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Characterisation of β_2-adrenoceptors, using the agonist [^{11}C]formoterol and positron emission tomography

Ton J. Visser [a,*], Aren van Waarde [a], Petra Doze [a], Philip H. Elsinga [a], Thomas W. van der Mark [b], Jan Kraan [b], Kees Ensing [c], Willem Vaalburg [a]

[a] *Positron Emission Tomography (PET) Center, Groningen University Hospital, P.O. Box 30001, 9700 RB Groningen, Netherlands*
[b] *Department of Pulmonary Diseases, Groningen University Hospital, P.O. Box 30001, 9700 RB Groningen, Netherlands*
[c] *Department of Analytical Chemistry and Toxicology, University Center for Pharmacy, Antonius Deusinglaan 1, 9713 AV Groningen, Netherlands*

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Abstract

The agonist radioligand *N*-[2-hydroxy-5-[1-hydroxy-2-[[2-(4-[^{11}C]-methoxyphenyl)-1-methylethyl]amino]ethyl]phenyl]formamide ([^{11}C]formoterol) was synthesised in order to test its ability to visualise pulmonary β_2-adrenoceptors in vivo, with positron emission tomography (PET). Formoterol was labelled via reaction of a dibenzyl-protected precursor with [^{11}C]CH_3I. Subsequent deprotection with Pd/C and H_2 yielded [^{11}C]formoterol in 5–15% (corrected for decay) and the specific activity ranged from 5.5–22.2 TBq mmol^{-1} (150–600 Ci mmol^{-1}), 60–70 min after end of bombardment. Biodistribution studies with [^{11}C]formoterol were performed in male Wistar rats which were either untreated or predosed with (D,L)-propranolol hydrochloride (2.5 mg kg^{-1}, β-adrenoceptor antagonist), erythro-DL-1-(7-methylindan-4-yloxy)-3-isopropylaminobutan-2-ol hydrochloride (ICI 118551, 0.15 mg kg^{-1}, β_2-adrenoceptor antagonist), isoprenaline (15 mg kg^{-1}, non-subtype selective β-adrenoceptor agonist) or (±)-(2-hydroxy-5-[2-((2-hydroxy-3-(4-((1-methyl-4-trifluoromethyl)1*H*-imidazol-2-yl-)phenoxy)propyl)amino)ethoxy]benzamide)monomethane sulfonate (CGP 20712A, 0.15 mg kg^{-1}, β_1-adrenoceptor antagonist). Lungs, heart, liver and plasma were analysed for radioactive metabolites. The kinetics of [^{11}C]formoterol in the lungs of male Wistar rats were investigated by means of a dynamic PET study. The biodistribution studies showed significant specific binding in tissues known to contain β_2-adrenoceptors (lungs, spleen, and heart). Binding in these organs was blocked by ICI 118551 and isoprenaline, but not by CGP 20712A. [^{11}C]Formoterol was rapidly metabolised in rats but lungs and heart did not substantially take up the labelled metabolites. The binding of [^{11}C]formoterol in various tissues of rats is consistent with the β_2-selectivity of formoterol. Whether [^{11}C]formoterol selectively binds to the high affinity state of β_2-adrenoceptors remains to be elucidated. [^{11}C]Formoterol is potentially useful for studying β_2-adrenoceptors with PET and this radioligand may provide new insights in the mechanisms underlying prolonged sympathomimetic action.

Keywords: [^{11}C]Formoterol; β_2-Adrenoceptor; Positron emission tomography; Lung

1. Introduction

N-[2-hydroxy-5-[1-hydroxy-2-[[2-(4-[^{11}C]-methoxyphenyl)-1-methylethyl]amino]ethyl]phenyl]formamide (formoterol) is a β_2-adrenoceptor agonist with interesting clinical features. Formoterol displays a high affinity (K_D = 1.05 ± 0.17 nM, in human lung membranes (Mak et al., 1994)) and selectivity (β_2:β_1 = 90:10) for the β_2-adrenoceptor (Roux et al., 1996). Addition of the non-hydrolysable GTP analogue, GTPγS gave a reduction of the K_D and B_{max}, suggesting that the receptors labelled by [^{3}H]formoterol are coupled to a guanine nucleotide binding protein and that formoterol labels the high affinity state (Mak et al., 1994). Formoterol causes a long lasting bronchodilation (at least 12 h) in man but only when the drug is administered by inhalation (Löfdahl and Svedmyr, 1989). It reduces asthma symptoms significantly and there is no evidence of tolerance or tachyphylaxis in long-term studies (Johnson, 1995; Steffensen et al., 1995). The mechanisms causing the sustained activity are not fully understood yet. In case of salmeterol, another long acting β_2-adrenoceptor agonist, it was explained by means of an exosite model (Bradshaw et al., 1987; Nials et al., 1993; Coleman et al., 1996) in which the ligand binds to an exosite close to the receptor, resulting in prolonged activation. Another theory

* Corresponding author. Tel.: +31-50-361-3183; Fax: +31-50-361-1687; E-mail: t.j.visser@pet.azg.nl

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is the plasmalemma diffusion micro kinetic model, based on the lipophilicity of formoterol and salmeterol (Anderson et al., 1994). But lipophilicity is probably not the only determinant, which governs the sustained action (Lindén et al., 1996). Knowledge about the in vivo pharmacokinetics and metabolism of formoterol is of major importance because delivery and metabolism may also influence the kinetics of the bronchodilatory effect (Jeppsson et al., 1989).

With positron emission tomography (PET) it is possible to study the behaviour and fate of radiolabelled (receptor) ligands in vivo, e.g., their biodistribution, the plasma and tissue kinetics, non-specific binding, and dose dependent changes therein. Receptors can be studied in their natural environment and their distribution and density can be assessed. Here, we describe the synthesis of a novel agonist radioligand [^{11}C]formoterol and its preclinical evaluation in experimental animals.

2. Materials and methods

2.1. Compounds

(D,L)-1-[(1-Methylethyl)amino]-3-(1-naphthalenyloxy)-2-propanol hydrochloride ((DL)-propranolol hydrochloride) was obtained from Janssen Chimica, Tilburg, the Netherlands. Erythro-DL-1-(7-methylindian-4-yloxy)-3-isopropylaminobutan-2-ol hydrochloride (ICI 118551 hydrochloride) was a gift from ICI pharmaceuticals, Macclesfield, UK. (±)-Isoprenaline was obtained from Sigma, St. Louis, MO. (±)-(2-hydroxy-5-[2-((2-hydroxy-3-(4-((1-methyl-4-trifluoromethyl)1*H*-imidazol-2-yl-)phenoxy)propyl)amino) ethoxy]benzamide)monomethane sulfonate (CGP 20712A) was a gift from Ciba Geigy, Basle, Switzerland.

2.2. The synthesis of [^{11}C]formoterol

[^{11}C]CH_3I was prepared from [^{11}C]CO_2, which was generated by the $^{14}N(p,\alpha)^{11}C$ nuclear reaction with 17 MeV protons. The [^{11}C]CO_2 was trapped in the reaction vessel which was previously filled with 0.3 ml of a diluted solution of lithium aluminum hydride ($LiAlH_4$) in tetrahydrofuran (THF, 0.15 ml of a saturated solution of $LiAlH_4$ in THF was diluted with THF to a final volume of 1 ml), using an Anatech robotic system (Scanditronix, Uppsala, Sweden). After evaporation of the solvent at 130°C, hydrogen iodide (57%, 0.8 ml) was added by the robot and the [^{11}C]CH_3I was formed. In this way 30 GBq [^{11}C]labelled methyliodide with a specific activity of 150 TBq mmol^{-1} was obtained 11 min after end of bombardment. The radiochemical yield was 70–90% (corrected for decay). The [^{11}C]CH_3I was trapped into a vial containing a solution of the desmethyl precursor 4-benzyloxy-3-formamido-α-[*N*-benzyl-*N*-(*p*-hydroxy-α-methylphenethyl)aminomethyl]-benzyl alcohol (1.0 mg, mixture of (*R*,*R*) and (*S*,*S*)-isomers) (Murase et al., 1977, 1978; Trofast et al., 1991) and potassium *tert*-butoxide (0.2 mg) in acetonitrile (0.8 ml) which was placed inside a heating block (room temperature). After the [^{11}C]CH_3I was trapped, the heating block was warmed up to 110°C (4 min) and the reaction mixture was allowed to react for 5 min at this temperature. To cool down, the reaction vial was placed into cold water for 1 min and acetic acid (1 μl) was added to destroy the base. Then the reaction mixture was added to a vessel containing a suspension of palladium on activated carbon (Pd/C, 30%, 3 mg) in ethanol (0.5 ml). Via a needle a flow of hydrogen (40 ml min^{-1}) was flushed through the suspension for 15 min. To remove the Pd/C, the suspension was filtered (Millex-HV, 0.45 μm, Millipore) and the filter was extracted with ethanol (1 ml). Subsequently the solvents were removed by rotary evaporation. The residue was dissolved in high performance chromatography (HPLC) eluent (1 ml) and injected onto the HPLC column (μ-Bondapak C-18, 5 μm 3.9 × 300 mm, Waters Millipore, mobile phase: water [with 0.9% sodium chloride (NaCl) and 2 mmol of sodium dihydrogen phosphate monohydrate (NaH_2PO_4), pH = 3]:ethanol = 85:15) running at 1.2 ml min^{-1}. The product had a retention time of 12 min. (An experimental sample of formoterol fumarate obtained from Astra Draco confirmed the identity of the

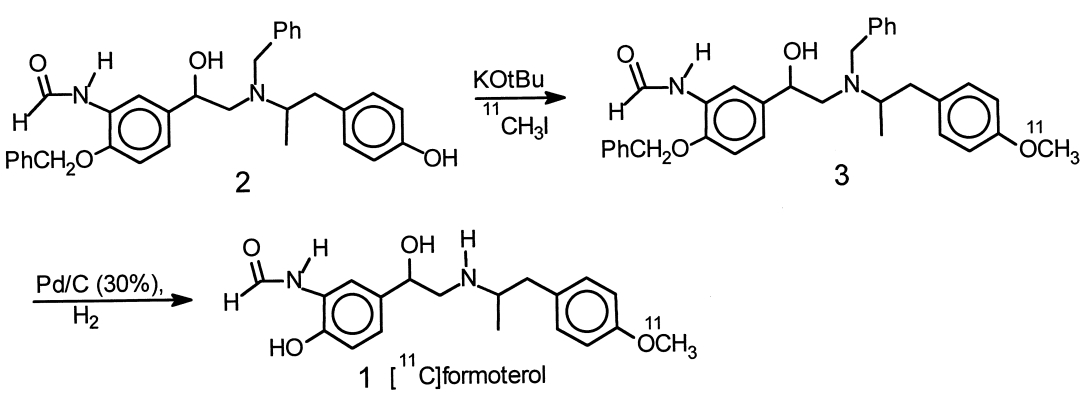

Fig. 1. Reaction scheme of [^{11}C]formoterol.

labelled product). The labelled product was collected and diluted with buffer (NaCl, 140 mmol l^{-1}; sodium phosphate (Na_3PO_4), 9.0 mmol l^{-1}; NaH_2PO_4, 1.3 mmol l^{-1}), before injection. The radiochemical yield was 5–15% (end of bombardment) and the specific activity ranged from 5.5–22.2 TBq $mmol^{-1}$ (150–600 Ci $mmol^{-1}$), 60–70 min after end of bombardment (Fig. 1).

2.3. Dynamic PET-study in male Wistar rats

Animal handling was in accordance with the Law on Animal Experiments of the Netherlands. PET-studies were carried out with a Siemens ECAT 951/31 positron camera. Data acquisition was performed using a dynamic protocol. The in-plane spatial resolution amounted to 6 mm full width half-maximum. During the reconstruction a zoom factor of 1.5 was applied and the matrix size was 128×128. The rats were anaesthetised with pentobarbital (60 mg kg^{-1} body-weight). The rats were positioned in the PET camera parallel to the transaxial plane of the tomograph, so sagittal sections were obtained. In six control rats a solution of [^{11}C]formoterol [1.85 MBq, specific activity 5.6–13 TBq $mmol^{-1}$] was administered by injection into the tail vein. Five (other) rats were pre-treated with propranolol (2.5 mg kg^{-1}), within 1 min followed by injection of a solution of the radioligand (1.85 MBq, specific activity 5.6–13 TBq $mmol^{-1}$). The following time frames were defined: 8 frames of 15 s, followed by 4 frames of 30 s, 4 frames of 1 min, 4 frames of 2 min, 6 frames of 4 min, and 2 frames of 10 min. Data analysis was performed using Siemens ECAT software (V6.5D) on a Sun workstation.

2.4. Biodistribution studies in male Wistar rats

[^{11}C]Formoterol (1.85–2.78 MBq, specific activity: 14.8–29.6 TBq $mmol^{-1}$) was intravenously injected into the tail vein of pentobarbital-anaesthetised male Wistar rats (242 ± 26 g, $N = 5$). In blocking experiments the rats were either pre-treated with propranolol (2.5 mg kg^{-1}, $N = 5$), ICI 118551 (0.15 mg kg^{-1}, $N = 5$), CGP 20712A (0.15 mg kg^{-1}, $N = 4$) or isoprenaline (15 mg kg^{-1}, $N = 4$) in saline, 1 min before injection of the radioligand (1.85–2.78 MBq, specific activity: 14.8–29.6 TBq $mmol^{-1}$). After 10 min the animals were sacrificed by extirpation of the heart and the following tissues were removed: cerebellum, cortex, fat, heart, intestine, kidney, liver, lung, muscle (skeletal), spleen, and trachea. Plasma and an erythrocyte pellet were obtained by centrifugation (6000 min^{-1}, 5 min). Next, the radioactivity was determined with a gamma counter (LKB-Wallac Compugamma 1282 CS, Turku, Finland) and the tissue samples were weighed.

2.5. Clearance from plasma and metabolism

The carotid artery of anaesthetised rats (258 ± 19 g, $N = 4$) was cannulated and arterial blood samples (0.2 to 0.3 ml) were drawn at 10, 20, 30, 40, 50, 60, 90, 120, 300, 600, 1200, and 2400 s after i.v. injection of 1.85 MBq of labelled formoterol (specific activity 11.1 TBq $mmol^{-1}$). Plasma and red blood cells were separated by centrifugation (2 min, 6000 min^{-1}, Hettich Zentrifugen, Tuttlingen, Germany). Plasma (100 μl) was then counted in a γ-counter. The clearance curve was analysed by means of a commercial non-linear regression program (EnzFitter, Elsevier Biosoft, Cambridge, UK).

Plasma samples for metabolite analysis were acquired from male Wistar rats ($N = 2$, 290 ± 21 g) as described above. Samples were drawn at 0.5, 1, 1.5, 2, 3, 5, (0.25 ml) and 10 min (0.5 ml) post injection of the radioligand (5.55 MBq, specific activity 11.1 TBq $mmol^{-1}$). Plasma and red blood cells were separated by centrifugation (2 min, 6000 min^{-1}, Hettich Zentrifugen, Tuttlingen, Germany). Subsequently, the plasma was deproteinized with two volumes of acetonitrile, centrifuged and the supernatant was injected onto an HPLC column (C-18 Radialpak, 5 μm, mobile phase: Na_2HPO_4 [50 mM]:ethanol = 66:34, pH = 3.0; flow rate 1.3 ml min^{-1}). Fractions (0.65 ml) of the eluate were collected during 15 min and counted

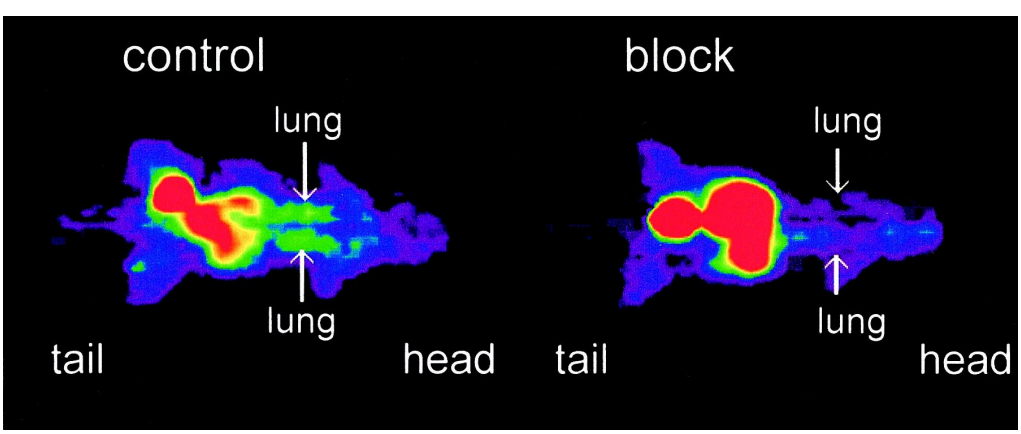

Fig. 2. PET images of β_2-adrenoceptors in the lungs of male Wistar rats acquired with [^{11}C]formoterol without (left image) and with (right image) propranol blocking.

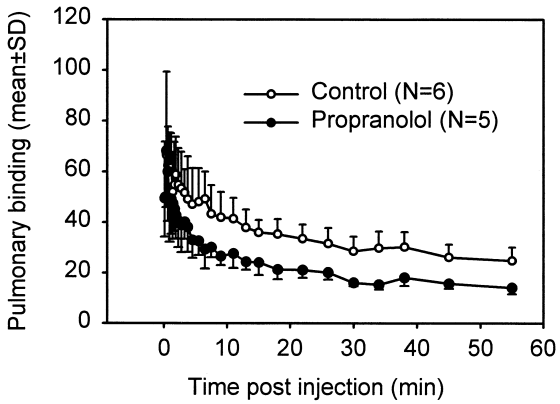

Fig. 3. Time activity curves generated from the lungs of rats acquired with [^{11}C]formoterol with or without propranolol pretreatment. Treated and untreated groups were significantly different (Student's *t*-test; $P < 0.05$).

in the gamma counter. The recovery from the column exceeded 90%.

For analysis of metabolites in lungs, heart, and liver, rats ($N = 2$, 244 ± 1 g) were injected with [^{11}C]formoterol (7.4 MBq, specific activity: 14.8 TBq mmol^{-1}) via a tail vein. After 10 min the rats were sacrificed by extirpation of the heart. Lungs, heart, and liver were quickly removed and homogenised in a mixture of water (1 vol.) and acetonitrile (2 vols.), using a Heidolph diax 600 apparatus (Hettich, Tuttlingen, Germany). Subsequently the extracts were centrifuged (5 min, 15 000 min^{-1}) and the supernatant was analysed by HPLC as described above.

3. Results

3.1. Dynamic PET study in male Wistar rats

In the PET study, lungs were visible in the untreated rats. After pre-treatment of animals with a non-selective beta-blocker (propranolol), the lungs were no longer visible (Fig. 2). The time activity curves generated from the lungs (control rats) showed a relatively rapid decline to a plateau (Fig. 3). After pre-treatment of rats with propranolol, pulmonary uptake was significantly suppressed (Student's *t*-test, $P < 0.05$). If tissue uptake of radioactivity after propranolol pre-treatment is considered to represent non-specifically bound radioligand only, ratios of total/non-specific binding can be calculated. Total/non-specific binding increased to 1.8 at 55 min post injection (Fig. 6).

3.2. Biodistribution studies

The biodistribution of [^{11}C]formoterol in male Wistar rats, both untreated and pre-treated with saturating doses of non-selective and subtype selective β-adrenoceptor agonists and antagonists, is shown in Table 1. Tissue uptake was determined by ex vivo counting, 10 min post injection, and expressed as a differential absorption ratio which is defined as (cpm recovered/g tissue)/(cpm injected/g body weight). Tissues rich in β_2-adrenoceptors (lung, spleen, heart) showed high accumulation of radioactivity. Uptake in cortex and cerebellum, was very low (0.03% of the injected dose per gram); here the tracer did not accumulate over plasma levels.

When the rats were pre-treated with propranolol (a lipophilic, non-selective β-adrenoceptor antagonist), radioactivity in heart, intestine, lung, skeletal muscle, red blood cells and spleen was significantly reduced. Isoprenaline (a hydrophilic, non-selective β-adrenoceptor agonist) and ICI 118551 (a lipophilic β_2-adrenoceptor antagonist) gave comparable results as propranolol. In contrast uptake in heart, lung, muscle, plasma, red blood cells and spleen was not affected after pre-treatment of the rats with CGP 20712A (a hydrophilic β_1-adrenoceptor antagonist).

Table 1
Biodistribution of [^{11}C]formoterol in rat[a]

Tissue	Control ($N = 5$)	Propranolol ($N = 5$)	ICI 118551 ($N = 5$)	CGP 20712A ($N = 4$)	Isoprenaline ($N = 4$)
Cerebellum	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Cortex	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Fat	0.24 ± 0.13	0.19 ± 0.12	0.17 ± 0.02	0.25 ± 0.07	0.24 ± 0.05
Heart	2.39 ± 0.23	1.82 ± 0.11[b]	1.70 ± 0.28[b]	2.34 ± 0.54	1.77 ± 0.31[b]
Intestine	1.45 ± 0.61	2.43 ± 1.08	1.81 ± 1.89	1.33 ± 0.12	0.93 ± 0.15
Kidney	10.7 ± 3.6	7.4 ± 2.8	5.5 ± 2.2[b]	10.0 ± 1.9	9.4 ± 1.7
Liver	1.19 ± 0.50	0.87 ± 0.24	0.88 ± 0.16	1.30 ± 0.12	1.32 ± 0.23
Lung	4.28 ± 0.39	2.07 ± 0.30[b]	1.86 ± 0.30[b]	4.27 ± 0.26	2.21 ± 0.57[b]
Muscle	0.53 ± 0.09	0.28 ± 0.03[b]	0.30 ± 0.07[b]	0.40 ± 0.05[b]	0.23 ± 0.04[b]
Plasma	0.45 ± 0.08	0.38 ± 0.03	0.31 ± 0.06[b]	0.40 ± 0.06	0.40 ± 0.07
Red blood cells	0.44 ± 0.07	0.27 ± 0.07[b]	0.28 ± 0.03[b]	0.38 ± 0.07	0.21 ± 0.03[b]
Spleen	3.11 ± 0.88	1.52 ± 0.16[b]	1.58 ± 0.30[b]	2.87 ± 0.14	1.76 ± 0.26[b]
Trachea	0.77 ± 0.18	0.68 ± 0.07	0.83 ± 0.23	0.93 ± 0.13	0.53 ± 0.15

[a]Tissue uptake is expressed as a differential absorption ratio and presented as a mean ± SD of N independent observations. Differences between treated and untreated groups were tested using Student's *t*-test; P = dual-tail probability.
[b]Significantly different from control rats, $P < 0.05$.

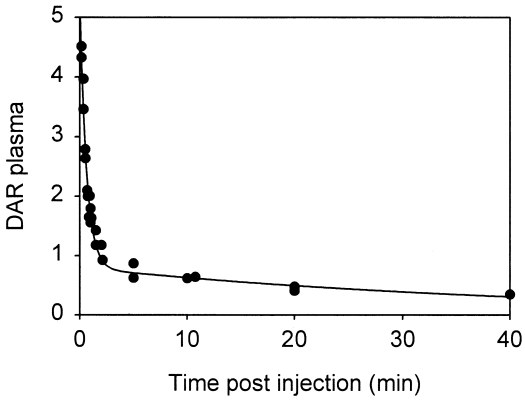

Fig. 4. The clearance of [^{11}C]formoterol from rat plasma. The solid line is a bi-exponential curve fit.

3.3. Clearance from plasma and metabolism

The initial clearance of i.v. injected [^{11}C]formoterol from plasma was very rapid (Fig. 4). The clearance showed a biphasic pattern with an initial rapid distribution phase (half-life: 0.43 min), representing 85% of the injected dose, and a slow elimination phase (half-life: 29 min). The time course of metabolism of the radioligand was also analysed. [^{11}C]Formoterol was substantially metabolised and radioactive metabolites appeared rapidly in plasma. The fraction of total plasma radioactivity representing unmodified radioligand decreased from more than 99.8% at 0 min to less than 15% at 10 min post injection (Fig. 5).

HPLC analyses of tissue extracts acquired 10 min post injection, indicated that respectively $>96\%$, $>94\%$ and $<8\%$ of radioactivity in lungs, heart, and liver represented the parent compound. The parent compound eluted with a retention time of 9 min; one hydrophilic radioactive metabolite was observed with a retention time of 3 min. To determine whether this metabolite is a glucuronide, two plasma samples were obtained 10 min post-injection. One was used as a control while the other was incubated with 100 μl of β-glucuronidase (solution in 50% glycerol, pH = 6.0, from *Escherichia coli*, Boehringer Mannheim, Germany) for 60 min at 22°C. HPLC analysis showed that the untreated control sample contained less than 10% of the parent compound while in the incubated sample this was more than 55%.

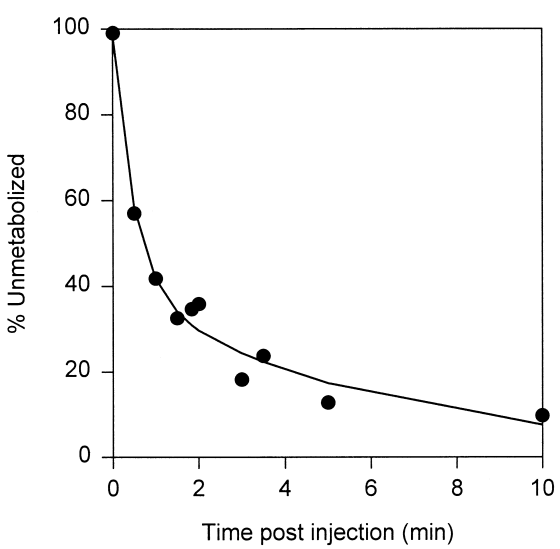

Fig. 5. Time-course of unchanged [^{11}C]formoterol in plasma. The fraction of total plasma radioactivity representing unmodified radioligand is plotted. The solid line is a bi-exponential curve fit.

4. Discussion

Formoterol was labelled with [^{11}C] in the methoxy group by reaction of a desmethyl precursor with [^{11}C]CH_3I. Because of the presence of a secondary amine and a phenolic hydroxyl group which are also able to react with [^{11}C]CH_3I, the benzyl-protected precursor **2** (Fig. 1) was synthesised as a mixture of (*R,R*) and (*S,S*)-isomers, according to a procedure as described by Murase et al. (1977, 1978). After labelling, compound **3** was deprotected with Pd/C (30%) and H_2 to afford [^{11}C]formoterol **1**. It may still be possible to further optimise the labelling/deprotection reaction conditions to obtain higher yields. For instance other protecting groups, which can be removed more rapidly, may be used.

The biodistribution data clearly demonstrate that the tissues rich in β_2-adrenoceptors (lung, spleen, heart) show high accumulation of [^{11}C]formoterol (Table 1). But probably due to its low lipophilicity, uptake of formoterol ($\log P = 0.4$, Jeppsson et al., 1989) in cerebral cortex and cerebellum, tissues with a high proportion of β-adrenoceptors (Minneman et al., 1979; Booze et al., 1989), was very low (0.03% of the injected dose per gram).

Propranolol pre-treatment resulted in a significant reduction of the uptake of [^{11}C]formoterol in lungs, heart, and spleen, suggesting that the accumulation in these organs is at least partially receptor-mediated (Table 1). ICI 118551 is lipophilic and shows a 100–300 times higher affinity for the β_2 than for the β_1-subtype (Bilski et al., 1983; Lemoine et al., 1985). After administration of this ligand the uptake in tissues containing β_2-adrenoceptors was significantly reduced. CGP 20712A is a hydrophilic β-adrenoceptor ligand which shows a high selectivity for the β_1 subtype (1000–10 000 fold) (Dooley et al., 1986). Pre-treatment of the rats with CGP 20712A did not significantly reduce uptake in the target organs with respect to the control values. Administration of the hydrophilic, non-selective β-adrenoceptor agonist, isoprenaline, before injection of the radioligand gave the same results in lungs, heart, and spleen as propranolol pre-treatment. Thus, the results of the blocking experiments indicate that formoterol uptake represents binding of the radioligand to β_2-adrenoceptors.

Metabolism of formoterol is fast. Sasaki et al. (1982) reported a somewhat slower metabolism after intravenous administration of [^{3}H]formoterol fumarate (50 μg kg^{-1}) to rats (41, 28, and 24% of radioactivity was unchanged parent compound at 5, 30, and 60 min). We found less than 15% unmetabolised ligand in rat plasma at 5 min post injection of the radioligand. The difference between Sasaki's and our results may be due to the fact that Sasaki et al. administered a more than 100 times higher dose in their experiments. Only one metabolite, the 2-*O*-glucuronide conjugate of formoterol was detected in urine and bile (Sasaki et al., 1982). We also found that glucuronidation is a major metabolic pathway. Since radioactivity in the target organs consists mainly (> 94%) of parent compound, the radioactive metabolites of formoterol seem to have negligible affinity to β-adrenoceptors and they do not accumulate in the lungs. Of course glucuronidation can be expected to reduce receptor affinity substantially and it results in rapid elimination of the ligand via the kidneys.

The ratios of total/non-specific binding of [^{11}C]formoterol in rat lung are much lower than those of the established β-adrenoceptor antagonist (*S*)-4-(3-*t*-butylamino-2-hydroxypropoxy)-2*H*-benzimidazol-2-[^{11}C]one ((*S*)-[^{11}C]CGP 12177) during the time-course of a PET scan. Whereas these ratios for [^{11}C]CGP 12177 rapidly increased to about 7 (Van Waarde et al., 1998), for [^{11}C]formoterol they rose to approximately 1.8 (Fig. 6). But a mixture of the (*R*,*R*) and (*S*,*S*) isomers of [^{11}C]formoterol was used in these experiments. From the four possible diastereoisomers of formoterol, the (*R*,*R*)-isomer is the most potent (Trofast et al., 1991). Therefore, in future studies we intend to use enantiomerically pure (*R*,*R*)-[^{11}C]formoterol, which may improve the target/non-target and tissue/plasma ratios. In experiments with radiolabelled muscarinic antagonists we noticed a doubling of the total/non-specific binding ratios under such conditions (Visser et al., 1997).

With these studies we have shown that it is possible to detect specific binding of the agonist radioligand [^{11}C]formoterol to β-adrenergic receptors in vivo with PET. Whether [^{11}C]formoterol selectively binds to the high affinity state of β_2-adrenoceptors remains to be elucidated. In vitro binding studies with [^{3}H]formoterol in human and guinea pig lung membranes revealed binding of formoterol to only one single class of receptors (Mak et al., 1994). Roux et al. (1996) demonstrated that formoterol displaces [^{125}I]iodocyanopindolol from guinea pig bronchial β_2-adrenoceptors and these competition curves were best characterised by a model for two-site interaction; the agonist binds to receptors that are in high ($pK_{i,high} = 9.62 \pm 0.4$) and low ($pK_{i,low} = 7.83 \pm 0.06$)-affinity states. If this model for two-site interaction also holds in vivo then it is likely (but difficult to prove) that the in vivo obtained PET images of the lungs of rats also represents binding of [^{11}C]formoterol to the high affinity state of β-adrenoceptors. Under the conditions of a PET study the low affinity state will not significantly contribute to total tissue binding since the binding potential of these receptors, that is the ratio of B_{max} and K_D, is much less than one (Scatchard, 1949).

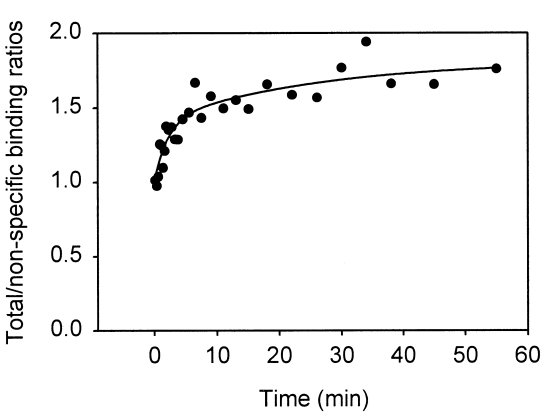

Fig. 6. Total/nonspecific binding ratios of [^{11}C]formoterol determined from the dynamic PET data in absence and presence of propranolol.

Since β_2-adrenoceptor agonists are potent relaxants of the airways, a dysfunction in the adrenergic system, e.g., a reduction in β-adrenoceptor density, and affinity, or a defect in the coupling of receptors to distal elements of the transduction chain, may lead to a less effective relaxation of the airways and may therefore be an important mechanism underlying asthma. Since antagonists do not induce high affinity states of receptors, antagonist radioligands will only provide information about the total number of receptors. Data on the high affinity state can be acquired only with agonist ligands. Such data can be of clinical interest because the fraction of receptors in the high affinity state may be altered by disease or after treatment. The efficacy of agonist drugs may be affected by alterations of the fraction of receptors in the high affinity state.

5. Conclusion

This is the first time that specific binding to β_2-adrenoceptors was observed in intact animals with PET, after i.v. administration of a radiolabelled agonist. With the introduction of this new radioligand a new research tool has become available to study pulmonary β_2-adrenoceptors and to examine the mechanisms underlying long duration of action in β-adrenergic bronchodilators.

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