

High non-specific binding of the β_1 -selective radioligand $2\text{-}^{125}\text{I}\text{-ICI-H}$

B. Riemann¹, M. P. Law^{1,2}, K. Kopka¹, St. Wagner¹, S. Luthra³, V. W. Pike⁴, J. Neumann⁵, U. Kirchhefer⁵, W. Schmitz⁵, O. Schober¹, M. Schäfers¹

Department of ¹Nuclear Medicine (Head: Prof. Dr. O. Schober), ⁵Institute of Pharmacology and Toxicology (Head: Prof. Dr. W. Schmitz), Münster University, Germany

²MRC Clinical Sciences Centre, Imperial College School of Science, Technology and Medicine (Head: Prof. Dr. P. G. Camici), ³Imaging Research Solutions Limited, Cyclotron Building (Head: Prof. Dr. J. Thornback), Hammersmith Hospital, London, United Kingdom

⁴Molecular Imaging Branch, National Institute of Mental Health, National Institute of Health, Bethesda, MD, USA

Keywords

SPECT, PET, β_1 -adrenoceptor radioligands, cardiac imaging

Summary

Aim: As results of cardiac biopsies suggest, myocardial β_1 -adrenoceptor density is reduced in patients with chronic heart failure. However, changes in cardiac β_2 -adrenoceptors vary. With suitable radiopharmaceuticals single photon emission computed tomography (SPECT) and positron emission tomography (PET) offer the opportunity to assess β -adrenoceptors non-invasively. Among the novel racemic analogues of the established β_1 -selective adrenoceptor antagonist ICI 89.406 the iodinated 2-ICI-H showed high affinity and selectivity to β_1 -adrenoceptors in murine ventricular membranes. The aim of this study was its evaluation as a putative subtype selective β_1 -adrenergic radioligand in cardiac imaging. **Methods:** Competition studies in vitro and in vivo were used to investigate the kinetics of 2-ICI-H binding to cardiac β -adrenoceptors in mice and rats. In addition, the radiosynthesis of $2\text{-}^{125}\text{I}\text{-ICI-H}$ from the silylated precursor 2-SiMe₃-ICI-H was established. The specific activity was 80 GBq/ μmol , the radiochemical yield ranged from 70 to 80%.

Results: The unlabelled compound 2-ICI-H showed high β_1 -selectivity and -affinity in the in vitro competition studies. In vivo biodistribution studies apparently showed low affinity to cardiac β -adrenoceptors. The radiolabelled counterpart $2\text{-}^{125}\text{I}\text{-ICI-H}$ showed a high degree of non-specific binding in vitro and no specific binding to cardiac β_1 -adrenoceptors in vivo. **Conclusion:** Because of its high non-specific binding $2\text{-}^{125}\text{I}\text{-ICI-H}$ is no suitable radiotracer for imaging in vivo.

Nuklearmedizin 2003; 42: 173–80

Schlüsselwörter

SPECT, PET, β_1 -Adrenoceptor Radioliganden, kardiale Bildgebung

Zusammenfassung

Ziel: Bei chronischer Herzinsuffizienz existieren biophysische Hinweise auf eine Verminderung der kardialen β_1 -Adrenozeptoren bei variablen Ergebnissen für die β_2 -Adrenozeptoren. SPECT und PET ermöglichen mit geeigneten Radiopharmaka, die β -Adrenozeptoren nicht invasiv zu quantifizieren. Von den neu synthetisierten racemischen Derivaten des β_1 -selektiven Adrenoceptor-Antagonisten ICI 89.406 zeigte in Untersuchungen an Membranpräparaten des Mausventrikels das iodierte Derivat 2-ICI-H eine hohe β_1 -Affinität und -Selektivität. Ziel dieser Studie war die Evaluation von 2-ICI-H als möglichen subtypeselektiven β_1 -Adrenoceptorradioliganden der kardialen Bildgebung. **Methoden:** Die Bindungskinetik des 2-ICI-H an β -Adrenozeptoren wurde in vitro und in vivo an Mäusen und Ratten in Konkurrenzstudien untersucht. Die Radiosynthese von $2\text{-}^{125}\text{I}\text{-ICI-H}$ aus dem silylierten Vorläufer 2-SiMe₃-ICI-H wurde etabliert (spezifische Aktivität: 80 GBq/ μmol ; radiochemische Ausbeute: 70-80%). **Ergebnisse:** Nicht radioaktives 2-ICI-H zeigte in In-vitro-Konkurrenzstudien eine hohe β_1 -Selektivität und -Affinität. Allerdings ergaben In-vivo-Biodistributionsstudien eine geringe apparente Affinität zu den kardialen β -Adrenozeptoren. Die entsprechende radioiodierte Verbindung ($2\text{-}^{125}\text{I}\text{-ICI-H}$) wurde in vitro fast und in vivo als völlig unspezifisch an kardiale β_1 -Adrenozeptoren gebunden. **Schlussfolgerung:** Die Vorläufersubstanz 2-SiMe₃-ICI-H wurde mit Iod-125 markiert, um den β_1 -Adrenoceptorantagonisten $2\text{-}^{125}\text{I}\text{-ICI-H}$ zu erhalten, der wegen seiner unspezifischen Bindung in vivo als Radiotracer ungeeignet scheint.

Hohe unspezifische Bindung des β_1 -selektiven Radioliganden $2\text{-}^{125}\text{I}\text{-ICI-H}$

Since the first classification of β -adrenoceptors three distinct subtypes (β_1 , β_2 , β_3) were identified and a fourth one was postulated (4):

- β_1 -adrenoceptor agonists increase heart rate and myocardial contractibility.
- β_2 -selective adrenoceptor agents cause vasodilation and bronchodilation (3).
- The so called atypical β_3 -adrenoceptors are involved in lipolysis (1).
- The putative subtype β_4 -adrenoceptor was identified in cardiac tissue (2).

In the healthy heart, β_1 -adrenoceptors play the major role in the adrenergic control of myocardial function. Biopsies and studies post mortem showed that in a number of heart diseases decreased myocardial β -adrenoceptor density is decreased with a proportionally more pronounced down-regulation of β_1 -adrenoceptors (3, 29). Imaging techniques, either single photon emission computed tomography (SPECT) or positron emission tomography (PET), with appropriate radioligands offer the possibility of assessing β -adrenoceptor density non-invasively in humans (19, 36).

Ligands such as radioiodinated carazolol or iodinated derivatives of CGP 12177 were prepared for imaging β -adrenoceptors with SPECT (7, 32). (S)-¹¹C-CGP 12177 was used with PET to quantify myocardial β -adrenoceptor density in the diseased heart (24, 25, 38). Recently, (S)-¹¹C-CGP 12388 was presented as a more easily synthesized radioligand for β -adrenoceptors (8, 9). None of these radioligands, how-

ever, are selective for distinct β -adrenoceptor subtypes.

The development of radioligands selective for β -adrenoceptor subtypes would offer the possibility of measuring the changes in the distribution of subtypes as well as the total β -adrenoceptor density. Although studies of isolated myocardial membranes showed that the β_1/β_2 -adrenoceptor ratio changes in heart disease, subtype selective radioligands are not available for routine use in medical imaging (4). A few β_1 -adrenoceptor selective radioligands, such as (+/-)- ^{11}C -HX-CH 44 (31) and (S)- ^{11}C -bisoprolol (27), however, are in development for PET (19).

The compound ICI 89.406 **1a** (18) is an example for a designed β_1 -selective adrenoceptor antagonist. It provokes effective β -adrenoceptor blockade during exercise in patients suffering from angina pectoris (14). It is characterized by high affinity and selectivity for β_1 -adrenoceptors as well as by moderate lipophilicity. Furthermore, it is amenable to labeling with ^{11}C , ^{18}F , or ^{123}I . We predicted that this molecule can be modified by radiolabelling of either of two regions without loss of β_1 -adrenoceptor affinity. It was selected, therefore, as basic compound for the development of new radiotracers for nuclear medical imaging.

Although it is known that the (S)-enantiomers of compounds with a 1-amino-3-aryloxy-2-propanol substructure are more potent β -adrenoceptor ligands than the corresponding (R)-enantiomers (16) we synthesized a number of racemic compounds with a 1-amino-3-aryloxy-2-propanol core in order to obtain a diverse range of compounds in a cost-effective and fast manner (data not shown).

Different substituents were inserted within both aromatic systems of ICI 89.406 **1a**. Affinities and selectivities were determined by using in vitro competition studies using ^{125}I -iodocyanopindolol (^{125}I -ICYP) with mouse ventricular membrane preparations. As a result we identified 2-I-ICI-H **1b** as potential ligand for in vivo use. After radiolabeling with ^{123}I and ^{11}C it might turn out as suitable for cardiac SPECT and PET imaging, respectively.

The aim of the study presented here was the evaluation of 2-I-ICI-H **1b** as putative subtype selective β_1 -adrenergic radioligand for cardiac imaging. Competition studies with (-)- ^3H CGP 12177 performed in vivo in rats confirmed the results obtained in vitro. Subsequently, the radiolabelled 2- ^{125}I -ICI-H **1d** was prepared to assess its binding to isolated myocardial membranes in vitro and its biodistribution in tissues after intravenous injection into rats.

Material, animals, methods

Reagents, synthesis of β_1 -adrenoceptor antagonists

(\pm)-CGP 12177-HCl was supplied by Tocris Cookson (Bristol, UK). (-)- ^3H -CGP 12177 was purchased from Amersham Pharmacia Biotech (Amersham, UK). ^{125}I -CYP was obtained from Perkin Elmer Life Sciences (Boston, USA). ^{125}I -NaI for the radioiodination of the precursor compound was obtained from Amersham Pharmacia Biotech (Amersham, UK) with a specific activity >0.6 TBq/mg iodide. All other chemicals, reagents and solvents were

of analytical grade and purchased from commercial sources.

The established β_1 -adrenoceptor antagonist ICI 89.406 **1a** (N-[2-[3-(2-cyano-phenoxy)-2-hydroxy-propylamino]-ethyl]-N'-phenyl-urea) (11, 20) and its iodinated analogue 2-I-ICI-H **1b** (N-[2-[3-(2-iodophenoxy)-2-hydroxy-propylamino]-ethyl]-N'-phenyl-urea) (Fig. 1) were prepared from N-(2-amino-ethyl)-N'-phenyl-urea hydrochloride, NaOH (10 mol/l), 2-(2-cyano-phenoxy)methyl-oxirane and 2-(2-iodo-phenoxy)methyl-oxirane, respectively. Synthesis was according to the procedure published elsewhere (18).

Preparation of 2- ^{125}I -ICI-H **1d** and myocardial membranes

2- ^{125}I -ICI-H **1d**, N-[2-[3-(2-[^{125}I]iodo-phenoxy)-2-hydroxy-propylamino]-ethyl]-N'-phenyl-urea, was prepared from the precursor 2-SiMe₃-ICI-H **1c**, N-[2-[3-(2-trimethylsilyl-phenoxy)-2-hydroxy-propylamino]-ethyl]-N'-phenyl-urea (39), by iododemetalation reaction with ^{125}I -NaI. 10 MBq n.c.a. ^{125}I -NaI and N-chlorosuccinimide (NCS) were added to 2-SiMe₃-ICI-H **1c** in acetic acid/NaOAc buffer, following known radioiodination procedures (12). The radiochemical purity of 2- ^{125}I -ICI-H **1d** was $>95\%$. HPLC analysis of the prepared radioligand did not show any radiochemical impurities within the γ -range and only the injection peak was detectable within the UV-range. The authenticity of the radiochemical product **1d** was confirmed by the addition of a small amount of the cold counterpart **1b** to the γ -fraction of **1d** and co-injection onto the HPLC column. After verification of the authenticity of the radiosynthesis the product fraction was evaporated to dryness and redissolved in varying volumes of a physiological buffer (10 nmol/l Tris/HCl, 154 nmol/l NaCl, 0.1 mmol/l ascorbic acid, pH 7.4) for pharmacological studies.

Membranes were prepared by homogenizing ventricles from DBA mice at 4°C for 90 s in 1 ml of a buffer A (pH 7.4) containing EDTA (10 mmol/l), HEPES (10 mmol/l), benzamidine (0.1 mmol/l), using a Polytron PT 3000 (Kinematica,

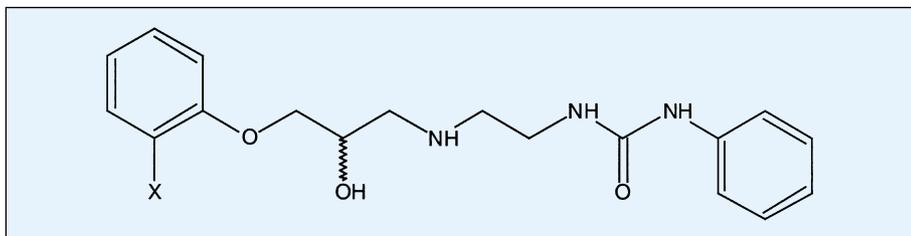


Fig. 1 Chemical structure of β_1 -adrenoceptor antagonists and precursors; X = CN (compound **1a**): ICI 89.406); X = I (compound **1b**): 2-I-ICI-H; X = SiMe₃ (compound **1c**): 2-SiMe₃-ICI-H; X = ^{125}I (compound **1d**): 2- ^{125}I -ICI-H

Lucerne, Switzerland) (15). Homogenates were centrifuged at 45 000 g for 15 min at 4°C. The pellets were resuspended in 1 ml of buffer B (pH 7.4) containing EDTA (1 mmol/l), HEPES (10 mmol/l), benzamidine (0.1 mmol/l) and recentrifuged at 45000 g for 15 min at 4°C. The pellets were resuspended in 1 ml of buffer B and centrifuged at 10000 g for 10 min at 4°C. The supernatants were recentrifuged at 45000 g for 15 min at 4°C. The pellets, partially enriched with membranes, were resuspended in buffer C (pH 7.4) containing Tris/HCl (50 mmol/l), MgCl₂ (5 mmol/l) and stored at -80°C.

Binding experiments and competition studies in vitro

The prepared membranes were resuspended in buffer D (pH 7.4) consisting of Tris/HCl (10 mmol/l), NaCl (154 mmol/l), ascorbic acid (0.1 mmol/l) for the measurement of β -adrenoceptor density using ¹²⁵I-CYP. Preliminary studies were carried out to determine the optimal protein content and incubation time of the binding assays. In all subsequent studies 15 μ g membrane protein were incubated with ¹²⁵I-CYP at 37°C for 60 min. Total β -adrenoceptor density was determined by incubating 15 μ g of membrane protein with increasing concentrations of ¹²⁵I-CYP (1-300 pmol/l) in buffer D. Non-specific binding was measured in the presence of alprenolol 20 μ mol/l). Reactions were stopped by filtering onto Whatman GF/B filters, washed, and the membrane bound activity was determined in a γ -counter. The maximum number of binding sites (B_{max}) and the dissociation constants (K_D) were calculated from plots according to the method of Scatchard (23).

The biological profile of the compound 2-I-ICI-H **1b** at β_1 - and β_2 -adrenoceptors was assessed. For these studies, membranes were incubated with a constant concentration of ¹²⁵I-CYP (80 pmol/l) and with varying concentrations of 2-I-ICI-H **1b** (1 pmol/l-100 μ mol/l). Competition binding curves were analyzed by nonlinear regression analysis as previously described (6, 10).

Statistical analysis was performed using Student's t test; $p < 0.05$ was considered as statistically significant.

Animals

Adult male Sprague Dawley rats (250-320 g) were used. Experiments were carried out by licensed investigators in accordance with the British Home Office's recommendations in "Guidance on the Operation of the Animals (Scientific Procedures) Act 1986" (HMSO, February 1990). Rats were anaesthetised by isoflurane/N₂O/O₂ and catheters (o.d. 1 mm) were inserted into the ventral tail artery and one lateral tail vein of each rat (13). Animals were allowed to recover from the anaesthesia for 2-3 h. During the studies rats were conscious but under light restraint.

Radioligand or unlabelled antagonist was injected as a bolus (1 μ l/g body weight) via the tail vein. Aliquots of each injectate were diluted in ethanol/saline and measured to determine the radioactivity injected into each animal. Six sequential arterial blood samples (~100 μ l) were drawn from each animal. An aliquot of whole blood was taken and the remainder centrifuged to separate the plasma. Animals were sacrificed by intravenous injection of sodium pentobarbitone (Euthatal) at 200 mg/kg body weight at 20 min and tissues rapidly removed.

The thorax was opened by incision on each side of the sternum. The heart and lungs were removed together. The heart was dissected into 5 regions (left and right atrial walls, left and right ventricular walls, interventricular septum) excluding the heart valves. The individual lobes of the lung were separated and samples (~50 mg) taken from each lobe were combined. Samples (100-200 mg) from liver, kidney, and striated muscle were also removed. A sample of urine was taken out of the bladder. Tissue samples were blotted and transferred to weighed vials for reweighing and measurement of radioactivity.

In vivo competition curves, biodistribution

Unlabelled drugs were dissolved in ethanol/saline (50/50, v/v). Appropriate concentrations of each antagonist were mixed with (\pm)-CGP 12177·HCl diluted in ethanol/saline to give final concentrations of unlabelled antagonists ranging from 1 nmol/ml to 10 μ mol/ml with (-)³H-CGP 12177 at ~1.48 MBq/ml, ~0.7 nmol/ml.

In one experiment, (-)³H-CGP 12177 was injected as a bolus (~0.7 nmol/kg, 1 μ l/g body weight) via the tail vein 5 min after pre-dosing animals with a large dose (10 μ mol/kg, 1 μ l/g) of each unlabelled antagonist (unlabelled (\pm)-CGP 12177·HCl, ICI 89.406 **1a** or 2-I-ICI-H **1b**). In all other experiments, (-)³H-CGP 12177, either in ethanol/saline or mixed with increasing concentrations of each unlabelled antagonist, (\pm)-CGP 12177·HCl, ICI 89.406 **1a** or 2-I-ICI-H **1b**, was injected as a bolus (1 μ l/g body weight) via the tail vein. Radioactivity in tissue samples was determined using an automated liquid scintillation counter (Beckman LS 6500). Radioactivity was expressed as dpm/g wet tissue.

Previous experiments (13) showed that tissue uptake of radioactivity was proportional to the radioactivity injected if the amount of (-)³H-CGP 12177 was <1 nmol/g wet tissue. Therefore, to correct for differences in animal body weight and injected dose, results were expressed as uptake index, defined as

$$\text{radioactivity}_{\text{tissue}}/\text{tissue wet weight} : \text{radioactivity}_{\text{injected}}/\text{body weight}.$$

2-[¹²⁵I]I-ICI-H **1d** (0.7-4.5 MBq/kg body weight) was injected as a bolus (1 μ l/g body weight) via the tail vein (13, 35, 37). Aliquots of each injectate were diluted in ethanol/saline and measured to determine the radioactivity injected into each animal. Animals were given unlabelled 2-I-ICI-H **1b** (1 μ mol·kg⁻¹) or ethanol:saline, the vehicle for injection, 5 min before injection of the radioligand. Six sequential arterial blood samples (~100 μ l) were taken from each animal at selected times after injection of radioligand. An aliquot of whole blood was taken and the remainder centrifuged to separate the plasma. Animals

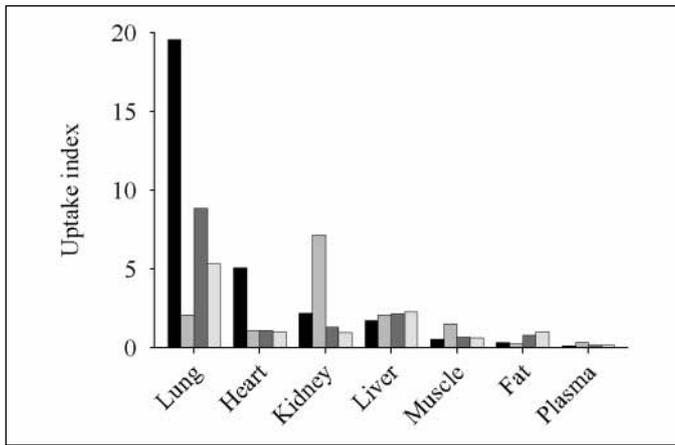


Fig. 2 Radioactivity in tissues 20 min after intravenous injection of (-)- ^3H -CGP 12177 (0.7 nmol/kg) into rats 5 min after injection (i.v.) of: ethanol/saline (black column), unlabelled (\pm) CGP 12177 (1 $\mu\text{mol}/\text{kg}$) (light gray), unlabelled ICI 89.406 1a (10 $\mu\text{mol}/\text{kg}$) (dark gray), unlabelled 2-I-ICI-H 1b (10 $\mu\text{mol}/\text{kg}$) (white)

were killed at 5-90 min and tissues rapidly removed.

In the first experiment (6 rats), the head was removed by large scissors, the brain separated from the cranium, and brain regions dissected. Brain dissection was not carried out in subsequent experiments (12 rats), but the thyroid was removed.

Other tissues were dissected as described above. Tissue samples were blotted and transferred to weighed vials for reweighing and measurement of radioactivity using an automated γ -counter (Wallac Wizard 3[™], Perkin Elmer Life Sciences, Boston, USA). Radioactivity was expressed as cpm/g wet tissue.

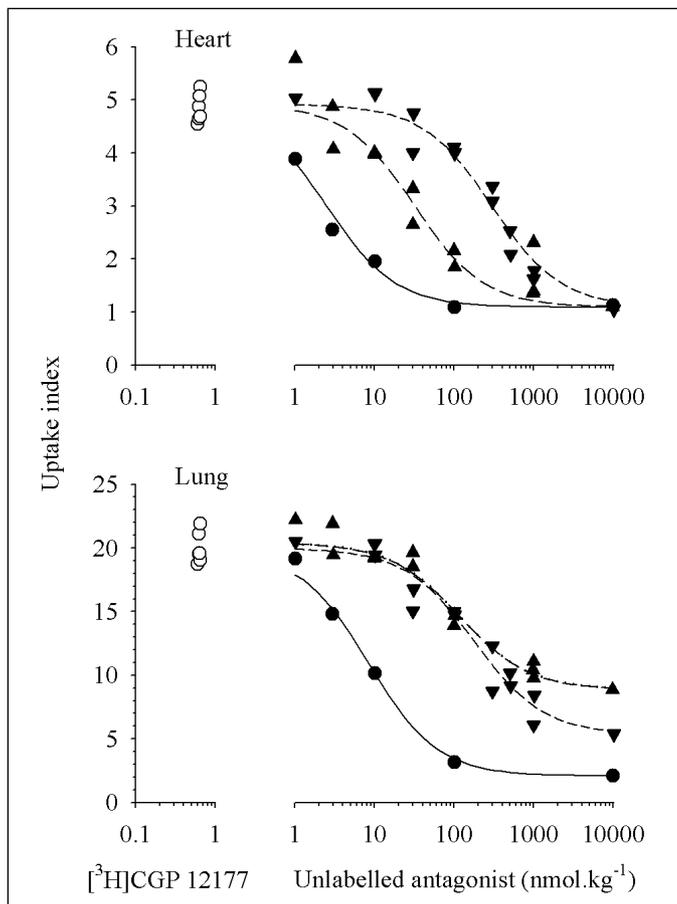


Fig. 3 Uptake of radioactivity in myocardium and lung of rat at 20 min after co-injection (i.v.) of (-)- ^3H -CGP 12177 (0.7 nmol/kg) with: ethanol/saline (\circ), increasing doses of unlabelled (\pm)CGP 12177/HCl (1 $\mu\text{mol}/\text{kg}$) (●), unlabelled ICI 89.406 1a (\blacktriangle), unlabelled 2-I-ICI-H 1b (\blacktriangledown) Each symbol represents the value for an individual rat. The solid lines indicate the values for the administered doses of unlabelled antagonists calculated using the parameters given in the text.

Results

Competition studies of β_1 -adrenoceptor analogues

In vitro studies

The Scatchard transformation of the binding of increasing concentrations of the non-selective β -adrenoceptor antagonist ^{125}I -CYP to murine myocardial membranes resulted in a linear curve. The correlation coefficient was >0.95 . K_D and B_{max} values determined from three experiments for the binding were 32.3 ± 1.9 pmol/l and 38.6 ± 2.9 fmol/mg protein, respectively.

In in vitro competition studies ICI 89.406 1a and its iodinated analogue 2-I-ICI-H 1b competed for ^{125}I -CYP binding sites in mouse ventricular membrane preparations. From these inhibition curves, the IC_{50} values of both compounds were calculated by nonlinear regression analysis. Then, these IC_{50} values were converted into the high- and low-affinity inhibition constants (K_{H1} and K_{H2}) using the K_D value of ^{125}I -CYP by the method of Cheng and Prusoff (5). Mean values (4 experiments) of K_{H1} and K_{H2} of ICI 89.406 1a were 1.3 ± 0.2 nmol/l and 160 ± 40 nmol/l, respectively. Corresponding values for 2-I-ICI-H 1b (6 experiments) were 0.045 ± 0.005 nmol/l and 12 ± 2 nmol/l, respectively. The ratios between K_{H2} and K_{H1} represent the β_1 -selectivities of the compounds tested. The selectivity of 2-I-ICI-H 1b ($K_{\text{H2}}/K_{\text{H1}} = 266 \pm 28$) was greater than that of ICI 89.406 1a ($K_{\text{H2}}/K_{\text{H1}} = 121 \pm 14$).

The compound 2-I-ICI-H 1b had a high affinity to β -adrenoceptors, was highly selective for β_1 -adrenoceptors but had a relatively high lipophilicity at physiological pH ($\log D = 1.19$). This compound and the key compound ICI 89.406 1a ($\log D = 0.44$) were assessed in vivo against (-)- ^3H -CGP 12177.

In vivo studies

The clearance of radioactivity from plasma after intravenous injection of (-)- ^3H -CGP 12177 into rats was rapid. Predosing rats with unlabelled (\pm)CGP 12177/HCl reduced the clearance. Unlabelled 2-I-ICI-

H **1b** or ICI 89.406 **1a** reduced the clearance of radioactivity from plasma but to a lesser extent (data not shown).

The distribution of radioactivity in rat tissues is illustrated in figure 2. Predosing with unlabelled (\pm)CGP 12177/HCl significantly decreased the radioactivity in myocardium and lung but not in the other analysed tissues. Predosing with 10 μ mol/kg unlabelled 2-I-ICI-H **1b** or ICI 89.406 **1a** reduced the uptake of radioactivity in the myocardium to a similar extent as (\pm)CGP 12177/HCl, but they exerted a weaker effect on uptake in the lung. This differential effect is compatible with published data showing that the proportion of β_1 - to β_2 -adrenoceptors in rat heart and lung differ. The majority of β -adrenoceptors in rat myocardium are of the subtype β_1 (61%) whereas in the lung the subtype β_2 is predominant (80%) (21, 30).

The effects of coinjecting unlabelled (\pm)CGP 12177/HCl, (\pm)ICI 89.406 **1a** or 2-I-ICI-H **1b** with ($-$)³H-CGP 12177 are illustrated in figure 3. Increasing the dose of (\pm)CGP 12177/HCl decreased the uptake of radioactivity in myocardium and lung, indicating competition for uptake sites in these tissues, but not in liver, kidney or muscle (data not shown).

Both ICI 89.406 **1a** and 2-I-ICI-H **1b** competed with ($-$)³H-CGP 12177 in the myocardium and in the lung. In the myocardium high doses of antagonists reduced the uptake of radioactivity to that observed after large doses of (\pm)CGP 12177/HCl but there was no evidence of differential binding to two subtypes of receptor. In the lung, however, neither ICI 89.406 **1a** nor 2-I-ICI-H **1b** reduced uptake to that observed for predosing with (\pm)CGP 12177/HCl.

To compare the binding potential of the three antagonists to myocardium, the data were fitted to an equation of the form:

$$\text{uptake index} = B + (B_{\max}/K_{\text{CGP 12177}}) * [1/(1 + C/K_{\text{CGP 12177}} + I/K_{\text{ICI 89.406}} + J/K_{\text{2-I-ICI-H}})]$$

B: non-displaceable background, B_{\max} : maximal number of binding sites (pmol/g wet tissue), C: injected (\pm)CGP 12177/HCl (nmol/kg body weight), I: injected ICI 89.406 (nmol/kg body weight), J: injected

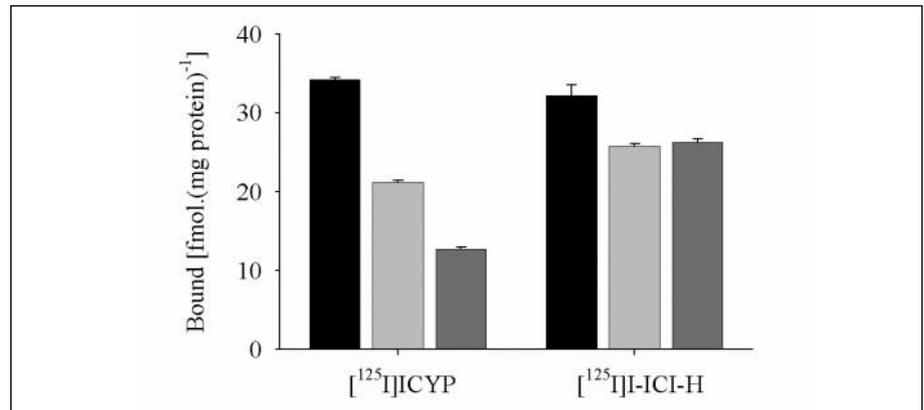


Fig. 4 Binding of ¹²⁵I-CYP and 2-¹²⁵I-ICI-H 1d to murine myocardial membranes incubated with: radioligand (black column), radioligand with 4.6 nmol/l of unlabelled 2-I-ICI-H 1d (light gray), 20 μ mol/l alprenolol (dark gray)

2-I-ICI-H (nmol/kg body weight), K_{CGP12177} : half saturation dose for (\pm)CGP 12177/HCl (nmol/kg body weight), $K_{\text{ICI89.406}}$: half saturation dose for ICI 89.406 (nmol/kg body weight), $K_{\text{2-I-ICI-H}}$: half saturation dose for 2-I-ICI-H (nmol/kg body weight).

Non-linear least-squares estimates of the parameters were obtained simultaneously using an iterative algorithm (17) with B as a constant, C, I, and J as independent variables, uptake index as the dependent variable and B_{\max} , K_{CGP12177} , $K_{\text{ICI89.406}}$, and $K_{\text{2-I-ICI-H}}$ as the fitted variables. Computed values for B_{\max} , K_{CGP12177} , $K_{\text{ICI89.406}}$, and $K_{\text{2-I-ICI-H}}$ with standard errors were 4.7 ± 0.9 pmol/g wet tissue, 0.58 ± 0.24 nmol/kg body weight, 15 ± 4 nmol/kg body

weight, and 140 ± 35 nmol/kg body weight, respectively. The lines of best fit are shown in figure 3.

A similar analysis could not be carried out for the data from the lung, since it was impossible to inject sufficient ICI 89.406 **1a** or 2-I-ICI-H **1b** to reduce radioactivity to background (B). Lung data obtained for (\pm)CGP 12177/HCl were fitted to the following equation:

$$\text{uptake index} = B + (B_{\max}/K_{\text{CGP 12177}}) * [1/(1 + (C/K_{\text{CGP12177}}))]$$

Computed values, with standard errors, for B_{\max} and K_{CGP12177} were 74 ± 10 pmol/g wet tissue, and 3.6 ± 0.6 nmol/kg body weight, respectively.

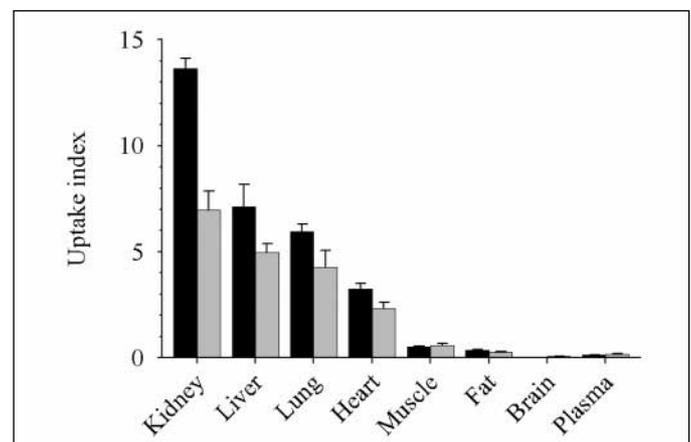


Fig. 5 Radioactivity in tissues 20 min after intravenous injection of 2-¹²⁵I-ICI-H 1d into rats (means with standard errors shown for 3 rats/group) 5 min after injection (i.v.) of: ethanol/saline (black column) or unlabelled 2-I-ICI-H **1b** (1 μ mol/kg) (gray)

In vitro and in vivo binding studies of 2-¹²⁵I-ICI-H 1d

The in vitro binding of the β_1 -selective adrenoceptor antagonist 2-¹²⁵I-ICI-H **1d** was compared to that of the nonselective β -adrenoceptor antagonist ¹²⁵I-CYP (Fig. 4). Using the three-fold K_D concentration total binding of both radioligands was

comparable. Radioligand binding in the presence of an excess of the nonselective β -adrenoceptor antagonist alprenolol (20 $\mu\text{mol/l}$) yielded a higher non-specific binding of 2-¹²⁵I-ICI-H **1d** as compared to ¹²⁵I-CYP. In addition, binding assays in the presence of 4.6 nmol/l unlabelled 2-I-ICI-H **1b**, a concentration at which β_1 - in contrast to β_2 -adrenoceptors are occupied, were

used to discriminate further between specific binding to β_1 - and β_2 -adrenoceptors. ¹²⁵I-CYP binding was reduced to a larger extent by alprenolol than by 2-I-ICI-H **1b** revealing specific binding to both β_1 - and β_2 -adrenoceptors. In contrast, 2-¹²⁵I-ICI-H **1d** binding was reduced to the same extent both in the presence of 2-I-ICI-H **1b** as well as in the presence of alprenolol indicating distinct binding to β_1 - but not β_2 -adrenoceptors.

The first in vivo study assessed the effect of increasing the amount of 2-¹²⁵I-ICI-H **1d** (0.75, 1.5, or 4.5 MBq/kg) injected either 5 min after ethanol/saline or 5 min after unlabelled 2-I-ICI-H **1b**. Rats were killed at 20 min after injection of radioligand. Uptake in all tissues increased linearly with injected dose, predosing with 2-I-ICI-H **1b** had no effect. Therefore, to correct for differences in body weight and injected dose, results were expressed as an uptake index, as defined above for (-)³H-CGP 12177.

The distribution of radioactivity in rat tissues at 20 min after injection of 2-¹²⁵I-ICI-H **1d** is illustrated in figure 5. Predosing with unlabelled 2-I-ICI-H **1b** did not decrease the uptake of radioactivity.

Figure 6 shows the radioactivity in plasma, myocardium, lung, and liver as a function of time after injection of 2-¹²⁵I-ICI-H **1d**. The clearance of radioactivity from plasma after intravenous injection of 2-¹²⁵I-ICI-H **1d** into rats was rapid and predosing rats with unlabelled 2-I-ICI-H **1b** had little effect. In the tissues, the maximal uptake was observed at 2 min after injection. Afterwards, a loss in radioactivity was observed. It was more rapid in liver and lung than in myocardium. Predosing with unlabelled 2-I-ICI-H **1b** had little or no effect on the uptake or loss of radioactivity.

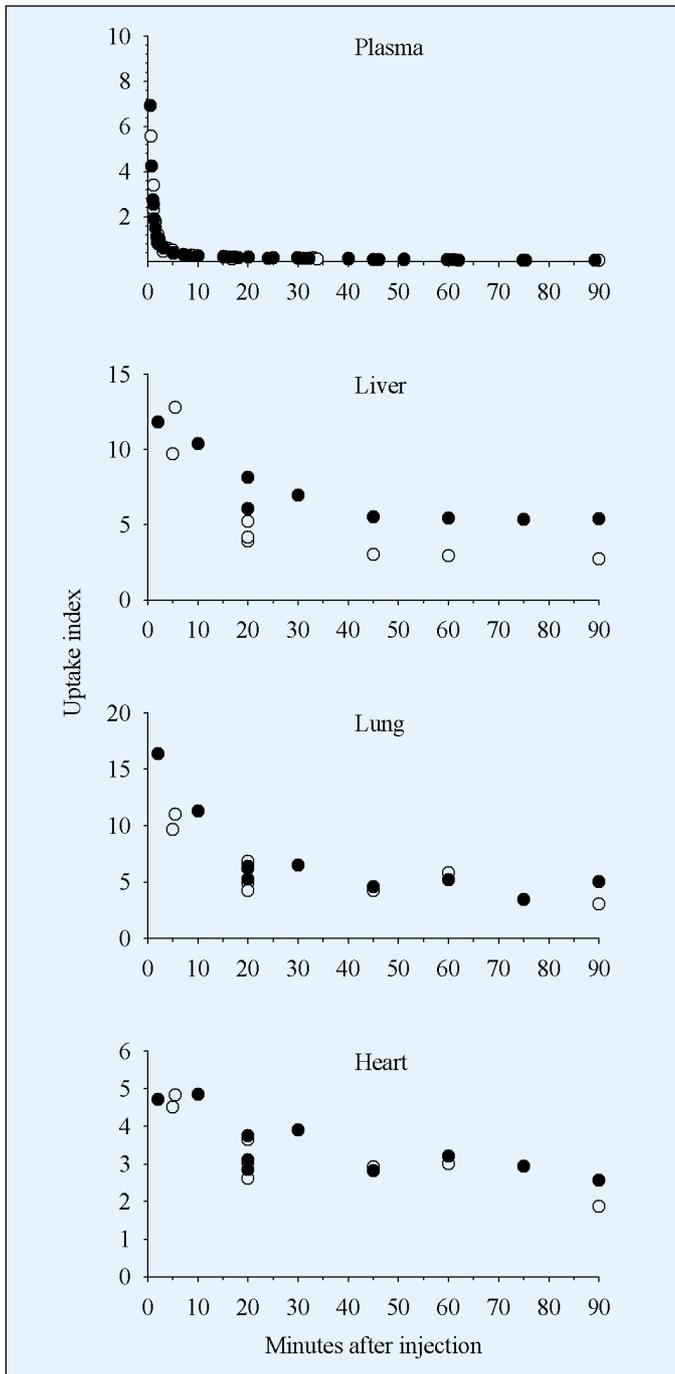


Fig. 6 Uptake of radioactivity in plasma, liver, lung, and heart after intravenous injection of 2-¹²⁵I-ICI-H **1d** into rats (each symbol is the datum point for an individual rat) 5 min after injection (i.v.) of: ethanol/saline (50/50, v/v) (●, 10 rats), unlabelled 2-I-ICI-H **1b** (1 $\mu\text{mol/kg}$) (○, 8 rats). Note the differences in the scales for uptake indices.

Discussion

In the adrenergic control of myocardial function β_1 -adrenoceptors play the major role and account for 70-80% of cardiac β -adrenoceptors. In cardiac biopsies of patients suffering from chronic heart failure a selective reduction in cardiac

β_1 -adrenoceptors was found while changes in cardiac β_2 -adrenoceptors varied (3, 28). Therefore, a procedure for visualization and quantification of β_1 -adrenoceptor populations rather than total β -adrenoceptor densities in the heart would be of great clinical interest (26). With suitable radiopharmaceuticals SPECT and PET offer the opportunity to probe β -adrenoceptors non-invasively (22).

First attempts were undertaken to develop β_1 -selective radioligands potentially suitable for cardiac receptor imaging. The most selective β_1 -adrenoceptor ligand known is CGP 26505, the (S)-isomer of CGP 20712A (33). However, ¹¹C-GP 26505 showed a relatively high non-specific binding and a rapid metabolism in vivo (34). Concordantly, the β_1 -selective radioligands ¹¹C-bisoprolol and (\pm)¹¹C-HX-CH 44 showed a high degree of non-specific binding in lung tissue that masked the heart uptake, thus limiting their use for heart studies with PET (27, 31). To sum up, no β_1 -selective radioligand suitable for the non-invasive assessment of cardiac β_1 -adrenoceptors is clinically established neither for SPECT nor for PET imaging.

We chose ICI 89.406 **1a** as the key compound for β_1 -adrenoceptor radioligand development on the basis of its high affinity and selectivity for β_1 -adrenoceptors, moderate lipophilicity (logD = 0.44) and amenability to labeling with ¹²⁵I and ¹¹C.

A promising β_1 -selective derivative of ICI 89.406 **1a** was the iodinated analogue 2-I-ICI-H **1b**. In vitro competition studies using mouse ventricular membrane preparations showed its high affinity to β_1 -adrenoceptors and its high selectivity for β_1 -adrenoceptors ($K_{12}/K_{11} = 265$). However, its lipophilicity (logD = 1.19) is higher than that of the parent compound (logD = 0.44). The key compound ICI 89.406 **1a** and 2-I-ICI-H **1b** were assessed in vivo in rats against (-)-³H-CGP 12177. Both ICI 89.406 **1a** and 2-I-ICI-H **1b** competed with (-)-³H-CGP 12177 in the myocardium and in the lung. In the myocardium a higher dose of 2-I-ICI-H **1b** (~10 μ mol/kg) than ICI 89.406 **1a** (~1 μ mol/kg) was needed to block the uptake of (-)-³H-CGP 12177 radioactivity but there was no evidence of differential binding to two subtypes of receptor.

In contrast to the myocardium, ICI 89.406 **1a** and 2-I-ICI-H **1b** had similar effects on the uptake of (-)-³H-CGP 12177 radioactivity in lung. Even at high doses, neither antagonist reduced lung uptake to that observed for predosing with (\pm)CGP 12177/HCl. These results are consistent, however, with both ICI 89.406 **1a** and its analogue 2-I-ICI-H **1b** being selective for β_1 -adrenoceptors as it is known that about 61% of β -adrenoceptors in rat myocardium are of the β_1 -subtype whereas around 80% of β -adrenoceptors in lung are of the β_2 -subtype (21, 30).

The silylated precursor, 2-SiMe₃-ICI-H **1c**, was synthesized and used to prepare radiolabelled 2-¹²⁵I-ICI-H **1d** as a potential radioligand for the non-invasive assessment of β_1 -adrenoceptors in the heart. The binding of this radioligand was assessed in vitro and in vivo. 2-¹²⁵I-ICI-H **1d** showed a considerably higher non-specific binding in vitro than the non-selective reference substance ¹²⁵I-CYP. These findings were confirmed in vivo at different concentrations of 2-¹²⁵I-ICI-H **1d**. In biodistribution studies in rats no detectable displacement of lung or heart radioactivity concentration was observed after pretreatment with propranolol or 2-I-ICI-H **1a**. This may be due in part to its relatively high lipophilicity after substitution of the cyano moiety by iodine. Therefore, the lack in specific binding of 2-¹²⁵I-ICI-H **1d** to the myocardium in vivo may be due to its high degree in non-specific binding.

Furthermore, the prepared 2-¹²⁵I-ICI-H **1d** was a racemic mixture. It is known that the (S)-enantiomers of compounds possessing the 1-amino-3-aryloxy-2-propanol substructure are more potent β -adrenoceptor ligands than the corresponding (R)-enantiomers (16). With respect to the non-selective β -adrenoceptor antagonist ³H-CGP 12177, specific uptake of both (\pm)-³H-CGP 12177 and (-)-³H-CGP 12177 to myocardium and lung was observed in vivo in rats (13). However, the racemic mixture of 2-¹²⁵I-ICI-H **1d** showed no specific binding at all. Therefore, it is unlikely that a sufficient specific binding will be obtained by its pure (S)-enantiomer.

Conclusion

The racemic β_1 -adrenoceptor radioligand 2-¹²⁵I-ICI-H **1d** has been evaluated both in vitro and in vivo. Because of its high non-specific binding it turned out as not suitable for SPECT or PET imaging in vivo using ¹²³I and ¹¹C, respectively. Future studies will concentrate on the preparation of the (S)-enantiomer of the more hydrophilic 2-¹²⁵I-ICI-COOH (logD = 0.77) and on further derivatives of ICI 89.406 **1a** as putative subtype selective β_1 -adrenergic radioligands for SPECT or PET.

Acknowledgements

This work was supported by a grant from the Jung-Stiftung für Wissenschaft und Forschung, Hamburg (Germany), the Sonderforschungsbereich 556 (Herzinsuffizienz und Arrhythmie) of the Deutsche Forschungsgemeinschaft DFG (Project C1), and the Royal Society, London, UK.

References

1. Arch JR, Ainsworth AT, Cawthorne MA et al. Atypical β -adrenoceptor on brown adipocytes as target for anti-obesity drugs. *Nature* 1984; 309: 163-5.
2. Bond RA, Clarke DE. Agonist and antagonist characterization of a putative adrenoceptor with distinct pharmacological properties from the alpha- and beta-subtypes. *Br J Pharmacol* 1988; 95: 723-34.
3. Brodde OE. β_1 - and β_2 -adrenoceptors in the human heart: properties, function, and alteration in chronic heart failure. *Pharmacol Rev* 1991; 43: 203-42.
4. Brodde OE, Michel MC. Adrenergic and muscarinic receptors in the human heart. *Pharmacol Rev* 1999; 51: 651-90.
5. Cheng Y, Prusoff WH. Relationship between the inhibition constant (K₁) and the concentration of inhibitor which causes 50 per cent inhibition of an enzymatic reaction. *Biochem Pharmacol* 1973; 22: 3099-108.
6. DeLean A, Hancock AA, Lefkowitz RJ. Validation and statistical analysis of radioligand binding data for mixtures of pharmacological receptor subtypes. *Mol Pharmacol* 1982; 21: 5-16.
7. Dubois EA, Somsen GA, van den Bos JC et al. Development of radioligands for the imaging of cardiac beta-adrenoceptors using SPECT. Part II: Pharmacological characterization in vitro and in vivo of new ¹²³I-labelled beta-adrenoceptor antagonists. *Nucl Med Biol* 1997; 24: 9-13.
8. Elsinga PH, van Waarde A, Jaeggi KA et al. Synthesis and evaluation of (S)-4-(3-(2'-[¹¹C]isopropylamino)-2-hydroxypropoxy)-2H-benzimidazol-2-one ((S)-[¹¹C]CGP 12388) and

- (S)-4-(3-((1'-[¹⁸F]-fluoroisopropyl)amino)-2-hydroxypropoxy)-2H-benzimidazol-2-one ((S)-[¹⁸F]fluoro-CGP 12388) for visualization of beta-adrenoceptors with positron emission tomography. *J Med Chem* 1997; 40: 3829-35.
9. Elsinga PH, Doze P, van Waarde A et al. Imaging of beta-adrenoceptors in the human thorax using (S)-[¹¹C]CGP12388 and positron emission tomography. *Eur J Pharmacol* 2001; 433: 173-6.
 10. Engel G, Hoyer D, Berthold R et al. [¹²⁵I]Iodocyanopindolol, a new ligand for β -adrenoceptors: identification and quantification of subclasses of β -adrenoceptors in guinea pig. *Naunyn Schmiedeberg's Arch Pharmacol* 1981; 317: 277-85.
 11. Erhardt PW, Woo CM, Matier WL et al. Ultra-short-acting β -adrenergic receptor blocking agents. 3. Ethylendiamine derivatives of (aryloxy)propranolamines having esters on the aryl function. *J Med Chem* 1983; 26: 1109-12.
 12. Knickmeier M, Matheja P, Wichter T et al. Clinical evaluation of no-carrier-added meta-[¹²⁵I]iodobenzylguanidine for myocardial scintigraphy. *Eur J Nucl Med* 2000; 27: 302-7.
 13. Law MP. Demonstration of the suitability of CGP 12177 for in vivo studies of β -adrenoceptors. *Br J Pharmacol* 1993; 109: 1101-9.
 14. Majid PA, Schreuder JE, de Feyter PJ et al. Clinical, electrocardiographic, and hemodynamic effects of ICI 89,406, a new cardioselective beta-adrenoceptor antagonist with intrinsic sympathomimetic activity, in patients with angina pectoris. *J Cardiovasc Pharmacol* 1980; 2: 435-44.
 15. Müller F, Lewin G, Matus M et al. Impaired cardiac contraction and relaxation and decreased expression of sarcoplasmic Ca^{2+} -ATPase in mice lacking the CREM gene. *FASEB J* 2002 (published online 10.1096/fj.02-0486fje).
 16. Mutschler E. *Arzneimittelwirkungen*. Stuttgart: Wissenschaftliche Verlagsgesellschaft mbH, 1996; 292.
 17. Nelder JM, Mead RA. Simple algorithm for function minimization. *Comput J* 1965; 7: 308-13.
 18. Patent CH 605666. Verfahren zur Herstellung von Alkanolaminderivaten. Imperial Chemical Industries Limited, London (UK).
 19. Pike VW, Law MP, Osman S et al. Selection, design and evaluation of new radioligands for PET studies of cardiac adrenoceptors. *Pharm Acta Helv* 2000; 74: 191-200.
 20. Pitha J, Milecki J, Czajkowska T et al. β -adrenergic antagonists with multiple pharmacophores: persistent blockade of receptors. *J Med Chem* 1983; 26: 7-11.
 21. Quast U, Vollmer KO. Binding of beta-adrenoceptor antagonists to rat and rabbit lung: special reference to levobunolol. *Arzneimittelforschung* 1984; 34: 579-84.
 22. Riemann B, Schäfers M, Law MP et al. Radioligands for imaging myocardial α - and β -adrenoceptors. *Nuklearmedizin* 2003; 42: 4-9.
 23. Scatchard G. The attraction of proteins for small molecules and ions. *Ann NY Acad Sci* 1949; 51: 660-72.
 24. Schäfers M, Dutka D, Rhodes CG et al. Myocardial presynaptic and postsynaptic autonomic dysfunction in hypertrophic cardiomyopathy. *Circ Res* 1998; 82: 57-62.
 25. Schäfers M, Lerch H, Wichter T et al. Cardiac sympathetic innervation in patients with idiopathic right ventricular outflow tract tachycardia. *J Am Coll Cardiol* 1998; 32: 181-6.
 26. Schäfers M, Riemann B, Levkau B et al. Current status and future applications of cardiac receptor imaging with positron emission tomography. *Nucl Med Commun* 2002; 23: 113-5.
 27. Soloviev DV, Matarrese M, Moresco RM et al. Asymmetric synthesis and preliminary evaluation of (R)- and (S)-[¹¹C]bisoprolol, a putative beta1-selective adrenoceptor radioligand. *Neurochem Int* 2001; 38: 169-180.
 28. Steinfath M, Lavicky J, Schmitz W et al. Regional distribution of β_1 - and β_2 -adrenoceptors in the failing and nonfailing human heart. *Eur J Clin Pharmacol* 1992; 42: 607-12.
 29. Steinfath M, Lavicky J, Schmitz W et al. Changes in cardiac β -adrenoceptors in human heart diseases: relationship to the degree of heart failure and further evidence for etiology-related regulation of β_1 and β_2 subtypes. *J Cardiothorac Vasc Anesth* 1993; 7: 668-73.
 30. Tumer N, Houck WT, Boehm C et al. Cardiac beta-adrenoceptor binding and characteristics with age following adrenal demedullation. *Br J Pharmacol* 1990; 99: 87-90.
 31. Valette H, Dolle F, Guenther I et al. Preliminary evaluation of 2-[4-[3-tert-butylamino)-2-hydroxypropoxy]phenyl]-3-methyl-6-methoxy-4(3H)-quinazolinone ([+/-]JHX-CH 44) as a selective beta1-adrenoceptor ligand for PET. *Nucl Med Biol* 1999; 26: 105-109.
 32. Van den Bos JC, van Doremalen PA, Dubois EA et al. Development of radioligands for the imaging of cardiac beta-adrenoceptors using SPECT. Part I: Asymmetric synthesis and structural characterization of five new iodine-containing beta-adrenoceptor antagonist derivatives. *Nucl Med Biol* 1997; 24: 1-7.
 33. Van Waarde A, Meeder JG, Blanksma PK et al. Suitability of CGP 12177 and CGP 26505 for quantitative imaging of beta-adrenoceptors. *Int J Rad Appl Instrum B* 1992; 19: 711-718.
 34. Van Waarde A, Meeder JG, Blanksma PK et al. Uptake of radioligands by rat heart and lung in vivo: CGP 12177 does and CGP 26505 does not reflect binding to β -adrenoceptors. *Eur J Pharmacol* 1992; 222: 107-112.
 35. Van Waarde A, Elsinga PH, Brodde OE et al. Myocardial and pulmonary uptake of S-1'-[¹⁸F]fluorocarazolol in intact rats reflects radioligand binding to β -adrenoceptors. *Eur J Pharmacol* 1995; 272: 159-168.
 36. Van Waarde A, Visser TJ, Elsinga PH et al. Imaging beta-adrenoceptors in the human brain with (S)-1'-[¹⁸F]fluorocarazolol. *J Nucl Med* 1997; 38: 934-939.
 37. Visser TJ, van Waarde A, Doze P et al. Characterization of β_2 -adrenoceptors, using the agonist [¹¹C]formoterol and positron emission tomography. *Eur J Pharmacol* 1998; 361: 35-41.
 38. Wichter T, Schäfers M, Rhodes CG et al. Abnormalities of cardiac sympathetic innervation in arrhythmogenic right ventricular cardiomyopathy: quantitative assessment of presynaptic norepinephrine reuptake and postsynaptic beta-adrenergic receptor density with positron emission tomography. *Circulation* 2000; 101: 1552-1558.
 39. Wilbur DS, Stone WE, Anderson KW. Regiospecific incorporation of bromine and iodine into phenols using (trimethylsilyl)phenols derivatives. *J Org Chem* 1983; 48: 1542-1544.

Correspondence to:

Burkhard Riemann, MD

Tel. +49/2 51/8 34 73 62

Fax +49/2 51/8 34 73 63

E-mail: riemanb@uni-muenster.de