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Validation of *S*-1′-[^{18}F]fluorocarazolol for in vivo imaging and quantification of cerebral β-adrenoceptors

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Abstract

S-1′-[^{18}F]fluorocarazolol (*S*-(−)-4-(2-hydroxy-3-(1′-[^{18}F]fluoroisopropyl)-aminopropoxy)carbazole, a non-subtype-selective β-adrenoceptor antagonist) has been investigated for in vivo studies of β-adrenoceptors. Previous results indicated that uptake of this radioligand in heart and lung can be inhibited by β-adrenoceptor agonists and antagonists. In the present study, blocking, displacement and saturation experiments were performed in rats, in combination with metabolite analysis to investigate the suitability of this radioligand for in vivo positron emission tomography (PET) imaging and quantification of β-adrenoceptors in the brain. The results demonstrate that, (i) the uptake of *S*-1′-[^{18}F]fluorocarazolol reflects specific binding to β-adrenoceptors, (ii) binding of *S*-1′-[^{18}F]fluorocarazolol to atypical or non-β-adrenergic sites is negligible, (iii) uptake of radioactive metabolites in the brain is less than 25% of total radioactivity, 60 min after injection, (iv) in vivo measurements of receptor densities (B_{max}) in cortex, cerebellum, heart, lung and erythrocytes are within range of densities determined from in vitro assays, (v) binding of *S*-1′-[^{18}F]fluorocarazolol can be displaced. In conclusion, *S*-1′-[^{18}F]fluorocarazolol seems to possess the appropriate characteristics to visualize and quantify β-adrenoceptors in vivo in the central nervous system using PET.

Keywords: PET (positron emission tomography); *S*-1′-[^{18}F]fluorocarazolol; β-Adrenoceptor; Brain; (Rat)

1. Introduction

Cerebral β-adrenoceptors are important in several physiological and behavioural responses, such as adaptation to stress (Stone and Platt, 1982; Stone et al., 1995), processing of visual stimuli (Maloteaux, 1986), respiratory control (Annane, 1991), glial proliferation (Sutin and Griffith, 1993; Hodges-Savola et al., 1996), memory function (Flexner et al., 1985) and motor learning (Heron et al., 1996). Abnormalities in β-adrenoceptor densities are associated with neurological pathologies like depression (De-Paermentier et al., 1990, 1992), schizophrenia (Joyce et al., 1992), alcoholism (Valverius et al., 1989), Alzheimer's disease (Kalaria et al., 1989) and Huntington's chorea (Waeber et al., 1991). Also in peripheral organs β-adrenoceptor densities are altered in various patho-physiological conditions, including hypertension (Michel et al., 1987), heart failure, ischemia (Brodde, 1991), airway infections, allergy and asthma (Innis et al., 1979).

With appropriate radioligands and experimental designs, positron emission tomography (PET) can be used for non-invasive measurement of regional densities of hormone and neurotransmitter receptors in the human body. Recently we evaluated the radioligand *S*-1′-[^{18}F]fluorocarazolol, a fluorinated analog of the potent, non-subtype selective β-adrenoceptor antagonist carazolol, which exhibits desirable properties for PET imaging. In humans, this compound was shown to bind to cerebral (Van Waarde et al., 1997), myocardial and pulmonary (Visser et al., 1997) β-adrenoceptors in vivo. In rats uptake of *S*-1′-[^{18}F]fluorocarazolol in lung, heart, brain and other organs containing β-adrenoceptors could be blocked stereoselectively by *S*-propranolol and was sensitive to selective β-adrenoceptor agonists and antagonists (Van Waarde et al., 1995).

To assess β-adrenoceptor densities (B_{max}) quantitatively with PET imaging and *S*-1′-[^{18}F]fluorocarazolol, an appropriate tracer-kinetic model is necessary. Several compartmental models have been described, using multiple injection techniques for quantification of receptor densities

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(Huang et al., 1989; Delforge et al., 1991). A most promising model was proposed by Delforge et al. (1991) in which three injections proved to be sufficient to identify the B_{max} and other model parameters of the β-adrenoceptor legend CGP 12177 in the heart. In order to apply this model to measurement of (cerebral) β-adrenoceptor densities with *S*-1′-[^{18}F]fluorocarazolol, knowledge is required about radioligand metabolism, adrenoceptor occupancy by the tracer, and reversibility of the binding.

The aim of the present study was to measure biodistribution and metabolism of *S*-1′-[^{18}F]fluorocarazolol in specific brain regions, and to investigate saturation and displacement of its binding to β-adrenoceptors in several β-adrenoceptor containing organs.

2. Materials and methods

2.1. Materials

S-Desisopropylcarazolol (enantiomeric excess $>98\%$) was prepared as reported previously (Elsinga et al., 1996). *S*-1′-[^{18}F]Fluorocarazolol (specific activity 18.5–185 TBq (500–5000 Ci)/mmol) was synthesized from [^{18}F]fluoroacetone and *S*-desisopropylcarazolol (Zheng et al., 1994). Non-radioactive *S*-1′-fluorocarazolol · HCl was similarly prepared, using commercially available fluoroacetone (Aldrich, Bornem, Belgium). Radiochemical purity of *S*-1′-[^{18}F]fluorocarazolol was $>99.9\%$ and chemical purity of unlabeled *S*-1′-fluorocarazolol · HCl was $>99\%$ as judged by reversed-phase high performance liquid chromatography (HPLC).

2.2. Animal handling

All animal studies were performed in compliance with the Law of Animal Experiments of The Netherlands. In distribution, displacement and metabolite studies, male Wistar rats (220 ± 20 g) were used which were supplied by Harlan, Lelystad, The Netherlands. Male rats (261 ± 12 g) from a locally bred Wistar strain (Department of Animal Physiology, Groningen University (RUG)) were used in saturation experiments. In each experiment the animals were anaesthetized with an intraperitoneal injection of sodium pentobarbital (60 mg/kg body weight) and a bolus injection of *S*-1′-[^{18}F]fluorocarazolol (1–1.85 MBq in 0.25 ml, 5–100 pmol/kg) was administered via the tail vein. Sixty minutes after radioligand injection, when specific binding in peripheral (Van Waarde et al., 1995) and cerebral tissues was maximal, the animals were killed by extirpation of the heart. A blood sample and several central and peripheral tissue samples were taken, after which tissue radioactivity was assessed by ex vivo counting, as published previously (Van Waarde et al., 1995). Uptake of ^{18}F was expressed as a 'body-weight-standardized value' or 'differential absorption ratio' (DAR): (cpm recovered/g tissue)/(cpm injected/g body weight).

2.3. Distribution and displacement

S-propranolol (0.15 mg/kg in 0.9% saline) was injected in the tail vein 5 min before the radioligand injection to study the distribution of specific binding. For displacement studies, a dose of 2.5 mg/kg *S*-propranolol was injected 5 min after the radioligand.

Peripheral organs were sampled as published previously (Van Waarde et al., 1995). The brain was dissected in more detail: two sections of the cortex were taken on the ventral side of the brain; the entorhinal cortex and the piriform cortex containing the amygdaloid nucleus. Olfactory bulbs were dissected and the brain was cut in three coronal slices. The first coronal cut was made ± 2 mm caudal to the olfactory bulbs (A3000 mm), the second just caudal to the optic chiasm (P1500 mm) (Palkovits and Brownstein, 1988). From the first slice anterior cingulate/frontopolar cortex was dissected. The second slice was used to isolate striatum and frontal cortex. Thalami could be scooped out from the third brainslice. In the same slice, the remainder of the cortex was peeled of, containing parietal, temporal and occipital areas, after which the hippocampi could be removed as well. Then the cerebellar hemispheres were removed from the hindbrain, and the pons and medulla were obtained by sectioning through the pons just caudal to the occipital cortex. Right and left samples of all brain regions were pooled.

Uptake of radioactivity was expressed as a DAR (see above). Specific binding was calculated by subtracting propranolol-pre-treated values from control values.

2.4. Saturation experiments

Thirty pentobarbital-anaesthetized rats were i.v. injected with 1 MBq *S*-1′-[^{18}F]fluorocarazolol and different amounts of unlabeled *S*-1′-fluorocarazolol in saline. Total mass of the drug was varied between 0.05 and 1000 nmol/kg body weight. Uptake data, with each value representing an average of at least 2 animals injected with a dose within the respective mass interval $(X - 10X)$, were examined using software for nonlinear curve fitting (EnzFitter, Elsevier Biosoft, Cambridge, UK). A logistic four-parameter model was fitted:

$$\text{Uptake} = \frac{(A - D)}{1 + (\text{Dose}/C)^{B}} + D$$

in which A = asymptotic maximum, B = slope parameter (pseudo-Hill slope), C = value at inflexion point (IC_{50}), and D = asymptotic minimum. Uptake was expressed as a DAR (see above) and Dose as nmol *S*-1′-fluorocarazolol/kg body weight.

Dose–response curves of tissue uptake were converted to hyperbolic saturation curves. First, specific binding was

calculated by subtracting the asymptotic minimum D (as determined above) from tissue radioactivity (DAR) observed after administration of *S*-1′-[^{18}F]fluorocarazolol. Specific binding was then expressed as pmol/g tissue wet weight using the following formula

Bound ligand (pmol/g) = Injected dose (nmol/kg)

× Specific tissue binding (differential absorption ratio)

Receptor-bound ligand was plotted as a function of the total amount of *S*-1′-fluorocarazolol (radioactive plus non-radioactive) injected, with each data point representing an average of at least two animals as described above. A hyperbolic (one-site) model was fitted to these data; the asymptotic maximum is the receptor density (B_{max}) expressed as pmol/g tissue.

2.5. Metabolite analysis

Radioligand metabolism was assessed by reversed-phase/HPLC analysis of tissue extracts, performed in a system consisting of a Waters 510 pump, Rheodyne injector type 7125, 1 ml sample loop, C18 guard column, Waters Radial-Pak C18 (100 × 8 mm i.d., 5 mm) and a Waters 486 tuneable absorbance detector.

The animals were sacrificed 60 min after injection of *S*-1′-[^{18}F]fluorocarazolol (1–1.85 MBq in 0.25 ml, 5–100 pmol/kg), after which heart, lung, cerebellum and cortex were isolated and extracted with 2 volumes of acetonitrile using a tissue homogenizer (Ultra Turrax). Protein was removed from the extracts by centrifugation (2 min, Hettich mikroliter centrifuge). The clear supernatant was injected onto the HPLC column. The mobile phase was prepared by dissolving 2.46 g anhydrous sodium acetate in 450 ml water, adding 550 ml acetonitrile, adjusting the pH to 6.5 with acetic acid and filtration (Millipore 0.45 mm FH). The flow rate was 2 ml/min. Twenty-four 0.6 ml fractions were collected over a period of 12 min using an automatic fraction collector. Radioactivity in each fraction was measured with a gamma counter. The fraction of radioactivity in the supernatant which still represented parent compound was determined.

3. Results

3.1. Regional tissue distribution

Pre-treatment with propranolol (0.15 mg/kg) caused a significant decrease in ^{18}F uptake in all brain regions (Table 1); central DARs varied in control animals from 0.27 ± 0.03 in medulla to 0.66 ± 0.17 in the parietal, temporal and occipital cortex, and in propranolol-pretreated animals from 0.18 ± 0.04 in medulla to 0.31 ± 0.07 in anterior cingulate/frontopolar cortex. The regional distribution of specific binding was calculated (not shown). This distribution was found to be heterogeneous, with most

Table 1
Regional distribution of ^{18}F in rat tissues in blocking (pre-treatment with 0.15 mg/kg *S*-propranolol, i.v.) and displacement studies (post-treatment with 2.5 mg/kg *S*-propranolol, i.v.), 60 min after *S*-1′-[^{18}F]fluorocarazolol injection

Tissue	Controls (n = 7–8)	Blocking (n = 3–5)	P: control vs. blocking	Displacement (n = 4)	P: control vs. displacement
Amygdala/piriform cortex	0.48 ± 0.06	0.24 ± 0.04	< 0.0005	0.37 ± 0.05	0.01
Cerebellum	0.55 ± 0.12	0.28 ± 0.08	0.001	0.43 ± 0.03	NS
Cingulate/frontopolar cortex	0.62 ± 0.04	0.31 ± 0.08	< 0.0005	0.44 ± 0.01	< 0.0005
Entorhinal cortex	0.50 ± 0.07	0.25 ± 0.04	< 0.0005	0.40 ± 0.03	< 0.05
Frontal cortex	0.63 ± 0.08	0.27 ± 0.01	< 0.0005	0.47 ± 0.06	< 0.01
Parietal/temporal/occipital cortex	0.66 ± 0.17	0.29 ± 0.02	0.001	0.48 ± 0.04	NS
Hippocampus	0.38 ± 0.04	0.26 ± 0.02	< 0.0005	0.34 ± 0.04	NS
Medulla	0.27 ± 0.03	0.18 ± 0.04	< 0.0005	0.26 ± 0.01	NS
Olfactory bulbs	0.36 ± 0.04	0.25 ± 0.07	< 0.005	0.33 ± 0.03	NS
Pons	0.30 ± 0.06	0.21 ± 0.03	< 0.005	0.30 ± 0.04	NS
Striatum	0.58 ± 0.05	0.30 ± 0.02	< 0.0005	0.43 ± 0.02	< 0.0005
Thalamus	0.39 ± 0.04	0.22 ± 0.03	< 0.0005	0.28 ± 0.02	< 0.0005
Heart	2.28 ± 0.22	0.68 ± 0.09	< 0.0005	1.41 ± 0.21	< 0.0005
Kidney	1.27 ± 0.23	0.97 ± 0.10	< 0.05	1.22 ± 0.12	NS
Liver	1.55 ± 0.26	2.56 ± 0.29	< 0.0005	2.67 ± 0.28	< 0.0005
Lung	17.66 ± 2.7	2.67 ± 0.56	< 0.0005	13.12 ± 1.7	0.01
Muscle	0.48 ± 0.12	0.43 ± 0.08	NS	0.47 ± 0.10	NS
Plasma	0.23 ± 0.04	0.35 ± 0.05	< 0.0005	0.38 ± 0.02	< 0.0005
RBC	1.12 ± 0.15	0.34 ± 0.03	< 0.0005	0.79 ± 0.08	< 0.005
Spleen	2.31 ± 0.27	0.67 ± 0.09	< 0.0005	1.47 ± 0.12	< 0.0005
Trachea	0.78 ± 0.16	0.39 ± 0.05	< 0.0005	0.56 ± 0.06	< 0.050

Tissue uptake is expressed as mean differential adsorption ratio (DAR; see Section 2) ± S.D. of n experimental animals.
Significant differences between control and experimental values were analysed using Student's t-test, with P as a dual-tail probability.
NS = not significant.

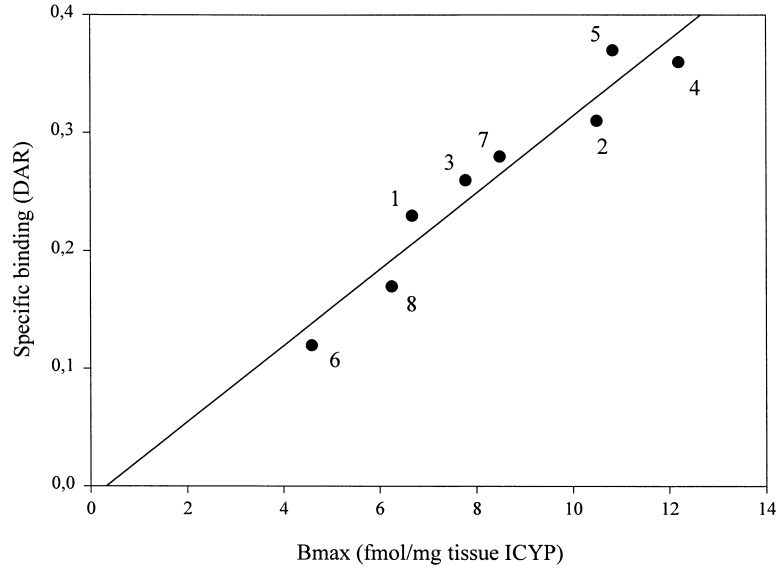

Fig. 1. Relationship between specific in vivo *S*-1′-[^{18}F]fluorocarazolol uptake (DAR), 60 min after injection, and β-adrenoceptor density determined by quantitative autoradiography of [^{125}I]iodocyanopindolol binding in several brain regions according to Grimm et al. (1992). The solid line represents a linear regression analysis of the data points which produced the following equation: DAR = $-0.01 + 0.0032$ ([^{125}I]iodocyanopindolol fmol/mg), $r = 0.93$, $P < 0.005$. Numbers indicate brain regions: (1) amygdala/piriform cortex; (2) anterior cingulate/frontopolar cortex; (3) entorhinal cortex; (4) frontal cortex; (5) parietal, temporal and occipital cortex; (6) hippocampus; (7) striatum and (8) thalamus.

binding occurring in frontal and parietal, temporal and occipital cortex. Slightly less specific binding was seen in the amygdala/piriform cortex, cerebellum, anterior cingulate/frontopolar cortex, entorhinal cortex, and striatum with moderate values in the thalamus. Pons, medulla, hippocampus and olfactory bulbs showed very little specific binding. In Fig. 1, the specific binding is compared with receptor densities obtained by in vitro autoradiography in rat brain using [^{125}I]iodocyanopindolol (ICYP) as the radioligand (Grimm et al., 1992). The specific *S*-1′-[^{18}F]fluorocarazolol uptake in different brain regions strongly correlated with values from these in vitro studies ($r = 0.93$; $P < 0.005$).

3.2. Displacement

A chase experiment with an intravenous injection of 2.5 mg/kg propranolol, showed that bound *S*-1′-[^{18}F]fluoro-

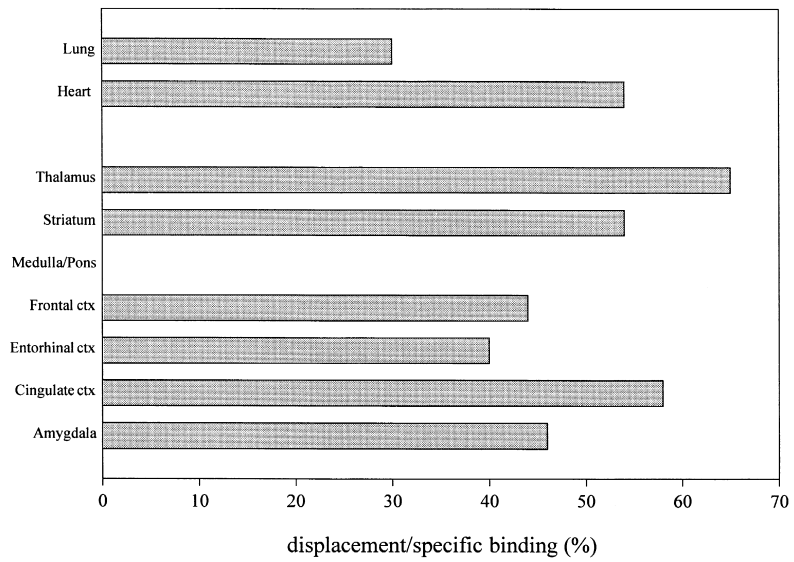

Fig. 2. Displacement of *S*-1′-[^{18}F]fluorocarazolol from 12 brain areas by an intravenous injection of 2.5 mg/kg *S*-propranolol ($n = 4$). Data is expressed as % displacement/specific binding, with specific binding calculated by subtracting *S*-propranolol-pre-treatment DAR values from control DAR values.

carazolol could be significantly displaced in several brain regions containing many β-adrenoceptors, namely the cingulate/frontopolar cortex, entorhinal cortex, frontal cortex, striatum and the thalamus (Table 1). No significant reversible binding was found in cerebral regions with little β-adrenoceptors (medulla and pons) and in the cerebellum. In peripheral regions, the radioligand could be displaced significantly in all tissues, except kidney, muscle, plasma and liver. Plasma and liver uptake DAR even increased. Uptake values varied in the periphery from 0.56 ± 0.05 in the trachea to 13.1 ± 1.69 in the lung, and in brain regions from 0.26 ± 0.01 in the medulla to 0.48 ± 0.03 in the parietal, temporal and occipital cortex. In Fig. 2 the radioligand uptake in several brain areas and in myocardial and pulmonary tissue is presented as the fraction of specific binding which is displaced within 60 min (expressed in %). These values were found to be highest in the anterior cingulate/frontopolar cortex, and the thalamus, slightly lower in the entorhinal and frontal cortex, amygdala/piriform cortex, striatum and heart, very low in the lung and zero in medulla and pons. Binding of *S*-1′-[^{18}F]fluorocarazolol in tissue containing mainly β_1-adrenoceptors, such as myocardial tissue, appeared to be more easily displaced than the binding in mainly β_2 subtype containing tissue such as the lung (55 and 30%, respectively). The same phenomenon was observed in the brain.

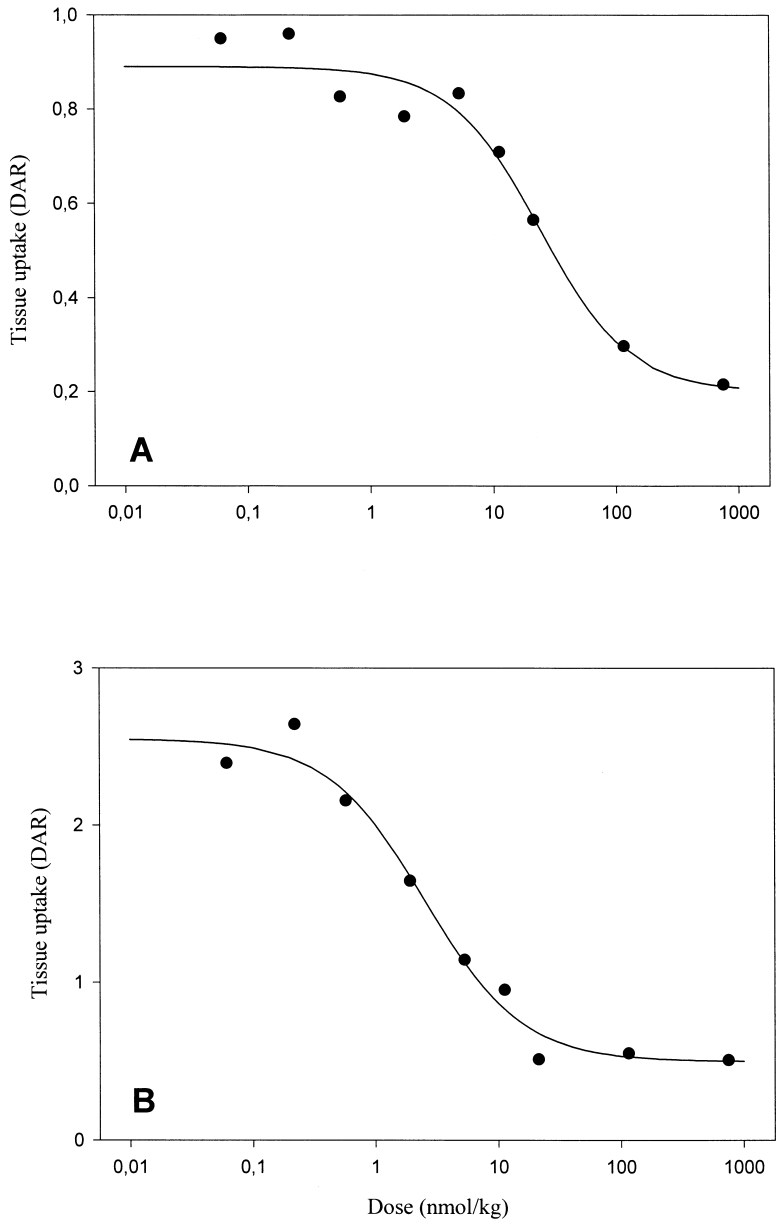

Fig. 3. Uptake of ^{18}F in (a) rat cerebral cortex and (b) rat myocardial tissue, after injection of 1 MBq of *S*-1′-[^{18}F]-fluorocarazolol and various doses of unlabeled *S*-1′-fluorocarazolol. The dose is expressed as the total (radioactive plus non-radioactive) amount of *S*-1′-fluorocarazolol injected. The solid lines are logistic curve fits.

Table 2
Curve fitting parameters of the in vivo saturation experiments with *S*-1′-[^{18}F]fluorocarazolol

Tissue	Maximal uptake (DAR)	Minimal uptake (DAR)	IC_{50} (nmol/kg body weight)	Pseudo–Hill slope	B_{max} (pmol/g tissue)
Cerebellum	0.88 ± 0.05	0.21 ± 0.02	7.4 ± 2.0	0.9 ± 0.2	4.6 ± 0.06
Cerebral cortex	0.89 ± 0.04	0.20 ± 0.02	24.1 ± 4.6	1.2 ± 0.2	12.3 ± 0.3
Heart	2.55 ± 0.15	0.50 ± 0.11	2.5 ± 0.7	1.1 ± 0.3	6.9 ± 0.6
Lung	21.9 ± 2.9	1.60 ± 0.16	0.8 ± 0.3	1.1 ± 0.2	14.4 ± 1.3
Red blood cells	1.47 ± 0.33	0.16 ± 0.10	0.93 ± 0.8	0.7 ± 0.3	3.2 ± 0.4

Parameters are expressed as mean ± S.E.M.
See Figs. 3 and 4 for the corresponding fits.

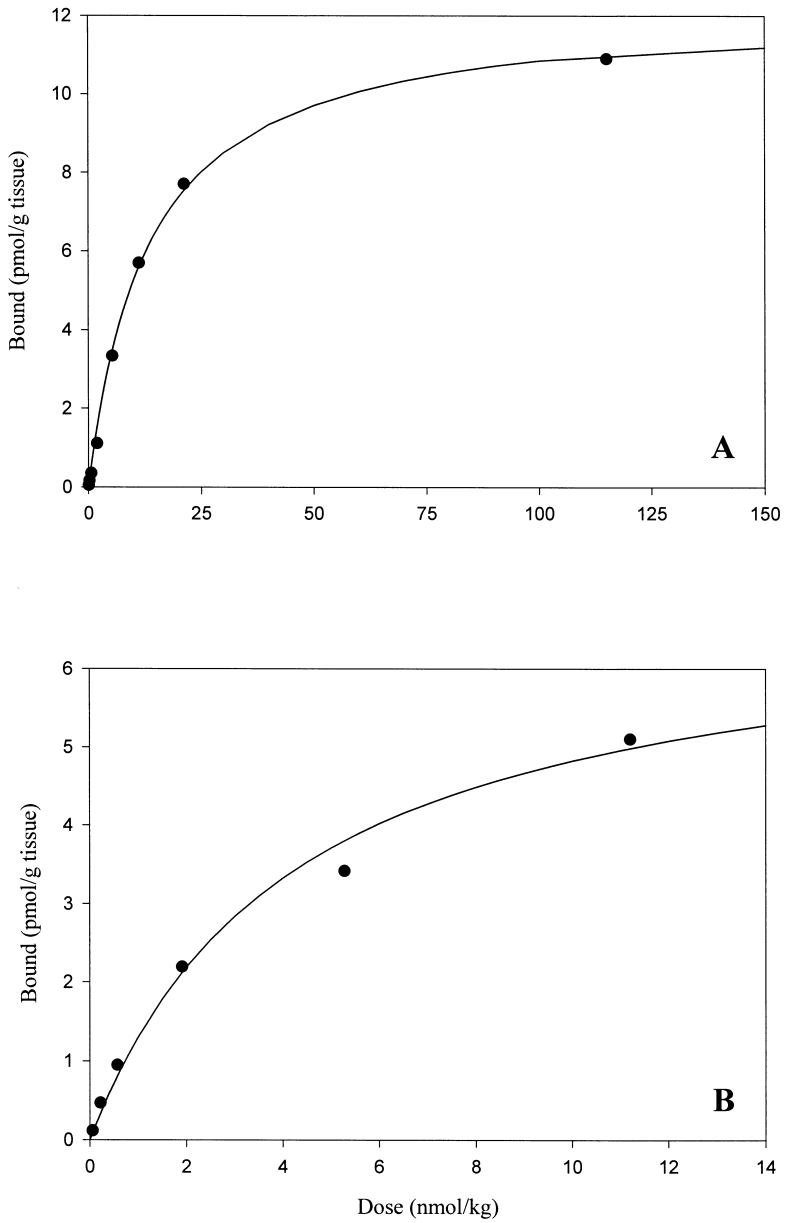

Fig. 4. Dose dependency of the specific binding of *S*-1′-fluorocarazolol in (a) rat cerebral cortex and (b) rat myocardial tissue. The concentration of bound ligand was calculated as described in Section 2; the solid lines are curve fits for a hyperbolic (one-site) model. Note that the scale on the horizontal axis for cerebral cortex and myocardial tissue is different since a higher dose was required to saturate β-adrenoceptors in the brain.

Uptake in the cortex, containing mainly β_1-adrenoceptors, seemed to be more easily reversible (40–58% displaced) than uptake in the cerebellum (not significantly reversible), which contains mainly β_2-adrenoceptors (Minneman et al., 1979; Rainbow et al., 1984; Grimm et al., 1992).

3.3. Saturation experiments

When *S*-1′-[^{18}F]fluorocarazolol (1 MBq/rat) was co-injected with non-radioactive *S*-1′-fluorocarazolol, and radioactivity in target tissues after 60 min was plotted against the total amount of drug injected, sigmoidal curves were acquired (Fig. 3). A logistic model was fitted to these data and parameters were estimated (Table 2). Values for IC_{50} could not be expressed as nM since the free ligand concentrations in the tissues were unknown and they could not be determined. Therefore, apparent IC_{50} values were calculated which were expressed in terms of mass injected per unit body weight. The estimated values were an order of magnitude lower in peripheral tissues (heart, lung, erythrocytes) than in the brain. Moreover, IC_{50} values in tissues containing mainly β_2-adrenoceptors (lung, cerebellum) were lower than those in tissues containing mainly β_1-adrenoceptors (heart, cerebral cortex). Note that the tissue uptake values for cortex determined in blocking and displacement studies are lower than those determined from these saturation data at doses of *S*-1′-fluorocarazolol comparable to those used in the biodistribution studies (5–100 pmol/kg).

Hyperbolic saturation data were acquired from the sigmoidal curves (Fig. 4). B_{max} values (pmol g^{-1} tissue wet weight) estimated from these curves are listed in Table 2. In Figs. 3 and 4 only the sigmoidal and hyperbolic saturations of adrenoceptors in the cerebral cortex and the heart are shown, which are representative for the curves of cerebellar and pulmonary tissue, and erythrocytes, although they exhibit different parameters (Table 2).

3.4. Metabolites of S-1′-[^{18}F]fluorocarazolol

Representative radioactivity profiles of tissue extracts are shown in Fig. 5. Radioactivity eluting with a retention time of about 7 min co-eluted with unchanged *S*-1′-[^{18}F]fluorocarazolol, whereas the peaks between 1.75 and 5.25 min represented metabolites. At 60 min after injection, 78.4 ± 4.3, 81.4 ± 3.8 and 75.5 ± 3.9% of radioactivity found in respectively cerebellum, cerebral cortex and remainder of the brain, was due to unmodified radioligand. Although the differences between the brain areas are not statistically significant, each animal seems to have a smaller fraction of parent compound in the remainder of the brain, than in the cortex, a region with relatively high β-adrenoceptor densities (Minneman et al., 1979; Rainbow et al., 1984; Grimm et al., 1992). In heart and lung the parent compound fractions (88.7 ± 2.3 and 95.1 ± 0.9% respectively) were similar to those determined in a previous study (Elsinga et al., 1996). In the brain, only one metabolite-peak was found, corresponding with the more polar one of the two metabolites found in peripheral organs (Fig. 5).

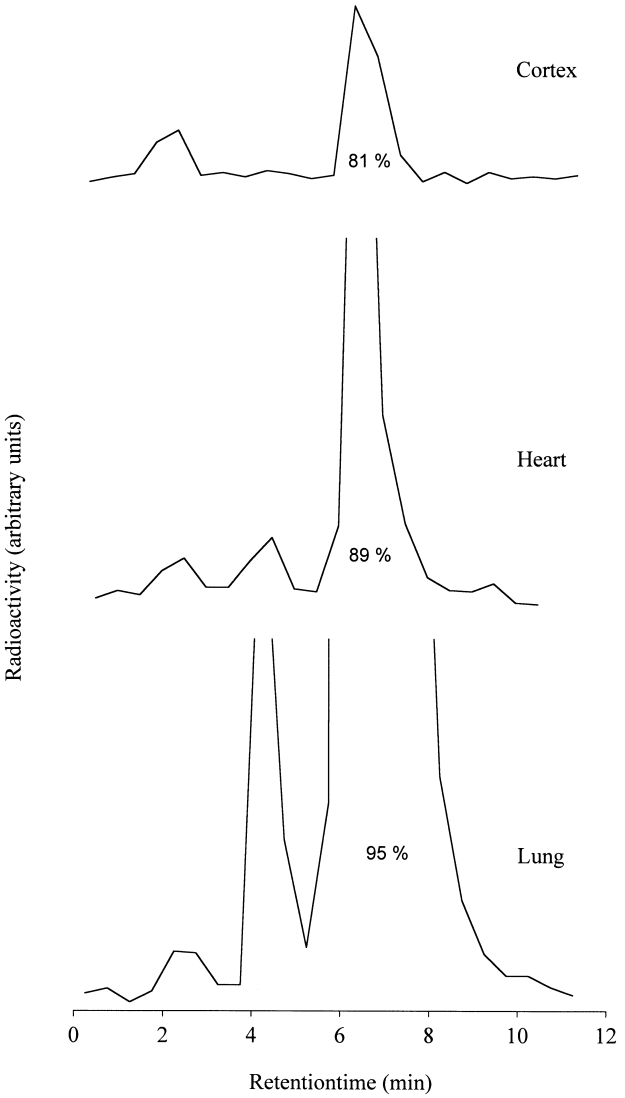

Fig. 5. Radiochromatogram of tissues extracted 60 min post injection. Representative profiles of cortex, heart and lung from one animal are shown. The chromatograms of cerebellum and the rest of the brain (not shown) were similar to that of the cortex. Note that the scale on the vertical axis is different for heart, lung and brain, in order to compare the retention times of the metabolites in central and peripheral tissue, while the fraction of the total radioactivity which belongs to the original compound is depicted in percentages.

4. Discussion

4.1. Suitability of S-1′-[^{18}F]fluorocarazolol for in vivo imaging of β-adrenoceptors

The in vivo visualization of hormone- and neuroreceptors with positron emission tomography (PET) requires

appropriate radioligands. Previous studies of *S*-1′-[^{18}F]fluorocarazolol have demonstrated that this radioligand exhibits desirable properties for in vivo imaging of β-adrenoceptors using PET. Myocardial, pulmonary (Visser et al., 1997) and cerebral (Van Waarde et al., 1997) β-adrenoceptors were clearly visualized in healthy volunteers, and binding of the radioligand to these receptors was inhibited after ingestion of pindolol. In the brain, uptake of *S*-1′-[^{18}F]fluorocarazolol delineated grey matter and was high in the posterior cingulate, precuneus and striatum, low in the thalamus and practically absent in white matter of the corpus callosum (Van Waarde et al., 1997).

Many β-adrenoceptor antagonists are known for their binding affinity to 5-hydroxytryptamine (5-HT) receptors. Fluorocarazolol however displays a 20–400 fold selectivity for β-adrenoceptors with respect to 5-HT$_1$ in in vitro assays (Van Waarde et al., 1997). Therefore, the contribution of binding to 5-HT$_1$ receptors to specific fluorocarazolol uptake in the brain can be neglected at a subnanomolar dose of the radioligand.

4.1.1. Specific binding

However, these reports do not demonstrate that uptake of *S*-1′-[^{18}F]fluorocarazolol reflects actual densities of β-adrenoceptors. Animal experiments with attempts to block uptake in peripheral target organs with subtype-selective antagonists indicated that *S*-1′-[^{18}F]fluorocarazolol is actually bound to β-adrenoceptors (Van Waarde et al., 1995). Moreover, the present report showed that inhibition of radioactivity uptake in peripheral organs after the administration of the non-subtype-selective β-antagonist, propranolol was high in tissues known to contain many β-adrenoceptors (heart and lung), and low in tissues containing few β-adrenoceptors (muscle) (see also: Van Waarde et al., 1995). The same phenomenon was observed in the brain. Furthermore, the regional distribution of the specific binding of *S*-1′-[^{18}F]fluorocarazolol in cerebral tissue corresponds to β-adrenoceptor densities determined from in vitro autoradiography with [^{125}I]iodocyanopindolol as radioligand (Grimm et al., 1992) ($r = 0.93$; $P < 0.005$), Fig. 1). This further indicates that specific binding of *S*-1′-fluorocarazolol in the brain of intact rats, calculated as described in Section 2, actually represents regional density of β-adrenoceptors. Because not all brain regions studied in the present report are represented in this in vitro assay using [^{125}I]iodocyanopindolol, data from cerebellum, medulla, olfactory bulbs and pons could not be compared. However, according to other in vitro data using [^{125}I]-iodopindolol (IPIN) as radioligand (Rainbow et al., 1984), very little specific binding is present in pons and medulla, in agreement with our small DARs measured in these regions.

S-1′-[^{18}F]fluorocarazolol, like other β-antagonists, may not only bind to β$_1$ and β$_2$ subtypes of adrenoceptors, but also to 'atypical' or β$_3$-adrenoceptors (Emorine et al., 1989, 1992) and to non-β-adrenergic sites (Goldie et al., 1986). Comparing the residual tissue uptake of radioactivity in the presence of 1 μmol/kg non-radioactive *S*-1′-fluorocarazolol with the uptake in animals pre-treated with 0.15 mg/kg *S*-propranolol reveals similar uptake values (compare blocking in Table 1 with minimal uptake in Table 2). Since binding to β$_3$- and non-β-adrenergic sites is insensitive to propranolol, in contrast to associations to β$_1$- and β$_2$-adrenoceptors (Emorine et al., 1989, 1992; Goldie et al., 1986), binding of *S*-1′-[^{18}F]fluorocarazolol to β$_3$-adrenoceptors and non-β-adrenergic sites seems negligible under our experimental conditions. Slightly, but not significantly lower uptake was found after treatment with 1 μmol/kg *S*-1′-fluorocarazolol than after 0.15 mg/kg *S*-propranolol, especially in pulmonary tissue (1.60 ± 0.16 vs. 2.67 ± 0.56) and erythrocytes (0.16 ± 0.10 vs. 0.34 ± 0.03). However, after pre-treating the animals with 2.5 mg/kg *RS*-propranolol residual uptake of ^{18}F in the lung (1.15 ± 0.13 (Van Waarde et al., 1995)) was similar to that seen after pre-treatment with 1 μmol/kg *S*-1′-fluorocarazolol.

Moreover, the pseudo-Hill coefficients did not appear to be significantly different from unity (Table 2) although the standard errors were large and a relatively shallow slope was found in red blood cells. In conclusion, *S*-1′-[^{18}F]fluorocarazolol did not bind to receptors other than β-adrenoceptors and it does not show appreciable binding to propranolol insensitive sites in brain, heart and lung, although there may be a minor contribution of such sites to total uptake in blood cells.

4.1.2. Apparent in vivo affinity

Knowing that the in vivo binding of *S*-1′-[^{18}F]fluorocarazolol to β-adrenoceptors is specific, it would be interesting to know the strength of this binding. Therefore the IC$_{50}$ was estimated from in vivo saturation experiments. These estimates of the apparent in vivo affinity of *S*-1′-[^{18}F]fluorocarazolol to β-adrenoceptors in erythrocytes, heart and lungs (values reported in Table 2) appeared to be lower than affinity constants determined in vitro ($K_i(\beta_1) = 0.4$ nM and $K_i(\beta_2) = 0.1$ nM, Van Waarde et al., 1997). Apparent affinities in the brain are still lower by an order of magnitude (Table 1).

Differences between the in vitro and the in vivo affinities of a radioligand are a common finding in PET studies (Nanoff et al., 1987; Law, 1993; Raffel, 1991; Sisson et al., 1991). Except for differences in experimental conditions such as (incubation) media and temperature, these discrepancies can also result from differences in radioligand availability for binding to receptors, possibly caused by the following mechanisms.

First, the free ligand concentration may not be distributed in a homogeneous manner because of the tissue heterogeneity and the lipophilicity of the radioligand (Delforge et al., 1994, 1996). If β-adrenoceptors are in a hydrophilic subcellular environment, the free ligand concentration in the neighbourhood of the receptor sites may

be much lower than the mean concentration in the tissue. This phenomenon will lead to an in vivo IC_{50} which is higher than the values measured in vitro, and this discrepancy will be even more pronounced when using a more lipophilic radioligand. Therefore, it is not surprising that the in vivo affinity for β-adrenoceptors of the lipophilic radioligand *S*-1′-[^{18}F]fluorocarazolol (log $P + 2.2$, pH 7.4; Zheng et al., 1994) is much lower than the in vitro affinity.

Second, binding to plasma proteins may cause a substantial increase in the apparent IC_{50} observed in vivo, since the amount of free ligand in plasma which is able to cross the endothelium will be diminished (Raffel et al., 1991). In a previous report, the free fraction of *S*-1′-[^{18}F]fluorocarazolol in rat plasma was determined by ultrafiltration, demonstrating that $> 70\%$ of the parent ligand is bound to proteins (Van Waarde et al., 1996). However it is not expected that *S*-1′-[^{18}F]fluorocarazolol binding to proteins will cause any substantial decrease in the availability of the ligand, because this binding reaches equilibrium probably very rapidly (Van Waarde et al., 1996) and will therefore not be the rate-limiting step for ligand uptake.

Third, specific pharmacokinetic features, like hepatic metabolism, diffusion barriers (blood–brain barrier), clearance or excretion (Raffel et al., 1991) may also result in a decrease of the availability of intact radioligand and cause a lower apparent affinity in vivo.

4.1.3. Subtype specificity

S-1′-fluorocarazolol exhibits subtype specificity for β_2-adrenoceptors; the in vitro affinity for β_1-adrenoceptors is 4 times smaller than for β_2-adrenoceptors ($pK_i(\beta_1) = 9.4$, $pK_i(\beta_2) = 10.0$) (Van Waarde et al., 1997). If the radioligand exhibits the same property in vivo, and considering that uptake of *S*-1′-fluorocarazolol really reflects specific binding to adrenoceptors, the amount of displacement and the affinity (IC_{50}) in vivo (this report), should correlate to the fraction of β_1-adrenoceptors as determined in vitro. Indeed, in tissue containing mainly β_1-adrenoceptors like the cortex (70–88% β_1: Minneman et al., 1979; Petrovic et al., 1983; Rainbow et al., 1984; Tiong and Richardson, 1989) and the heart (66–83% β_1: Minneman et al., 1979; Vago et al., 1984; Tumer et al., 1990; Cerbai et al., 1995), a greater displacement and higher IC_{50} values (Fig. 2 and Table 2) were found than in tissue containing only a small fraction of β_1-adrenoceptors, like the cerebellum (2–15% β_1: Minneman et al., 1979; Pittman et al., 1980; Rainbow et al., 1984; Levin and Dunn-Meynell, 1989) and the lungs (15–34% β_1: Barnett et al., 1978; Minneman et al., 1979; Whitsett et al., 1981; Winter et al., 1986).

4.2. Suitability of S-1′-[^{18}F]fluorocarazolol for in vivo quantification of β-adrenoceptor densities

In order to be suitable for quantitative imaging of receptors, a radioligand should display certain characteristics.

First, the radioligand should metabolize as slowly as possible and its metabolites should accumulate very little in target tissues. Previous studies with *S*-1′-[^{18}F]fluorocarazolol have demonstrated that at 10 min p.i., only 20% (Elsinga et al., 1996) and 47% (Van Waarde et al., 1997) of the total radioactivity in respectively rat and human plasma co-eluted with the parent compound. Sixty min after injection only 5% (Elsinga et al., 1996) and 20% (Van Waarde et al., 1997) of the original compound is present in rat and human plasma, respectively. This suggests a rapid metabolism of *S*-1′-[^{18}F]fluorocarazolol by the liver. Therefore, the tracer-kinetic model should account for plasma metabolites in order to obtain a correct input function.

In the brain, relatively small amounts of radioactive *S*-1′-[^{18}F]fluorocarazolol-metabolites are found (18.6 to 24.5%), 60 min after injection. However, these amounts are larger than those found in heart and lung (respectively 11.3 and 4.9%). The fraction of parent compound (75.5 to 81.4%) in the rat brain is in the range of those of other well-known radioligands, like ^{76}Br-labelled quinuclidinyl phenylacetate (QNP) isomers (70–100% (Strijckmans et al., 1997)).

Some suggestions can be made about the nature of the radioactive metabolites. Cerebellum, cortex and the rest of the rat brain showed only one metabolite, corresponding to the most polar one of the two compounds found in myocardial and pulmonary tissue (Fig. 5) (see also Elsinga et al., 1996). This suggests that the *S*-1′-[^{18}F]fluorocarazolol metabolites, found in the rat 60 min after injection, are formed in the liver and that the more polar compound penetrates the blood–brain barrier, while the other metabolite is unable to reach the brain, which is surprising since polar compounds are usually hardly transported over the blood–brain barrier. A common metabolic pathway of β-antagonists with an isopropyl group like propranolol, is the oxidative N-dealkylation, yielding *N*-desisopropylpropranolol in combination with acetone (Bakke et al., 1973; Vu and Abramson, 1980). Therefore, it is possible that the most polar radioactive metabolite of *S*-1′-[^{18}F]fluorocarazolol is fluoroacetone, which can cross the blood–brain barrier. Since the contribution of labelled metabolites to radioactivity in the brain is low and within range of those of well-known radioligands and the polar metabolite will not bind to β-adrenoceptors, we suppose that tracer metabolism does not preclude quantitative receptor imaging in the brain.

The second criterion for an appropriate model concerns the possibility to saturate the receptors by addition of non-radioactive *S*-1′-fluorocarazolol and occurrence of low occupancy of adrenoceptors by the tracer at the non-carrier added level. Indeed *S*-1′-fluorocarazolol was able to saturate β-adrenoceptors. Furthermore, the saturation curves clearly show that a higher dose was required to saturate β-adrenoceptors in cerebral tissue than in peripheral organs (Figs. 3 and 4), which must be considered when

designing the multiple injection protocol. However, the rat data cannot be applied directly in the protocol, because these saturation data are merely an indication of the occupancy of the receptors. Moreover, pharmacokinetic characteristics differ between species, so extrapolation of rat data to human studies should be performed with the necessary caution. Furthermore, it should be noted that at doses of *S*-1′-fluorocarazolol comparable to those used in the biodistribution studies (5–100 pmol/kg), the tissue uptake values for cortex in these saturation studies are higher than those determined from blocking and displacement studies. This discrepancy could be attributed to the use of different rat strains (see Section 2).

Our values for B_{max}, determined from in vivo saturation studies, are expressed as pmol g^{-1} tissue wet weight (Table 2). Literature data based on in vitro binding assays are usually expressed as fmol mg^{-1} protein and are therefore not directly comparable. However, a few authors have published receptor densities in terms of pmol g^{-1} tissue wet weight, which enables comparison with our data.

In vitro and in vivo assays have indicated a β-adrenoceptor density of 5–10 pmol g^{-1} wet weight in rat heart (U'Prichard et al., 1978; Raffel et al., 1990; Sisson et al., 1991; Law, 1993) which compares favourably with our estimation of 6.9 pmol g^{-1} (Table 2). Literature data for rat lung show a very large variation: from 3.4 pmol g^{-1} (U'Prichard et al., 1978) to 45 pmol g^{-1} wet weight (Law, 1993); our value of 14.4 pmol g^{-1} (Table 2) is intermediate. β-Adrenoceptor densities per gram packed erythrocytes have not been reported in the literature. In vitro assays have indicated a B_{max} of 6–20 pmol g^{-1} tissue in rat cerebral cortex (Bylund and Snyder, 1976; Bergstrom and Kellar, 1979; Schweitzer et al., 1979; Wagner et al., 1979; Abel et al., 1983; Byerley et al., 1988; Grimm et al., 1992); our estimation of 12.3 pmol g^{-1} is in the middle of this range. For rat cerebellum, data per gram tissue are not available, but it is known that β-adrenoceptor densities in this part of the brain are one-third to one-half of those in cerebral cortex (U'Prichard et al., 1980; Biegon and Israeli, 1986; O'Donnell, 1988; Brannan et al., 1995). Thus, our estimate of 4.6 pmol g^{-1} cerebellum fits with the 12.3 pmol g^{-1} which we found in cerebral cortex.

Third, for the experimental design of the model described by Delforge et al., 1991, the radioligand should dissociate from the receptor. In dynamic PET studies it was shown that in control rats *S*-1′-[^{18}F]fluorocarazolol uptake in the head occurs within 2 min (PET study not shown), so a chase experiment with an excessive dose of propranolol, 5 min after radioligand injection, is appropriate to demonstrate reversibility of the radioligand binding. In this study *S*-1′-[^{18}F]fluorocarazolol could be significantly displaced from tissues containing β-adrenoceptors. Brain regions known to have little β-adrenoceptors, such as medulla and pons (Rainbow et al., 1984) showed no reversible uptake, indicating that the uptake in these regions mainly consists of non-specific binding. After a propranolol chase levels of radioactivity in plasma and liver were increased. This can be expected since the displaced radioligand is transported to excretory organs.

Considering the above, it can be concluded that *S*-1′-[^{18}F]fluorocarazolol is a suitable radioligand for in vivo measurement of β-adrenoceptor densities, fulfilling the criteria necessary for the tracer-kinetic model proposed by Delforge et al., 1991. However, since such a model requires multiple- and possibly carrier-added administration of radioligand, a toxicological study should be performed before a multiple-injection protocol is applied in humans. Naturally, differences between the pharmacokinetic features of the radioligand in rats and humans, should also be considered when applying the tracer-kinetic model.

The aim of this study was to investigate the suitability of *S*-1′-[^{18}F]fluorocarazolol as a PET radioligand for visualizing and quantifying central β-adrenoceptors in vivo. *S*-1′-[^{18}F]fluorocarazolol proved to be an appropriate radioligand for imaging of central β-adrenoceptors since: (i) *S*-1′-[^{18}F]fluorocarazolol uptake reflects specific binding to β-adrenoceptors; ligand accumulation in the brain can be blocked by pre-treating rats with *S*-propranolol, and regional distribution of ^{18}F in these brain regions corresponds to regional β adrenoceptor densities known from in vitro autoradiography with [^{125}I]iodocyanopindolol; (ii) Binding of *S*-1′-[^{18}F]fluorocarazolol to atypical or non-β-adrenergic sites seems negligible.

Furthermore, *S*-1′-[^{18}F]fluorocarazolol is suitable for quantification of central β-adrenoceptors in vivo with an appropriate tracer-kinetic model, since (i) levels of ^{18}F-labeled metabolites in cerebral tissue are relatively low, (ii) values for tissue B_{max} determined from in vivo saturation experiments are within the range of those measured in in vitro binding assays, (iii) bound *S*-1′-[^{18}F]fluorocarazolol can be displaced, indicating that *S*-1′-[^{18}F]fluorocarazolol binding is reversible.

In conclusion, its specificity for β-adrenoceptors, relatively minor formation of metabolites and reversible binding, in combination with its potential to penetrate the blood–brain barrier, indicate that *S*-1′-[^{18}F]fluorocarazolol is a suitable radioligand for quantitative imaging of β-adrenoceptors in vivo, with PET. This radioligand may be used in scientific and clinical research of central β-adrenoceptors and their properties in (patho-) physiological conditions.

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References

Abel, M.S., Villegas, F., Abreu, J., Gimino, F., Steiner, S., Beer, B., Meyerson, L.R., 1983. The effect of rapid eye movement sleep

deprivation on cortical β-adrenergic receptors. Brain Res. Bull. 11, 729–734.

Annane, D., 1991. Médiation β-adrénergique de la commande central de la ventilation: mythe ou réalité?. J. Toxicol. Clin. Exp. 11, 325–336.

Bakke, O.M., Davies, D.S., Dollery, C.T., 1973. Metabolism of propranolol in rat: the fate of the *N*-isopropyl group. Life Sci. 13, 1665–1675.

Barnett, D.B., Rugg, E.L., Nahorski, S.L., 1978. Direct evidence of two types of β-adrenoceptor binding site in lung tissues. Nature 273, 166–168.

Bergstrom, D.A., Kellar, K.J., 1979. Effect of electroconvulsive shock on monoaminergic receptor binding sites in rat brain. Nature 278, 464–466.

Biegon, A., Israeli, M., 1986. Localization of the effects of electroconvulsive shock on β-adrenoceptors in the rat brain. Eur. J. Pharmacol. 123, 329–334.

Brannan, S.K., Miller, A., Jones, D.J., Kramer, G.L., Petty, F., 1995. β-Adrenergic receptor changes in learned helplessness may depend on stress and test parameters. Pharmacol. Biochem. Behav. 51, 553–556.

Brodde, O.E., 1991. β_1- and β_2-adrenoceptors in the human heart: properties, function and alterations in chronic heart failure. Pharmacol. Rev. 43, 203–342.

Byerley, W.F., McConnell, E.J., McCabe, R.T., Dawson, T.M., Grosser, B.I., Wamsley, J.K., 1988. Decreased β-adrenergic receptors in rat brain after chronic administration of the selective serotonin uptake inhibitor fluoxetine. Psychopharmacology (Berl.) 94, 141–143.

Bylund, D.B., Snyder, S.H., 1976. β-Adrenergic receptor binding in membrane preparations from mammalian brain. Mol. Pharmacol. 12, 568–580.

Cerbai, E., Guerra, L., Varani, K., Barbieri, M., Borea, P.A., Mugelli, A., 1995. β-Adrenergic receptor subtypes in young and old rat ventricular myocytes: a combined patch-clamp and binding study. Br. J. Pharmacol. 116, 1835–1842.

Delforge, J., Syrota, A., Lançon, J.P., Nakajima, K., Loc'h, C., Janier, M., Vallois, J.M., Cayla, J., Crouzel, C., 1991. Cardiac β-adrenergic receptor density measured in vivo using PET, CGP 12177, and a new graphical method. J. Nucl. Med. 32, 739–748.

Delforge, J., Bendriem, B., Mazière, B., 1994. Correlation between the ligand lipophilicity and the ratio in vivo over in vitro Kd estimates. J. Nucl. Med. 35, 255, (Abstract).

Delforge, J., Syrota, A., Bendriem, B., 1996. Concept of reaction volume in the in vivo ligand–receptor model. J. Nucl. Med. 37, 118–125, [Abstract].

DePaermentier, F., Cheetam, S.C., Crompton, M.R., Katona, C.L., Horton, R.W., 1990. Brain β-adrenoceptor binding sites in antidepressant-free depressed suicide victims. Brain Res. 525, 71–77.

DePaermentier, F., Crompton, M.R., Katona, C.L., Horton, R.W., 1992. β-adrenoceptors in brain and pineal from depressed suicide victims. Pharmacol. Toxicol. 71 (Suppl1), 86–95.

Elsinga, P.H., Vos, M.G., Van Waarde, A., Braker, A.H., De Groot, T.J., Anthonio, R.L., Weemaes, A.M.A., Brodde, O.E., Visser, G.M., Vaalburg, W., 1996. (*S,S*)- and (*S,R*)-1′-[^{18}F]fluorocarazolol, ligands for the visualization of pulmonary β-adrenergic receptors with PET. Nucl. Med. Biol. 23, 159–167.

Emorine, L.J., Marullo, S., Briend Sutren, M.M., Patey, G., Tate, K., Delavier Klutchko, C., Strosberg, A.D., 1989. Molecular characterization of the human β3-adrenergic receptor. Science 245, 1118–1121.

Emorine, L.J., Feve, B., Pairault, J., Briend Sutren, M.M., Nahmias, C., Marullo, S., Delavier Klutchko, C., Strosberg, D.A., 1992. The human β_3-adrenergic receptor: relationship with atypical receptors. Am. J. Clin. Nutr. 55, 8S–215S.

Flexner, J.B., Flexner, L.B., Chuch, A.C., Rainbow, T.C., Brunswick, D.J., 1985. Blockade of β_1 but not of β_2-adrenergic receptors replicates propranolol suppression of the cerebral spread of an engram in mice. Proc. Natl. Acad. USA 82, 7458–7461.

Goldie, R.G., Spina, D., Rigby, P.J., Paterson, J.W., 1986. Autoradiographic localisation of ascorbic acid-dependent binding sites for [^{125}I]iodocyanopindolol in guinea-pig trachea. Eur. J. Pharmacol. 124, 179–182.

Grimm, L.J., Blendy, J.A., Kellar, K.J., Perry, D.C., 1992. Chronic reserpine administration selectively up-regulates β_1- and α_{1b}-adrenergic receptors in rat brain: an autoradiographic study. Neuroscience 47, 77–86.

Heron, C., Gould, T.J., Bickford, P., 1996. Acquisition of a runaway motor learning task is impaired by a β-adrenergic antagonist in F344 rats. Behav. Brain Res. 78, 235–241.

Hodges-Savola, C., Rogers, S.D., Ghilardi, J.R., Timm, D.R., Mantyh, P.W., 1996. β-Adrenergic receptors regulate astroglyosis and cell proliferation in the central nervous system in vivo. Glia 17, 52–62.

Huang, S.C., Bahn, M.M., Barrio, J.R., Hoffman, J.M., Satyamurthy, N., Hawkins, R.A., Mazziotta, J.C., Phelps, M.E., 1989. A double injection technique for in vivo measurement of dopamine D2-receptor densities in monkeys with 3-(2′-[^{18}F]Fluoroethyl)spiperone and dynamic positron emission tomograpy. J. Cereb. Blood Flow Metab. 9, 850–858.

Innis, R.B., Correa, F.M., Snyder, S.H., 1979. Carazolol, an extremely potent β-adrenergic blocker: binding to β-receptors in brain membranes. Life Sci. 24, 2255–2264.

Joyce, J.N., Lexow, N., Kim, S.J., Artymyshyn, R., Senzon, S., Lawrence, D., Cassanova, M.F., Kleinmann, J.E., Bird, E.D., Winokur, A., 1992. Distribution of β-adrenergic receptor subtypes in human postmortem brain: alterations in limbic regions of schizophrenics. Synapse 10, 228–246.

Kalaria, R.N., Andorn, A.C., Tabaton, M., Whitehouse, P.J., Harik, S.I., Unnerstall, J.R., 1989. Adrenergic receptors in ageing and Alzheimer's disease: increased β_2-receptors in prefrontal cortex and hippocampus. J. Neurochem. 53, 1772–1781.

Law, M.P., 1993. Demonstration of the suitability of CGP 12177 for in vivo studies of β-adrenoceptors. Br. J. Pharmacol. 109, 1101–1109.

Levin, B.E., Dunn-Meynell, A., 1989. Noradrenergic innervation does not affect chronic regulation of [^{125}I]pindolol receptors in fetal rat brain transplants or host neocortex. Brain Res. 494, 325–338.

Maloteaux, J.M., 1986. Drug and transmitter binding in human brain; characterization and localization of serotonin, dopamine and adrenergic receptors. Acta Neurol. Belg. 86, 61–129.

Michel, M.C., Wang, X.L., Schlicher, E., Gothert, M., Beckeringh, J.J., Brodde, O.E., 1987. Increased β_2-adrenoceptor density in heart, kidney and lung of spontaneously hypertensive rats. J. Auton. Pharmacol. 7, 41–51.

Minneman, K.P., Hegstrand, L.R., Molinoff, P.B., 1979. Simultaneous determination of β_1 and β_2-adrenergic receptors in tissues containing both receptor subtypes. Mol. Pharmacol. 16, 34–46.

Nanoff, C., Freissmuth, M., Schütz, W., 1987. The role of a low β_1-adrenoceptor selectivity of [^{3}H]CGP-12177 for resolving subtype-selectivity of competitive ligands. NS Arch. Pharmacol. 336, 519–525.

O'Donnell, J.M., 1988. Psychopharmacological consequences of activation of β-adrenergic receptors by SOM-1122. J. Pharmacol. Exp. Therap. 246, 38–46.

Palkovits, M., Brownstein, M.J., 1988. Maps and Guides to Microdissection of the Rat Brain. Elsevier, New York.

Petrovic, S.L., McDonald, J.K., Snyder, G.D., McCann, S.M., 1983. Characterization of β-adrenergic receptors in rat brain and pituitary using a new high-affinity ligand, [^{125}I]iodocyanopindolol. Brain Res. 261, 249–259.

Pittman, R.N., Minneman, K.P., Molinoff, P.B., 1980. Ontogeny of β_1- and β_2-adrenergic receptors in rat cerebellum and cerebral cortex. Brain Res. 188, 357–368.

Raffel, D.M., 1991. In vivo receptor pharmacology studies with β-adrenergic receptors in isolated rat heart. PhD thesis.

Raffel, D.M., Holden, J.E., Endres, C.J., 1990. Two-injection determination of β-adrenergic receptor density and receptor-ligand affinity in isolated perfused rat heart. J. Nucl. Med. 31, 709–710, (Abstract).

Raffel, D.M., Holden, J.E., De Jesus, O.T., 1991. Plasma free fraction as determinant of in vivo vs. in vitro equilibrium dissociation constants

of receptor binding ligands: direct experimental validation. J. Nucl. Med. 32, 1004–1005, (Abstract).

Rainbow, T.C., Parsons, B., Wolfe, B.B., 1984. Quantitative autoradiography of β_1- and β_2-adrenergic receptors in rat brain. Proc. Natl. Acad. Sci. USA 81, 1585–1589.

Schweitzer, J.W., Schwartz, R., Friedhoff, A.J., 1979. Intact presynaptic terminals required for β-adrenergic receptor regulation by desipramine. J. Neurochem. 33, 377–379.

Sisson, J.C., Wieland, D.M., Koeppe, R.A., Normolle, D., Frey, K.A., Bolgos, G.L., Johnson, J.W., Van Dort, M.E., Gildersleeve, D.L., 1991. Scintigraphic portrayal of β receptors in the heart. J. Nucl. Med. 32, 1399–1407.

Stone, E.A., Platt, J.E., 1982. Brain adrenergic receptors and resistance to stress. Brain Res. 237, 405–414.

Stone, E.A., Najimi, M., Quartermain, D., 1995. Potentiation by propranolol of stress-induced changes in passive avoidance and open-field emergence tests in mice. Pharmacol. Biochem. Behav. 51, 297–300.

Strijckmans, V., Bottlaender, M., Luo, H., Ottaviani, M., McPherson, D.W., Loc'h, C., Fuseau, C., Knapp, F.F., Mazière, B., 1997. Positron emission tomographic investigations of central muscarinic cholinergic receptors with three isomers of [^{76}Br]BrQNP. Eur. J. Nucl. Med. 24, 475–482.

Sutin, J., Griffith, R., 1993. β-adrenergic receptor blockade suppresses glial scar formation. Exp. Neurol. 120, 214–222.

Tiong, A.H., Richardson, J.S., 1989. Characterization of rat cerebral cortical β adrenoceptor subtypes using (−)-[^{125}I]-iodocyanopindolol. J. Recept. Res. 9, 495–508.

Tumer, N., Houck, W.T., Boehm, C., Roberts, J., 1990. Cardiac β-adrenoceptor binding characteristics with age following adrenal demedullation. Br. J. Pharmacol. 99, 87–90.

U'Prichard, D.C., Bylund, D.B., Snyder, S.H., 1978. (±)-[^{3}H]Epinephrine and (−)[^{3}H]dihydroalprenolol binding to β_1- and β_2-noradrenergic receptors in brain, heart, and lung membranes. J. Biol. Chem. 253, 5090–5102.

U'Prichard, D.C., Reisine, T.D., Mason, S.T., Fibiger, H.C., Yamamura, H.I., 1980. Modulation of rat brain α- and β-adrenergic receptor populations by lesion of the dorsal noradrenergic bundle. Brain Res. 187, 143–154.

Vago, T., Bevilacqua, M., Dagani, R., Meroni, R., Frigeni, G., Santoliss, C., Norbiato, G., 1984. Comparison of rat and human left ventricle β-adrenergic receptors: subtype heterogeneity delineated by direct radioligand binding. Biochem. Biophys. Res. Commun. 121, 346–354.

Valverius, P., Borg, S., Valverius, M.R., Hoffman, P.L., Tabakoff, B., 1989. β-Adrenergic receptor binding in brain of alcoholics. Exp. Neurol. 105, 280–286.

Van Waarde, A., Elsinga, P.H., Brodde, O.E., Visser, G.M., Vaalburg, W., 1995. Myocardial and pulmonary uptake of *S*-1′-[^{18}F]fluorocarazolol in intact rats reflects radioligand binding to β-adrenoceptors. Eur. J. Pharmacol. 272, 159–168.

Van Waarde, A., Visser, T.J., Posthumus, H., Elsinga, P.H., Anthonio, R.L., Weemaes, A.M.A., Visser, G.M., Beaufort-Krol, G.C.M., Paans, A.M.J., Vaalburg, W., 1996. Quantification of the β-adrenoceptor ligand, *S*-1′-[^{18}F]fluorocarazolol, in plasma of humans, rats and sheep. J. Chromatogr. B 678, 253–260.

Van Waarde, A., Visser, T.J., Elsinga, P.H., De Jong, B.M., Van der Mark, T.W., Kraan, J., Ensing, K., Pruim, J., Willemsen, A.T.M., Brodde, O.E., Visser, G.M., Paans, A.M.J., Vaalburg, W., 1997. Imaging β-adrenoceptors in the human brain with (S)-1′-[^{18}F]fluorocarazolol. J. Nucl. Med. 38, 934–939.

Visser, T., Van Waarde, A., Van der Mark, T.W., Kraan, J., Elsinga, P.H., Pruim, J., Ensing, K., Jansen, T., Willemsen, A.T.M., Fransen, E.J.F., Visser, G.M., Paans, A.M.J., Vaalburg, W., 1997. Characterization of pulmonary and myocardial β-adrenoceptors with *S*-1′-[Fluorine-18]Fluorocarazolol. J. Nucl. Med. 38, 169–174.

Vu, V.T., Abramson, F.P., 1980. The pathways of propranolol metabolism in dog and rat liver 10,000× *g* supernatant fractions. Drug Metab. Disp. 8, 300–304.

Waeber, C., Rigo, M., Chinaglia, G., Probst, A., Palacios, J.M., 1991. β-Adrenergic subtypes in the basal ganglia of patients with Huntington's chorea and Parkinson's disease. Synapse 8, 270–280.

Wagner, H.R., Crutcher, K.A., Davis, J.N., 1979. Chronic estrogen treatment decreases β-adrenergic responses in rat cerebral cortex. Brain Res. 171, 147–151.

Whitsett, J.A., Manton, M.A., Darovec-Beckerman, C., Adams, K., 1981. β-Adrenergic receptors and catecholamine-sensitive adenylate cyclase in the developing rat lung. Life Sci. 28, 339–345.

Winter, R.J., Dickinson, K.E., Rudd, R.M., Sever, P.S., 1986. Tissue specific modulation of β-adrenoceptor number in rats with chronic hypoxia with an attenuated response to down-regulation by salbutamol. Clin. Sci. 70, 159–165.

Zheng, L.B., Berridge, M.S., Ernsberger, P., 1994. Synthesis, binding properties, and ^{18}F labelling of fluorocarazolol, a high-affinity β-adrenergic receptor antagonist. J. Med. Chem. 37, 3219–3230.