in vivo labeling of the β_1- and β_2-subtypes of adrenoceptors by *S*-[^{11}C]CGP-12388. Similar results were obtained in blocking experiments with the radioligand *S*-[^{3}H]CGP-12177 (Van Waarde et al., 1992b).

Clearance, metabolism and tissue kinetics of *S*-[^{11}C]CGP-12388 in Wistar rats were comparable to those of *S*-[^{11}C]CGP-12177 (Figs. 2 and 3; Tables 2 and 3). After injection of 0.15 nmol *S*-[^{3}H]CGP-12177, 97% of the injected dose disappeared from plasma with a half-life of 0.18 min and the remaining 3% with a half-life of 17 min (Van Waarde et al., 1992a). Corresponding values for 0.2 nmol *S*-[^{11}C]CGP-12388 were: 95% with a half-life of 0.26 min and 5% with a half-life of 30.9 min. After injection of CGP-12177, the fraction of unmetabolized ligand in rat plasma decreased from $>$ 99.8% at time zero to 67% at $t = 80$ min (Van Waarde et al., 1995; Luthra et

Table 3
Properties of the beta-adrenoceptor ligands

	S-[^{11}C]CGP-12177	*S*-[^{11}C]CGP-12388	*S*-1-[^{18}F]fluoro-CGP 12388	*S*-1-[^{18}F]fluorocarazolol
Practical yield (GBq) (EOS), not corrected for decay)	1.85	1.85	0.37	0.37
Specific activity (TBq/mmol)	0.37–18.5, often very low	22–30	74 or greater	74 or greater
Physical half-life (min)	20.4	20.4	109.8	109.8
Affinity β_1-subtype (nM)	0.33[a]	sub-nM[b]	not determined	0.41[c]
Affinity β_2-subtype (nM)	0.90[a]	sub-nM[b]	not determined	0.10[c]
Total/nonspecific binding 10 min post injection[d]	3.2[e]	4.4	3.1	2.9[f]
Total/nonspecific binding 60 min post injection[d]	7.1[e]	7.2	1.8	4.9[f]

[a] Data from Nanoff et al. (1987).
[b] Equally or slightly ($<$ 2-fold) less potent as CGP-12177 in functional tests (Jaeggi, not previously published).
[c] Data from Van Waarde et al. (1997).
[d] In rat lung, calculated from PET images of untreated and propranolol-pretreated rats (see Section 2).
[e] Van Waarde, not previously published.
[f] Data from Elsinga et al. (1996).

al., 1992). Corresponding values for CGP-12388 were: 99.9% at time zero and 61% at $t = 40$ min.

Data on the metabolism of β-adrenoceptor ligands prepared in our laboratory are presented in Table 2. It is evident that the hydrophilic ligands, *S*-[^{11}C]CGP-12177 and *S*-[^{11}C]-CGP-12388 are relatively slowly metabolized, in contrast to the lipophilic ligand *S*-1′-[^{18}F]fluorocarazolol.

Ratios of total/nonspecific binding in rat lung can be calculated on the assumption that tissue uptake in propranolol-pretreated animals represents nonspecific binding. From PET images of untreated and propranolol-treated rats, we determined such ratios after injection of 0.1–0.2 nmol of four different radiotracers: *S*-[^{11}C]CGP-12177, *S*-[^{11}C]CGP-12388, *S*-1′-[^{18}F]fluorocarazolol and [^{18}F]fluoro-CGP-12388 (see Table 3). The fraction of specific binding rapidly increased and reached a relatively stable plateau when CGP-12177, CGP-12388 or fluorocarazolol were administered. However, in the case of [^{18}F]fluoro-CGP-12388, an initial increase was followed by a continuous decline at intervals greater than 5 min post injection. Maximal ratios of total/nonspecific binding were ca. 7 in the case of CGP-12388 and CGP-12177, but only 5 in the case of fluorocarazolol (Table 3).

In biodistribution studies, cardiac uptake at 60 min post injection was 2.7 ± 0.2 for CGP-12388 (Table 1) and 3.0 ± 0.3 for CGP-12177 (Van Waarde et al., 1992a); these values were reduced to 0.8 ± 0.2 and 0.9 ± 0.2 after treatment of animals with *RS*-propranolol. Pulmonary uptake at 60 min post injection was 10 ± 1 for CGP-12388 (Table 1) and 14 ± 3 for CGP-12177 (Van Waarde et al., 1992a); propranolol-treatment lowered these values to 0.9 ± 0.2 and 2.0 ± 0.3.

Thus, biodistribution studies indicated similar uptake of CGP-12177 and CGP-12388 in the target organs and slightly superior ratios of specific/nonspecific binding for CGP-12388. PET images showed similar ratios of total/nonspecific binding in rat lung after injection of CGP-12177 and CGP-12388. Lower ratios were observed after injection of fluorocarazolol. Specific binding of these antagonists in rat lung increased during a period of 40–45 min and then reached a relatively stable plateau. Totally different kinetics were observed after injection of the fluoro analog of CGP-12388. Here, the ratio of total/nonspecific binding initially increased to a maximum of 3.5 which was reached at 5 min post injection and was followed by a rapid decay. This result may indicate that fluoro-CGP-12388 has intrinsic sympathomimetic activity, i.e. that on binding the receptor is transformed from a high-affinity state into a state with low affinity for the radioligand. The relatively poor ratio of total/nonspecific binding and the transient nature of the binding of fluoro-CGP-12388 suggest that this ligand will not be very suitable for PET studies in humans.

In conclusion, CGP-12388 shows similar in vivo behavior as CGP-12177 but it is much more easily prepared. The facile and reliable preparation of *S*-[^{11}C]CGP-12388 could make it the tracer of choice for clinical studies of β-adrenoceptors.

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