Demonstration of pulmonary β_2-adrenergic receptor binding in vivo with [^{18}F]fluoroethyl-fenoterol in a guinea pig model

A. Helisch[1], E. Schirrmacher[1], O. Thews[2], R. Schirrmacher[1], H. G. Buchholz[1], W. Dillenburg[2], S. Höhnemann[3], J. Tillmanns[2], I. Wessler[4], R. Buhl[5], F. Rösch[3], P. Bartenstein[1]

[1] Department of Nuclear Medicine, University Hospital, Langenbeckstrasse 1, 55101 Mainz, Germany
[2] Institute of Physiology and Pathophysiology, University of Mainz, Mainz, Germany
[3] Institute of Nuclear Chemistry, University of Mainz, Mainz, Germany
[4] Institute of Pharmacology, University of Mainz, Mainz, Germany
[5] Pulmonary Division, University Hospital, Mainz, Germany

Received: 4 May 2005 / Accepted: 14 July 2005 / Published online: 31 August 2005

Abstract. *Purpose:* The new β_2 radioligand *(R,R)(S,S)* 5-(2-(2-[4-(2-[^{18}F]fluoroethoxy)phenyl]-1-methylethylamino)-1-hydroxyethyl)-benzene-1,3-diol ([^{18}F]FE-fenoterol; [^{18}F]FEFE), a fluoroethylated derivative of racemic fenoterol, was evaluated in vivo and ex vivo using a guinea pig model.
Methods: Dynamic PET studies over 60 min with [^{18}F]FEFE were performed in nine Hartley guinea pigs in which a baseline (group 1, *n*=3), a predose (group 2, *n*=3; 2 mg/kg fenoterol 5 min prior to injection of [^{18}F]FEFE) or a displacement study (group 3, *n*=3; 2 mg/kg fenoterol 5 min post injection of [^{18}F]FEFE) was conducted.
Results: In all animal groups, the lungs could be visualised and semi-quantified separately by calculating uptake ratios to non-specific binding in the neck area. Premedication with non-radioactive fenoterol and displacement tests showed significant reduction of lung uptake, by 94% and 76%, respectively.
Conclusion: These data demonstrate specific binding of the new radioligand to the pulmonary β_2-receptors in accordance with ex vivo measurements. Therefore, [^{18}F]FEFE seems to be suitable for the in vivo visualisation and quantification of the pulmonary β_2-receptor binding in this animal model.

Keywords: β_2-receptor agonist – [^{18}F]fluoroethyl-fenoterol – Specific pulmonary binding – Small animal imaging

Eur J Nucl Med Mol Imaging (2005) 32:1324–1328
DOI 10.1007/s00259-005-1914-9

A. Helisch (✉)
Department of Nuclear Medicine,
University Hospital,
Langenbeckstrasse 1,
55101 Mainz, Germany
e-mail: helisch@nuklear.klinik.uni-mainz.de
Tel.: +49-6131-172448, Fax: +49-6131-175650

Introduction

Pulmonary β-receptors play a central role in the regulation of muscular, glandular and epithelial lung function. In humans, predominantly the β_2 subtype is represented, which occurs on airway smooth muscles, on submucosal glands and on the bronchial epithelium. Rapid-acting β_2-receptor agonists such as fenoterol are widely used as rescue medication for patients with chronic obstructive pulmonary disease (COPD) or with asthma. Different responses to β_2-receptor agonists in both patient groups may be secondary to differences in pathogenesis and β_2-receptor density [1]. Additionally, there is some evidence that long-term therapy with β_2-receptor agonists could modulate the density and/or the function of the peripheral β-receptor system [2]. Non-invasive imaging of β_2-receptors would allow for a better understanding of such issues concerning pathogenesis, therapy and prognosis of obstructive lung diseases. Positron emission tomography (PET) is frequently used for in vivo evaluation of biodistribution and quantification of biological processes and would therefore be the method of choice for this issue. Other groups have described the labelling and evaluation of the long-acting β_2 agonist [^{11}C]formoterol [3], the β_2 antagonist [^{11}C]ICI 118551 [4] or the non-selective β-antagonist [^{18}F]carazolol [5]. Since fenoterol is a highly selective hydrophilic β_2 agonist which binds with a rapid onset time to the receptor, its pharmacological properties seem to be superior for imaging pulmonary β_2-receptors compared with other compounds. The possibility of labelling fenoterol with ^{18}F offers the opportunity of an extended measurement period for the quantification of pulmonary β_2-receptor binding. Here we present data on the evaluation of *(R,R)(S,S)* 5-(2-(2-[4-(2-[^{18}F]fluoroethoxy)phenyl]-1-methylethylamino)-1-hydroxyethyl)-benzene-1,3-diol ([^{18}F]FEFE), a fluoroethylated derivative of the racemic fenoterol, in vivo and ex vivo. Previously published ex vivo data comparing IC_{50} values of *(R,R)(S,S)*-fenoterol and the *(R,R)(S,S)*-fluoroethylated derivative

demonstrated similar β_2-adrenergic receptor binding affinity of both compounds [6].

Materials and methods

Chemistry

[^{18}F]Fluoride was purchased from several institutions as an aqueous solution. *(R,R)(S,S)*-fenoterol liberated from *(R,R)(S,S)*-fenoterol hydrobromide (provided by Boehringer Ingelheim Pharma, Germany) and 2-[^{18}F]fluoroethyltosylate were used for radioactive labelling of *(R,R)(S,S)*-[^{18}F]fluoroethyl-fenoterol as described previously [6].

PET studies

In vivo evaluation of the radiotracer was conducted in nine Hartley guinea pigs. The animals were anaesthetised with ketamine (10 mg/kg) and xylazine (0.6 mg/kg). We defined three study groups, each consisting of three animals: the first group was used in a baseline study, the second was a group that received pretreatment with 2 mg/kg fenoterol and the third group was used in a displacement study, with administration of 2 mg/kg fenoterol 5 min post injection of the radiotracer. Dynamic PET studies were acquired in 2D mode with a mean activity of 12 MBq [^{18}F]FEFE using an ECAT EXACT 922 scanner (Siemens/CTI, Knoxville, Tenn.). A 15-min transmission scan prior to injection was performed for attenuation correction using an external ^{68}Ga/^{68}Ge ring source. In 2D mode the axial resolution in the central field of view of this PET scanner is 5 mm [7]. During the PET study the guinea pigs were placed in the supine position and breathed spontaneously through a tracheal tube. We defined the following time frames up to 60 min p.i.: six frames of 10 s, four frames of 1 min and 11 frames of 5 min. *(R,R)(S,S)*-[^{18}F]FEFE and the non-radioactive compound were administered as a bolus injection via the left jugular vein. PET data were reconstructed by iterative processing using an ordered subset expectation maximisation algorithm (two iterations, eight subsets) on a Sun workstation. Regions of interest (ROIs) for both lungs and for the right neck area were drawn manually on three consecutive transverse PET transmission images for all guinea pigs. Here the lungs are distinguishable from heart and adjacent abdominal organs, especially the liver. The size and the position of the ROIs were chosen in such a manner that the border was at least 10 mm away from heart and liver (Fig. 1). The right neck area was selected as a reference region of non-specific binding. Then the lung and neck ROIs were copied to the corresponding emission images and used for time-activity curve analysis. For in vivo semi-quantification of β_2-receptor binding we calculated the uptake ratios of the lungs and of the right neck area for all study groups.

Ex vivo organ measurements

At 65 min p.i. the animals were sacrificed, and the organ samples rapidly removed and dissolved in KOH (4*N*) at 75°C for 30 min. Additional blood samples up to 10 ml were taken immediately post mortem from each animal and blood plasma was separated by centrifugation (10 min, 3,000×*g*). Measurement of organ and plasma activity was conducted with a calibrated gamma counter (Wallac 1480, Turku, Finland) and calculated as % of injected activity per gram tissue (%ID/g) for all specimens (Table 1).

Results

All animals could be examined in the PET camera over the total scan time of 60 min p.i. No life-threatening side-effects of the i.v. administration of fenoterol or of the radiolabelled analogue were seen. Using 2D mode, the spatial resolution of the PET camera allowed the visualisation of both lungs separately in each case (Figs. 1, 2). Time-activity curves of the lungs (Fig. 3) demonstrated nearly stable pulmonary uptake of *(R,R)(S,S)*-[^{18}F]FEFE from 20 to 60 min p.i. in the baseline study. In contrast, after premedication with 2 mg/kg non-radioactive fenoterol, lung uptake was inhibited (mean 94%, range 85–97%) up to the end of the PET measurements. In the displace-

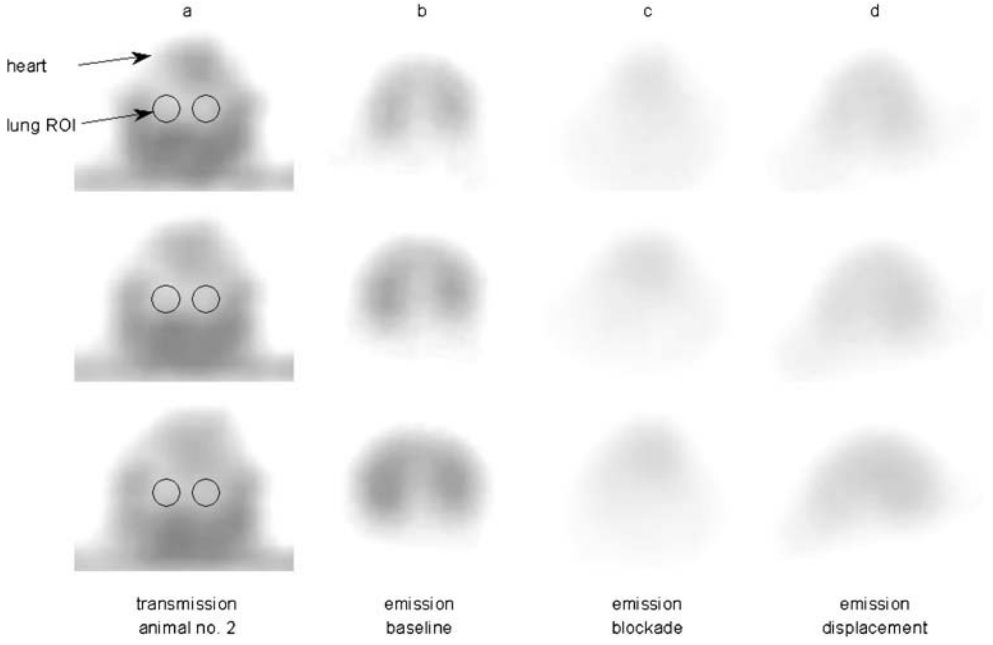

Fig. 1. Consecutive transverse transmission (**a**, with pulmonary ROIs) and emission slices (**b**–**d**, 56–60 min p.i.), slice thickness 3.4 mm

Table 1. Ex vivo ^{18}F measurements of the explanted organs and plasma samples in guinea pigs, expressed as %ID/g±SD

Organ	Baseline (n=3)	Blockade (n=3)	Displacement (n=3) 5 min p.i.
Lung	1.30±0.20	0.31±0.09	0.52±0.15
Heart	0.46±0.06	0.20±0.07	0.27±0.04
Liver	0.85±0.17	0.57±0.25	0.64±0.10
Kidney	0.69±0.09	1.56±1.30	2.17±0.24
Plasma	0.24±0.05	0.29±0.08	0.20±0.002

ment study (group 3) after injection of non-radioactive fenoterol mean pulmonary uptake decreased by 76% (range 58–88%) compared with the baseline group. Corresponding time-activity curves for mean pulmonary binding are shown in Fig. 3. As expected, the reference ROIs (right neck area) contained low activity in all three study groups, representing non-specific activity. The average lung to neck ratio decreased in the baseline study between 10 and 60 min p.i. from 6.5 to 4.3. The respective values for the blockade group were 1.4 (10 min p.i.) and 1.2 (60 min p.i.), demonstrating that almost no specific uptake was detectable under this condition. In the displacement study a rapid decrease in the ratio was detectable following injection of non-radioactive fenoterol (10 min p.i.: 3.6; 60 min p.i.: 1.8). As expected, in vivo cardiac uptake of *(R,R)(S,S)*-[^{18}F]FEFE was low (Fig. 1) and the comparison of in vivo tracer kinetics over the heart did not reveal relevant differences between the three studies which would produce in vivo evidence of specific cardiac uptake. This indicates high β_2 selectivity of the compound in equivalence to fenoterol.

Mean ex vivo ^{18}F measurements of the lungs in the baseline group resulted in an organ uptake of *(R,R)(S,S)*-[^{18}F]FEFE of 1.30±0.20%ID/g. Premedication (study 2) and displacement (group 3) with non-radioactive fenoterol reduced the mean pulmonary ^{18}F activity to 0.31±0.09 and 0.52±0.15%ID/g, respectively. Details concerning the other ex vivo organ measurements are shown in Table 1.

Discussion

The highly specific pulmonary β_2-receptor agonist fenoterol, labelled with ^{18}F, was evaluated in vivo and ex vivo in a guinea pig model. Pulmonary binding of the racemic compound was displaceable and could be inhibited by non-radioactive fenoterol.

Previous PET studies describing β-adrenergic compounds differ relevantly from our study concerning animal model, affinity profile, β_2 selectivity and analysis of pulmonary binding. Visser et al. [3] used the long-acting β_2-receptor agonist formoterol labelled with ^{11}C in rats. Pulmonary binding was analysed in vivo with ratios of lung uptake in baseline and blockade studies (propranolol). These calculations resulted in a total/non-specific pulmonary binding ratio of 1.8 at 55 min p.i. Moresco et al. [4] labelled the β_2-receptor antagonist ICI 118551 with ^{11}C, demonstrating pulmonary tracer accumulation in rats and monkeys. However, lung uptake was displaceable neither with propranolol nor with ICI 118551. The authors concluded that pulmonary uptake of [^{11}C]ICI 118551 is predominantly non-specific and therefore this compound seemed not to be suitable for in vivo evaluation of β_2-receptors. Van Waarde et al. [5] found specific pulmonary binding of the non-selective β-receptor antagonist carazolol labelled with ^{18}F, adequate for in vivo evaluation in rats. In contrast, we selected fenoterol for this issue as a highly selective β_2-receptor agonist, widely used as rapid-acting rescue medication for patients with asthma or COPD. Guinea pigs were chosen due to the similarity of the pulmonary β-adrenoceptor system to that in humans, especially concerning β_1/β_2 distribution and functional selectivity of β_2-receptors [8]. Considering these methodological differences, the almost complete suppression of pulmonary binding by fenoterol pretreatment (mean 94%) and the negligible cardiac uptake indicate that [^{18}F]FEFE has superior binding properties compared with the previously described compounds and therefore seems to be most suitable for use in humans.

In our limited animal sample the ex vivo measurements of pulmonary ^{18}F organ activity also confirmed [^{18}F]FEFE

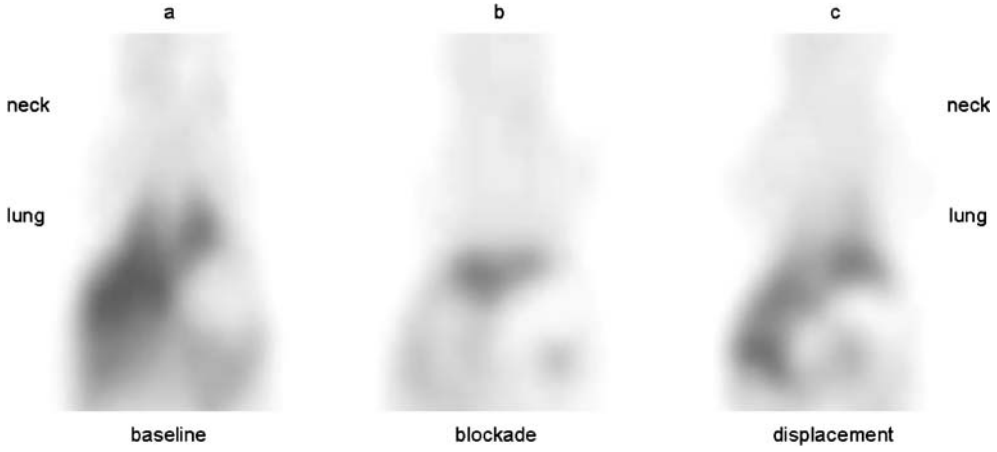

Fig. 2. Pulmonary tracer uptake of *(R,R)(S,S)*-[^{18}F]FEFE in three animals, shown as maximum intensity projections 56–60 min p.i. Specific binding (**a**) was inhibited by pretreatment (**b**) and was displaceable by injection of fenoterol (**c**)

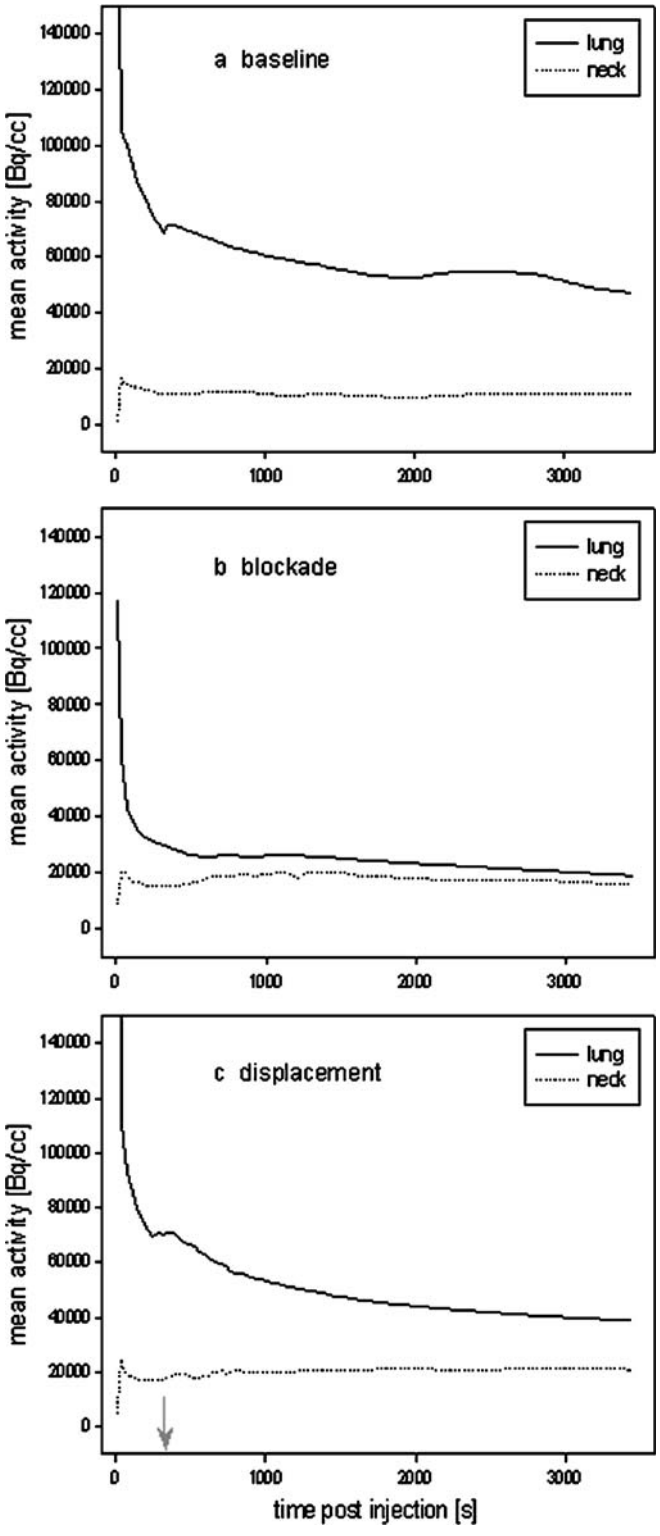

Fig. 3. Averaged (n=3) time-activity curves of the lungs and the neck areas in the study groups (**a**–**c**). The *arrow* in **c** indicates the time of injection of non-radioactive fenoterol: 2 mg/kg fenoterol 5 min post injection of [^{18}F]FEFE

to be an adequate ligand for the quantification of pulmonary β_2-receptors. However, the explanted lungs in groups 2 (blockade) and 3 (displacement) contained a somewhat higher relative ^{18}F activity compared with the ROI-based in vivo data. A possible explanation is that the ex vivo ^{18}F lung activity measured in all three groups was contaminated by an increased post-mortem load of blood in pulmonary capillaries containing [^{18}F]FEFE that could not be removed completely by bleeding of the guinea pigs. The same may be true for the explanted animal hearts, which revealed a somewhat lower ^{18}F activity in the blockade and displacement study compared with baseline, indicative of some specific binding. However, relevant specific binding was not confirmed in vivo, which is in line with high β_2 selectivity of the compound.

Since no adequate reference tissue is available for comparison to lung tissue (especially concerning the blood flow component) we abstained from proper kinetic modelling. Instead we decided to determine changes in specific uptake in a robust way by calculating uptake ratios between the lung and the right neck area, under the assumption that uptake in this area with no specific tracer binding would be stable throughout the experiments.

Labelling with ^{18}F has potential advantages over labelling with ^{11}C, although it does not allow for multiple radiotracer injections in the same patient during one day. Beside the commercial aspect with the possibility of distribution, ^{18}F labelling offers additional opportunities in a pure scientific setting. For instance, the extended scanning time allows the tracer kinetics to be followed for longer after a pharmacological or other intervention. This enables more accurate characterisation of the dynamics of the induced displacement.

The PET scanner that we used has limitations in terms of spatial resolution in the context of small animal imaging. Nevertheless, the size of the animal lungs was sufficient to assess global pulmonary uptake with this PET camera (Fig. 1). Previously published phantom data described recovery coefficients for different sphere volumes using the ECAT EXACT camera [9]. However, correcting for this partial volume effect would not change the ratios of lung and neck activities, since equivalent acquisition geometry was given throughout the different experiments. Although specific binding of the racemic compound was apparently very high, using the pure enantiomer *(R,R)*-[^{18}F]FEFE with higher specific binding [10] will most probably improve quantification of the uptake kinetics owing to homogeneous affinity. We are currently developing methods for the purification and labelling of this enantiomer.

Conclusion

Our data indicate that the new PET ligand [^{18}F]FEFE exhibits specific and displaceable binding to pulmonary β_2-receptors in guinea pigs. These promising results justify the further evaluation of the radiotracer in this animal model, concentrating on the high-affinity pure enantiomer *(R,R)*-[^{18}F]FEFE. This issue is currently being addressed.

Acknowledgements. This study was supported by the German Research Foundation (DFG grant FOR 474). Additionally, the authors thank Boehringer Ingelheim Pharma, Germany, for providing *(R,R)(S,S)*-fenoterol hydrobromide. Studies had previously been approved by the regional ethics committee and were conducted according to the German Law for Animal Protection.

References

1. O'Byrne PM, Postma DS. The many faces of airway inflammation. Asthma and chronic obstructive pulmonary disease. Asthma Research Group. Am J Respir Crit Care Med 1999; 159:41–63
2. Katsunuma T, Fujita K, Mak JC, Barnes PJ, Ueno K, Iikura Y. Beta-adrenergic agonists and bronchial hyperreactivity: role of beta2-adrenergic and tachykinin neurokinin-2 receptors. J Allergy Clin Immunol 2000;106:104–8
3. Visser TJ, van Waarde A, Doze P, Elsinga PH, van der Mark TW, Kraan J, et al. Characterisation of beta2-adrenoceptors, using the agonist [^{11}C]formoterol and positron emission tomography. Eur J Pharmacol 1998;361:35–41
4. Moresco RM, Matarrese M, Soloviev D, Simonelli P, Rigamonti M, Gobbo C, et al. Synthesis and in vivo evaluation of [^{11}C]ICI 118551 as a putative subtype selective beta2-adrenergic radioligand. Int J Pharm 2000;204:101–9
5. Van Waarde A, Elsinga PH, Brodde OE, Visser GM, Vaalburg W. Myocardial and pulmonary uptake of S-1′-[^{18}F]fluorocarazolol in intact rats reflects radioligand binding to beta-adrenoceptors. Eur J Pharmacol 1995;272:159–68
6. Schirrmacher E, Schirrmacher R, Thews O, Dillenburg W, Helisch A, Wessler I, et al. Synthesis and preliminary evaluation of *(R,R)(S,S)* 5-(2-(2-[4-(2-[^{18}F]fluoroethoxy)phenyl]-1-methylethylamino)-1-hydroxyethyl)-benzene-1,3-diol ([^{18}F]FEFE) for the in vivo visualisation and quantification of beta2-adrenergic receptor status in lung. Bioorg Med Chem Lett 2003;13:2687–92
7. Wienhard K, Eriksson L, Grootoonk S, Casey M, Pietrzyk U, Heiss WD. Performance evaluation of the positron scanner ECAT EXACT. J Comput Assist Tomogr 1992;16:804–13
8. Carswell H, Nahorski SR. Beta-adrenoceptor heterogeneity in guinea-pig airways: comparison of functional and receptor labelling studies. Br J Pharmacol 1983;79:965–71
9. Helisch A, Thews O, Buchholz HG, Schreiber W, Karg A, Vaupel P, et al. Small animal imaging with a conventional PET scanner: application of partial volume correction validated with ex vivo measurements. Eur J Nucl Med Mol Imaging 2002; 29:138P
10. MaloneyHuss K, Lybrand TP. Three-dimensional structure for the beta-2 adrenergic receptor protein based on computer modeling studies. J Mol Biol 1992;225:859–71