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Synthesis and in vivo evaluation of [^{11}C]ICI 118551 as a putative subtype selective β_2-adrenergic radioligand

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Abstract

Erytro-(±)-1-[2,3-(dihydro-7-methyl-1H-inden-4-yl)oxy]-3-[*iso*-propylamino]-2-butanol (ICI 118551) a potent clinically used β_2 adrenergic antagonist, was labelled with carbon-11 ($t_{1/2} = 20.4$ min) as a potential radioligand for the non-invasive assessment of β_2 adrenergic receptors in the lung with positron emission tomography (PET). The radiolabelled compound was prepared by reductive *N*-alkylation of its des-isopropyl precursor with [2-^{11}C]acetone. (±)-[^{11}C]ICI 118551 was obtained in greater than 98% radiochemical purity in 30 min with a radiochemical yield of 15 ± 5% (non-decay corrected) and a specific radioactivity 2.5 ± 0.5 Ci/μmol. The biological evaluation of racemic erythro (±)-[^{11}C]ICI 118551 in rats and *Macaca Nemestrina* shows a high radioactivity uptake in lung and heart. However, in both animal models no detectable displacement of lung radioactivity concentration was observed after pre-treatment with propranolol or ICI 118551, which indicates that in this organ, radioligand uptake is mostly due to non-specific binding. The biological data suggest that erythro (±)-[^{11}C]ICI 118551 is not adequate to be further developed as a tracer for β_2 adrenergic receptor imaging in vivo.

Keywords: (±)-[^{11}C]ICI 118551; β_2-Adrenergic receptors; Emission tomography; Rats; Monkeys

1. Introduction

Pulmonary beta adrenergic receptors exert a fundamental role not only in the regulation of airway caliber, but also in other physiological processes related to respiration such as surfactant production, alveolar-capillary fluid balance, mast cells and other inflammation cell functions and in glandular secretion. On the basis of their ligand specificity, beta adrenergic receptors have been classified at least in two subtypes: β_1 which are mainly expressed in myocite, and β_2 that are predominant in airway smooth muscles, pulmonary alveoli and inflammatory cells. Modifications in β_2 adrenergic receptor function have been demonstrated in cystic fibrosis, obstructive pulmonary disease and emphysema.

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Several positron emitting β-ligands have been synthesised for the in vivo study of peripheral β-adrenergic receptors by positron emission tomography (PET; Boullais et al., 1985; Antoni et al., 1989; Brady et al., 1990; Hammadi and Crouzel, 1990, 1991; Aigbirhio et al., 1992; Berridge et al., 1992; Kinsey et al., 1992; De Groot et al., 1993; Berridge et al., 1994; Zheng et al., 1994; Van Waarde et al., 1998). Among these [^{11}C]CGP 12177 and (*S*)-1-[^{18}F]carazolol, are suitable ligands for the in vivo PET measurement of β adrenergic receptors in the heart and lung of human subjects (Brady et al., 1990; De Groot et al., 1993). However, these tracers are not subtype selective. [^{11}C]CGP 12177 has only a slightly higher affinity for β_1 receptors (Brady et al., 1990) and (*S*)-1-[^{18}F]carazolol is slightly more active on β_2-sites (Elsinga et al., 1996). Thus using these tracers is not possible to separate the single receptor sub-populations. In both cardiovascular and respiratory system at least two distinct beta adrenoreceptor binding sites coexist, i.e. β_1 and β_2 subtypes, but the proportion of these subtypes differs between tissues and is strictly species specific. In addition due to their differences in cellular distribution, second messenger coupling and pharmacological behaviour, their relative expression may be selectively modified during pathology or after drug treatment (Rugg et al., 1978). Thus in order to distinguish the two beta adrenergic receptor sub-population PET tracers with higher in vivo selectivity are requested.

Recently [^{11}C-methyl]-formoterol, a β_2-selective adrenoceptor agonist ($K_d = 1{,}05$; $\beta_2/\beta_1 = 90$), has been developed as a tracer for the in vivo measurement of the high affinity state of β_2-adrenoceptors. The lung uptake of racemic [^{11}C-methyl]-formoterol in rats was displaceable by propranolol and ICI 118551, reaching values of total to non-specific binding ratio of 2.3 at 10 min. However, the tracer is highly metabolised and undergoes a rapid blood clearance (85% of injected dose with half-life of 0.43 min; Visser et al., 1997). ICI 118551 is the most selective β_2 receptor antagonist with a β_1 over β_2 selectivity over 100 and with an in vitro K_d below 1 nM (Hieble et al., 1995). The development of ICI 118551 as a PET tracer may allow the selective measurement of β_2 receptor expression in lungs, heart and brain of human subjects. The purpose of this study was the labelling of racemic erythro (±)ICI 118551 with C-11 and its evaluation as a potential radioligand for the in vivo study of β_2 adrenergic receptors in humans using PET.

2. Materials and methods

Reagents and solvents were obtained from Aldrich Italia S.p.A., Milano, Italy, and used without further purification unless otherwise noted. An authentic sample of (±)-ICI 118551 (250 mg), was obtained from ZENECA S.p.A., Milano, Italy. The des-*iso*-propyl precursor of (±)-ICI 118551 was prepared by Argus chemicals, Prato, Italy, following the procedures described in the European Patent Application (Tucker, 1979). [^{11}C]Carbon dioxide was produced by the ^{14}N(P,α)^{11}C reaction on a CTI-Siemens RDS-112 cyclotron, using 11.5 MeV proton beam currents of 10–30 μA, and trapped in a hollow stainless steel loop cooled with liquid nitrogen. [2-^{11}C]Acetone was synthesised in 5 min with over 50% radiochemical yield (EOS, not corrected for decay) by the reaction of [^{11}C]carbon dioxide with 0.07 M MeLi solution in THF (freshly distilled from sodium and benzophenone 1:15 v/v) using the selective two-step quenching reaction published elsewhere (Soloviev et al., 1997; Studenov et al., 1999).

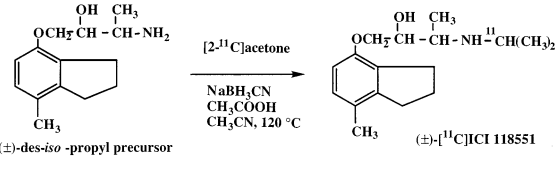
Radiochemical syntheses were performed on the modified fully automated synthesis module PET Tracer Synthesiser, Nuclear Interface Datentechnik GmbH, Münster, Germany, following the procedure described by Berridge et al. (1992). HPLC was performed with Waters Millennium system equipped with UV absorbance detector set at 225 nm, a flow radioactivity detector (Bioscan), and a reversed phase analytical HPLC column Shandon Hypersil BDS C-18, 5 μm, 250 × 4.6 mm. The course of [^{11}C]alkylation was assessed by radio HPLC using acetonitrile:100 mM sodium dihydrogen orthophosphate in water at 1 ml/min flow rate; pH of the solutions was measured on pH-meter Schott Geräte.

2.1. Radiosynthesis of [iso-propyl-^{11}C]ICI 118551

[2-^{11}C]Acetone was trapped at -50°C into the reaction vessel containing 2–3 mg (0.009–0.13 μmol) of precursor in a mixture of 120 μl of methanol with 2 mg of sodium cyanoborohydride and 4 μl acetic acid (Scheme 1). The mixture was heated for 6 min in a sealed vessel at 120°C and purified by HPLC after dilution with 0.8 ml of mobile phase. Purification and radiopharmaceutical formulation of (±)-[^{11}C]ICI 118551 was accomplished using a semi-preparative reverse phase column (Shandon Hypersil BDS C-18, 5 μm, 250 × 10 mm I.D.) eluted with acetonitrile:30 mM disodium dihydrogen orthophosphate (3/2; v/v; 6 ml/min). Retention times were confirmed before each radiosynthesis by comparison with authentic standards and correspond to 8.5 and 3.6 min for [*iso*-propyl-^{11}C]ICI 118551 and des-*iso*-propyl precursor, respectively. The retention times of unreacted [2-^{11}C]acetone under these conditions was 2 min, thus allowing a sufficient separation from labelled (±)-[^{11}C]ICI 118551 and other unknown impurities. The effluent from the column corresponding to (±)-[^{11}C]ICI 118551 was passed through a Sep-Pak C-18 (Millipore) which had been pre-activated with ethanol (10 ml) and sterile water (10 ml). The Sep-Pak was washed with water (10 ml) before eluting with ethanol (0.5 ml) in a vial containing 10 ml of saline solution. The final solution was sterilised through a sterile 0.22-μm filter (Gelman Acrodisc). The final solution was neutral.

2.2. Determination of specific radioactivity, chemical and radiochemical purity

The final solution of known volume was assayed for total radioactivity and a 20-μl aliquot was applied to an analytical column, eluted with a



Scheme 1. Radiosynthesis of (±)-[iso-propyl-^{11}C]ICI 118551.

mobile phase of acetonitrile:100 mM sodium dihydrogen orthophosphate (55/455; v/v; at 1 ml/min). Under these conditions, the retention time of (±)-[^{11}C]ICI 118551 was 4 min, whereas desmethyl precursor and [2-^{11}C]acetone eluted at 3 and 2 min, respectively. The amount of carrier was calculated from the UV absorbance peak area by means of the external standard calibration plot. The minimal detectable concentration was 83 ng/ml. Chemical purity of the final product was assessed from the UV trace. The presence of minor unidentified impurities was verified at the sub-nanomolar level. In the typical experiment starting from 400 to 600 mCi of [^{11}C]carbon dioxide, 20–40 mCi of (±)-[^{11}C]ICI 118551 ready for injection was obtained at the end of 30 min radiosynthesis in greater than 98% radiochemical purity with specific radioactivity ranging from 0.6 to 2.5 Ci/μmol.

2.3. Animals

Albino male CD rats (225–250 g), were obtained from Charles River (Italy). Erytho (±)-[^{11}C]ICI 118551 or cold drugs were diluted in saline to a final volume of 300 μl and injected in a tail vein using a 0.7 × 30-mm syringe needle. The biodistribution of (±)-[^{11}C]ICI 118551 was assayed at 5, 15, 30, 60 and 90 min (number of rats = 3, 4, 3, 4, 3, respectively) after injection of 426 ± 177 μCi (range 115–617) of (±)-[^{11}C]ICI 118551 corresponding to 0.43 ± 0.244 nmol of (±)-ICI 118551 (range: 0.08–0.68). Immediately after killing a blood sample was collected into heparinazed tube. Various organs (heart, lung, trachea, spleen, kidney, adrenal gland, intestine, muscle, testis) were removed and transferred to a pre-weighed tube. Heart tissue was sampled at the level of left ventricular wall near the apex and pulmonary parenchima at the apex of the left lung. The brains were removed from the skull, the cerebellum and cortex were dissected out, weighed, and placed in a gamma counter for radioactivity assay. Radioactivity concentration in plasma were obtained after centrifugation of blood samples

To evaluate the specificity of (±)-[^{11}C]ICI 118551 binding to β_2 adrenergic receptors, the

inhibition of (±)-[^{11}C]ICI 118551 uptake was studied by pretreating rats with DL-Propranolol (2.5 mg/kg, i.v.; $n=6$) or erythro (±)-ICI 118551 (0.15 mg/kg, i.v.; $n=4$) and saline for control ($n=4$, i.v.), 5 min prior to tracer injection and measuring radioactivity biodistribution at 30 min after the injection of (±)-[^{11}C]ICI 118551. At these doses DL-Propranolol and erythro (±)-ICI 118551 completely inhibits the in vivo binding of [^{18}F]carazolol to β_2 receptors (Van Waarde et al., 1995). Each group of animal, i.e. control, propranolol or erythro (±)-ICI 118551 pretreated rats, received approximately the same doses of (±)-[^{11}C]ICI 118551 (pmoles injected: control = 0.32 ± 0.2; DL-Propranolol pretreated = 0.39 ± 0.15, $P=0.4$; (±)-ICI 118551 pre-treated = 0.34 ± 0.27, $P=0.9$). The uptake of radioactivity in brain and periphery was calculated as a percentage of the injected dose per gram of tissue (% I.D./g).

2.4. Monkeys

The distribution of (±)-[^{11}C]ICI 118551 in heart and lung was also studied by means of PET in two female *Macaca Nemestrina* monkeys weighing 8 and 7.5 kg (animal 1 and 2, respectively). The animals were treated with 10 mg/kg of ketamine 30 min before the beginning of PET studies. Anesthesia was then maintained adding 0.5 mg/kg of Xylazine at 30-min intervals. PET studies were performed with a 14-ring, whole body positron emission tomograph (model GE ADVANCE, General Electric Medical Systems). The animals were positioned on the scanner bed centring the field of view of the tomograph at the thorax level. One 10-min transmission scan was carried out with an external ^{68}Ge ring source. At the end of the transmission scan animals were injected with 1.1 and 1.4 mCi of (±)-[^{11}C]ICI 118551, respectively (0.55 and 0.46 nmol of cold ligand). Monkey #2 was pre-treated with 0.075 mg/kg of DL-Propranolol 5 min before (±)[^{11}C]ICI 118551 injection. Immediately after tracer injection, five sequential PET scans were acquired during the following 30 min. Data from 35 transaxial planes covering an axial field of view of 15 cm were obtained simultaneously at each acquisition. The scans were reconstructed using a Hanning filter, with a cut-off frequency of 0.5 cycle per pixel.

(±)-[^{11}C]ICI 118551 uptake was defined on PET images containing lungs, heart and ventricular cavity. Images acquired between 15 and 30 min after tracer injection were summed by time to better define anatomical regions of interests. Irregular regions of interest (ROIs) were drawn on lungs (divided into anterior and posterior), and heart (left ventricle). Blood concentration was evaluated the same images by positioning a circular region of interest in the ventricular cavity. ROIs were then copied to the entire sequence of images to generate time course curves of radioactivity. The resulting curves were corrected for the physical decay of carbon-11 from the time of injection to midpoint of the imaging scans. Mean counts per pixel were normalised for time and converted into units of concentration (nCi/ml) with a calibration factor. (±)-[^{11}C]ICI 118551 uptake was calculated as percentage of the injected dose per gram of blood or tissue (% I.D./g).

Drug effects were evaluated using a Student's *t*-test for unpaired comparison. Statistical significance of the difference was set at $P<0.05$.

3. Results

3.1. Biodistribution in rats

The kinetics of radioactivity biodistribution after the injection of (±)-[^{11}C]ICI 118551 in rats is shown in Table 1. Radioactivity concentration of (±)-[^{11}C]ICI 118551 in plasma was stable during the entire period of observation (0.036 ± 0.016 until 90 min). The tracer preferentially accumulated in blood where radioactivity concentration was higher than in plasma indicating that (±)-[^{11}C]ICI 118551 binds to red blood cells. The in vivo whole blood to plasma ratio slowly decreased during time, from 1.97 at 5 min after injection to 1.17 at the end of the study. Radioactivity concentration progressively accumulated in liver and intestine until the end of the study. The tracer is rapidly taken up in tissues known to contain β_2 adrenergic receptors such as lungs, spleen and

Table 1
Biodistribution of radioactivity in rats after (±)-[^{11}C]ICI 118551 injection[a]

Tissue	5′ ($n=3$)	15′ ($n=4$)	30′ ($n=3$)	60′ ($n=4$)	90′ ($n=3$)
Blood	0.07 ± 0.03	0.09 ± 0.03	0.08 ± 0.01	0.05 ± 0.01	0.04 ± 0.02
Plasma	0.04 ± 0.01	0.047 ± 0.01	0.05 ± 0.02	0.04 ± 0.01	0.04 ± 0.02
Heart	0.74 ± 0.39	0.55 ± 0.17	0.36 ± 0.15	0.28 ± 0.17	0.13 ± 0.06
Lung	6.27 ± 3.00	6.08 ± 2.17	4.32 ± 1.20	2.50 ± 1.55	1.19 ± 0.50
Trachea	0.23 ± 0.07	0.24 ± 0.02	0.21 ± 0.06	0.24 ± 0.06	0.12 ± 0.04
Liver	0.25 ± 0.16	1.14 ± 0.18	1.64 ± 0.71	1.65 ± 0.27	1.32 ± 0.57
Adrenal gland	0.71 ± 0.35	1.57 ± 0.46	1.07 ± 0.26	0.89 ± 0.30	0.36 ± 0.12
Kidney	0.93 ± 0.55	0.93 ± 0.20	0.82 ± 0.39	0.61 ± 0.17	0.40 ± 0.20
Spleen	0.42 ± 0.22	0.84 ± 0.05	0.94 ± 0.08	0.69 ± 0.16	0.40 ± 0.04
Testis	0.08 ± 0.04	0.16 ± 0.01	0.22 ± 0.12	0.30 ± 0.10	0.22 ± 0.09
Intestine	0.30 ± 0.06	0.81 ± 0.5	0.95 ± 0.56	1.58 ± 0.90	0.50 ± 0.35
Muscle	0.30 ± 0.19	0.27 ± 0.08	0.26 ± 0.16	0.27 ± 0.08	0.19 ± 0.16
Cerebellum	0.48 ± 0.25	0.66 ± 0.114	0.67 ± 0.24	0.53 ± 0.28	0.25 ± 0.11
Cortex	0.59 ± 0.30	0.81 ± 0.15	0.89 ± 0.34	0.82 ± 0.10	0.36 ± 0.15

[a] Data are expressed as %I.D./g and are mean ± S.D. of number independent observations.

adrenal glands and heart, where it reached its maximum uptake between 15 and 30 min (Fig. 1). At 15 min after injection, lung radioactivity concentration was approximately ten times higher than that measured in the heart (6.09 ± 2.17% I.D./g and 0.55 ± 0.17% I.D./g, respectively).

As expected from the pharmacokinetics and the pharmacodynamic profile of (±)-ICI 118551, the tracer permeates the blood brain barrier and accumulates in brain where it reaches its maximum concentration between 15 and 30 min after injection. Brain distribution did not follow the pattern of β_2 receptor expression. The radioactivity concentration of the tracer was higher in the cerebral cortex, a region with prevalent expression of β_1 receptors (β_1:β_2 = 63:37; 80:20) than in cerebellum which mainly expresses the β_2 subtype (β_1:β_2 = 9:91; 20:80; Beer et al., 1988; Erdtsieck-Ernste et al., 1991) although differences between regions were not statistically significant.

The results of inhibition experiments are summarised in Table 2 and Fig. 2. Neither DL-Propranolol or (±)ICI 118551 significantly inhibited (±)-[^{11}C]ICI 118551 uptake in lungs, whereas, a 30% inhibition ($P < 0.05$) of radioligand uptake was observed in the spleen, kidney and blood of animals pretreated with DL-Propranolol. In addition, a reduction in whole blood to plasma ratio was observed in rats pre-treated with DL-Propranolol (30%, $P < 0.05$) or (±)ICI 18551 (35%).

3.2. PET studies in monkeys

Results of PET studies in monkeys, indicate that (±)-[^{11}C]ICI 118551 rapidly accumulates in lungs, (particularly in the posterior regions) and heart but it is also rapidly cleared. At 2.5 min after tracer injection the %I.D./g was found to be 0.1, 0.09, 0.19 and 0.06, respectively, in heart, posterior lungs, anterior lungs and blood. Tissue

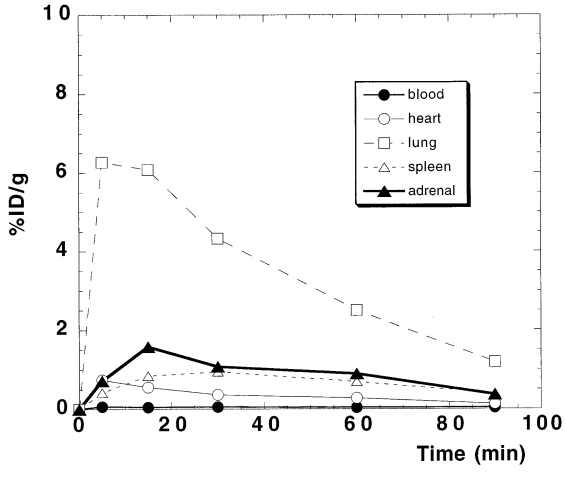


Fig. 1. Time course of radioactivity distribution at various times after the injection of (±)-[^{11}C]ICI 118551 in rats. Data are presented as mean ± S.D. of the number of independent observations and expressed as %I.D./g.

Table 2
Biodistribution of (±)-[^{11}C]ICI 118551 in control rats and in rats pre-treated with DL-Propranolol or (±)ICI 118551, at 30 min after tracer injection[a]

Tissue	Controls (saline; $n=4$)	DL-Prop. (2.5 mg/kg; $n=6$)	(±)ICI 118551 (0.15 mg/kg; $n=4$)
Blood	0.07 ± 0.01	0.04 ± 0.01*	0.05 ± 0.02
Plasma	0.04 ± 0.01	0.03 ± 0.01	0.05 ± 0.02
Heart	0.34 ± 0.07	0.30 ± 0.09	0.35 ± 0.08
Lung	3.15 ± 0.78	2.82 ± 1.33	3.87 ± 0.50
Trachea	0.21 ± 0.06	0.14 ± 0.04*	0.26 ± 0.09
Liver	1.52 ± 0.54	1.18 ± 0.75	1.69 ± 0.40
Adrenal	1.19 ± 0.25	1.15 ± 0.50	1.38 ± 0.37
Kidney	0.69 ± 0.14	0.55 ± 0.17*	0.84 ± 0.33
Spleen	1.04 ± 0.15	0.72 ± 0.20*	1.09 ± 0.26
Testis	0.20 ± 0.08	0.16 ± 0.06	0.26 ± 0.09
Intestine	0.76 ± 0.43	0.44 ± 0.12	0.65 ± 0.10
Muscle	0.21 ± 0.09	0.17 ± 0.09	0.30 ± 0.14
Cerebellum	0.54 ± 0.14	0.47 ± 0.17	0.80 ± 0.25
Cortex	0.73 ± 0.21	0.64 ± 0.25	1.13 ± 0.29

[a] Data are presented as mean ± S.D. of the number of independent observations and expressed as %I.D./g. Drugs or saline were administered i.v. 5 min before tracer injection. DL-Propranolol (2.5 mg/kg, i.v.; $n=6$) or (±)-ICI 118551 (0.15 mg/kg, i.v.; $n=4$) or saline ($n=4$).

* Significantly different from control rats, $P<0.05$.

to blood ratios increased slowly over time, reaching maximum values at 30 min. At this time the normalised tissue uptake was in the order: posterior lung > anterior lung > heart (corresponding tissue to blood ratios: 4.2, 2.6 and 1.7).

As observed in rats, DL-Propranolol administration failed to inhibit the accumulation of (±)-[^{11}C]ICI 118551 in the lungs of a *Macaca Nemestrina* (Fig. 3). In the animal pre-treated with 0.075 mg/kg of DL-Propranolol, tissue to blood ratios calculated at 30 min were similar to those obtained when the tracer was injected alone, being 1.7, 2.5 and 4.7 for heart, anterior lungs and posterior lungs, respectively (Fig. 3).

4. Discussion

In this study we have developed and evaluated in rats and monkey a novel putative radioligand for the in vivo measurement of β_2 adrenergic receptors using PET. Biodistribution studies of (±)-[^{11}C]ICI 118551 in rats indicate that the tracer preferentially accumulates in regions expressing β_2 receptors such as spleen, adrenal gland, lungs and erythrocytes as suggested by blood to plasma ratios higher then unit. Radioactivity concentration in lung was ten times higher than in the heart, where more than 70% of β receptors are of the β_1 subtype. This pattern of distribution was partially confirmed in monkey (*Macaca Nemestrina*) experiment, although radioactivity concentration in lungs was only two times higher than that found in the heart. However PET data were not corrected for tissue density and thus, in monkey experiments, lung radioactivity concentration was underestimated.

Despite the fact that regional distribution of (±)-[^{11}C]ICI 118551 partially follows that of β_2 receptors, no detectable inhibition of radiotracer binding was observed in rats and monkey in any of the examined region except for blood, kidney, spleen and trachea of rats pretreated with D,L Propranolol. However also in these organs, only 30% of the total radioactivity was blocked by D,L Propranolol preadministration. For the agonist [^{11}C]formoterol, it was previously observed by Visser et al. (1998), that lung radioactivity concentration was only slightly higher than spleen or heart concentration, being lung to spleen and lung to heart ratios equal to 1.37 and 1.8, respectively. On the contrary, the uptake of [^{11}C]ICI 118551 in

lungs is four times higher than in spleen and eight times higher than in heart. This observation uniting with the results of inhibition studies, suggests that a large proportion of tracer radioactivity, especially in lung tissue, reflects flow dependent delivery of the tracer or non-specific binding. On this basis the high (±)-[^{11}C]ICI 118551 accumulation in lungs might be explain by tracer interaction with biological components different from β_2 adrenergic receptors such as proteins involved in the facilitated transport of aminic drugs into endothelial cells or alveolar macrophages (Dollery and Junod, 1976; Kornhauser et al., 1980; Vestal et al., 1980; Hemsworth and Street, 1981).

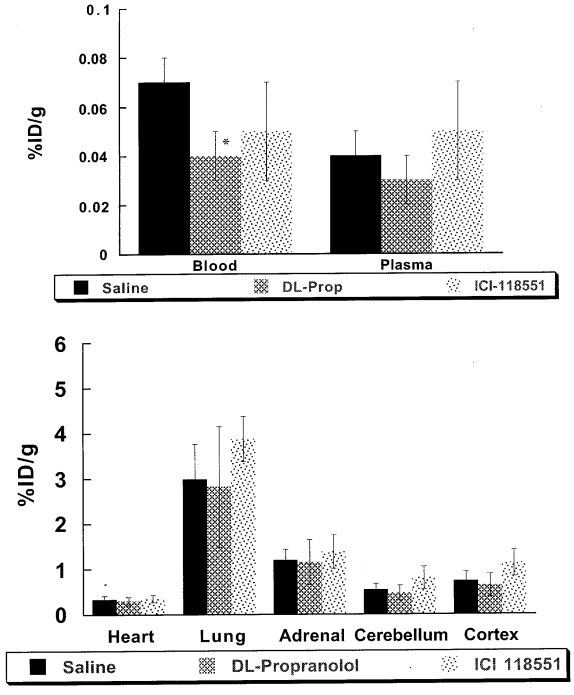


Fig. 2. Effect of pre-treatment with DL-Propranolol or (±)-ICI 118551 on radioactivity concentration in blood (top) or tissue (bottom) measured at 30 min after the injection of (±)-[^{11}C]ICI 118551. Data are presented as mean ± S.D. of the number of independent observations and expressed as %I.D./g.

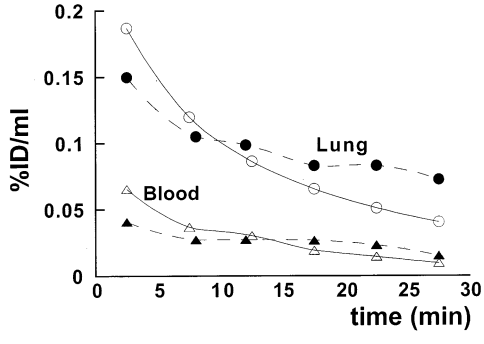


Fig. 3. Time course of radioactivity distribution in lungs, heart and blood of two *Macaca Nemestrina*, measured at various times after the injection of either (±)-[^{11}C]ICI 118551 alone or (±)-[^{11}C]ICI 118551 plus DL-Propranolol.

In the brain, (±)-[^{11}C]ICI 118551 uptake did not follow the distribution of β_2 receptors and tracer accumulation was not reduced by the administration of saturating doses of (±)-ICI 118551 or D,L Propanolol, indicating that (±)-[^{11}C]ICI 118551 uptake did not reflect cerebral β_2 adrenergic expression.

The in vitro K_d of erythro (±)-[^{11}C]ICI 118551 (1 nM; Hieble et al., 1995) is lower than that of [^{18}F]Carazolol (0.41 nM at β1 and 0.1 at β2; Van Waarde et al., 1997) or [^{11}C]CGP12177 (0.3 nM at β1 and 0.9 at β2; Nanoff et al., 1987). Thus one may argue that an in vitro K_d in the 1.0 nM range is not adequate for the in vivo measurement of β_2 adrenoreceptors using tracer with high levels of non-specific binding such as (±)-[^{11}C]ICI 118551. In addition tracer metabolism which was not evaluated in the present study could increase the fraction of circulating radioactivity in all regions examined thus increasing the fraction of non specific binding. Finally, the use of the racemic form of [^{11}C]ICI 118551 instead of the active enantiomer, could additionally reduce the fraction of specific binding observed in this study.

In conclusion, pre-clinical studies in rats and monkeys indicate that despite the promising pharmacological properties (±)-[^{11}C]ICI 118551 displays a high fraction of non-specific binding, especially in lung tissue, which might preclude its suitability for the in vivo imaging of β_2 adrenergic receptors. This issue may be clarified on further experiments provided that adequate animal models of β_2 adrenorereceptors impairment will become available.

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