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Review

Receptor imaging in the thorax with PET

Philip H. Elsinga*, Aren van Waarde, Willem Vaalburg

Groningen University Hospital, PET-center, P.O. Box 30001, 9700 RB Groningen, The Netherlands

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Abstract

This review focuses on positron emission tomography (PET)-imaging of receptors in the sympathetic and the parasympathetic systems of heart and lung and highlights the human applications of PET. For the α-adrenoceptor, only [^{11}C]GB67 (*N*2-{6-[(4-amino-6,7-dimethoxy-2-quinazolinyl)(methyl)amino]hexyl}-*N*2-[^{11}C]methyl-2-furamide hydrochloride) has been developed. Its potential for application in patients needs to be assessed. For both the β-adrenergic and the muscarinic systems, potent PET radioligands have been prepared and evaluated in patients. It has been possible to measure receptor densities quantitatively in human heart {[^{11}C]MQNB: [^{11}C]methylquinuclidinyl benzilate, [^{11}C]CGP12177: *S*-(3′-*t*-butylamino-2′-hydroxypropoxy)-benzimidazol-2-[^{11}C]one and [^{11}C]CGP12388: (*S*)-4-(3-(2′-[^{11}C]isopropylamino)-2-hydroxypropoxy)-2H-benzimidazol-2-one} and qualitatively in lung {[^{11}C]VC002: *N*-[^{11}C]-methyl-piperidin-4-yl-2-cyclohexyl-2-hydroxy-2-phenylacetate and [^{11}C]CGP12177}. Besides these subtype nonselective radioligands, the development of compounds that are selective for one subtype are ongoing and have not found successful application in humans yet.

Keywords: α-Adrenoceptor; β-Adrenergic; PET radioligands

Contents

* Corresponding author. Tel.: +31 50 3613247; fax: +31 50 3611687.
E-mail address: p.h.elsinga@pet.azg.nl (P.H. Elsinga).

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1. Introduction

Neurotransmission involves processes in the nerve terminals, the synaptic cleft, postsynaptic sites and second messenger systems. These processes regulate the function of tissues and organs. Important organs in the thorax are heart and lung. The myocardial nerves are important for functions such as the heart rhythm, conduction and repolarization (James, 1983). Neural control of airway smooth muscle resulting in contraction and relaxation is an important determinant of airway caliber in health and disease (Barnes, 1992; Rogers, 2002).

Well-studied forms of neurotransmission in heart and lung are the sympathetic and the parasympathetic systems with (−)-norepinephrine and acetylcholine as the corresponding endogenous neurotransmitters (Carrio, 2001; Langer and Halldin, 2002). Activation of the noradrenergic system results in increased heart rate (HR) and contractile force. Activation of the cholinergic system decreases these parameters and causes inhibition of conduction (Carrio, 2001). In the lung, acetylcholine acts on muscarinic receptors (bronchoconstriction) and (nor)epinephrine on both α (bronchoconstriction) and β-adrenoceptors (bronchorelaxation; Barnes, 1992). These neural mechanisms may be involved in the pathophysiology of asthma, chronic obstructive pulmonary disease, heart failure and cardiomyopathy. A noninvasive in vivo diagnostic tool to investigate the aforementioned abnormalities could improve the understanding of the neurotransmission mechanisms leading to better treatment of patients. Nuclear medicine techniques like positron emission tomography (PET) are useful imaging modalities for this purpose.

Radiopharmaceuticals for the imaging of the presynaptic elements of neurotransmission can be aimed to measure uptake of neurotransmitters (noradrenaline or acetylcholine) in the neurons, storage of the neurotransmitter or its binding to specific sites within the nerve terminal. Radioligands for postsynaptic receptors include antagonists and agonists for the α- and β-adrenoceptors and muscarinic cholinergic receptors. Finally, it is possible to investigate functionality of the receptor system by mapping the formation or the activity of second messengers. Some studies have been performed to map cAMP (DaSilva et al., 2002), diacyl glycerol, protein kinase C and inositol phosphate (Imahori et al., 2002). The possibility to map the cascade of processes within neurotransmission in vivo can improve the insight of their functions and roles in disease. Because an enormous amount of research has been done in this field (Carrio, 2001; Langer and Halldin, 2002; Riemann et al., 2003; Elsinga et al., 1998; Pike et al., 2000), this review will focus on PET imaging of postsynaptic receptors and will highlight the human applications of PET.

2. Alpha-adrenoceptors

α-Adrenoceptors are G-protein coupled and can be subdivided into postsynaptic α_1- and pre- and postsynaptic α_2-adrenoceptors (Starke, 1981). In the heart, α-adrenoceptors are considered to act as a backup for β_1-adrenoceptors. Normally, β_1-adrenoceptors play the major role in the adrenergic control of myocardial function. In disease, the number of β-adrenoceptors decreases. In this case, an increase in α_1-adrenoceptors may be a compensatory mechanism for maintaining the function of the heart (Heusch, 1990; Bristow et al., 1988). Increases in α_1-adrenoceptor densities are thus generally observed whenever the number of β-adrenoceptors is decreased. Another function of α-adrenoceptors is the regulation of cardiovascular functions, such as noradrenaline release (α_1) and coronary vasoconstriction (both α-subtypes) (Drew, 1976; Yamaguchi and Kopin, 1980). About 15% of cardiac adrenoceptors are α_1-adrenoceptors. Because of activation of α_1-adrenoceptors, phospholipase C is activated and the second messengers inositol phosphate and diacylglycerol are formed. Cardiac α_2-adrenoceptors are negatively coupled to adenylate cyclase and thus inhibit cAMP formation (Tsien, 1977; Berridge, 1987).

α-Adrenoceptors that mediate bronchoconstriction have been shown to exist in airways of several animal species, but there is considerable doubt about the role of α-adrenoceptors in the regulation of human airway tone because it is difficult to demonstrate their presence experimentally. α-Adrenoceptors may play a role in the regulation of pulmonary blood flow and there is some evidence that α-adrenoceptor agonists may reduce airway narrowing in exercise-induced asthma. In dog trachea, the α_2-subtype predominates over the α_1-subtype (α_1: 11 and α_2: 51 fmol/mg protein), whereas in canine peripheral lung, the α_1-subtype predominates (α_1: 47 and α_2: 4 fmol/mg protein; Barnes et al., 1983). For human lung, no data on α-adrenoceptor densities are available.

2.1. Radioligands for the α_1-adrenoceptor

A single radioligand for the α_1-adrenoceptor has been developed for human application. *N*2-{6-[(4-amino-6,7-dimethoxy-2-quinazolinyl)(methyl)amino]hexyl}-*N*2-methyl-2-furamide hydrochloride (GB67; Fig. 1), a structural and pharmacological analogue of the α_1-adrenoceptor

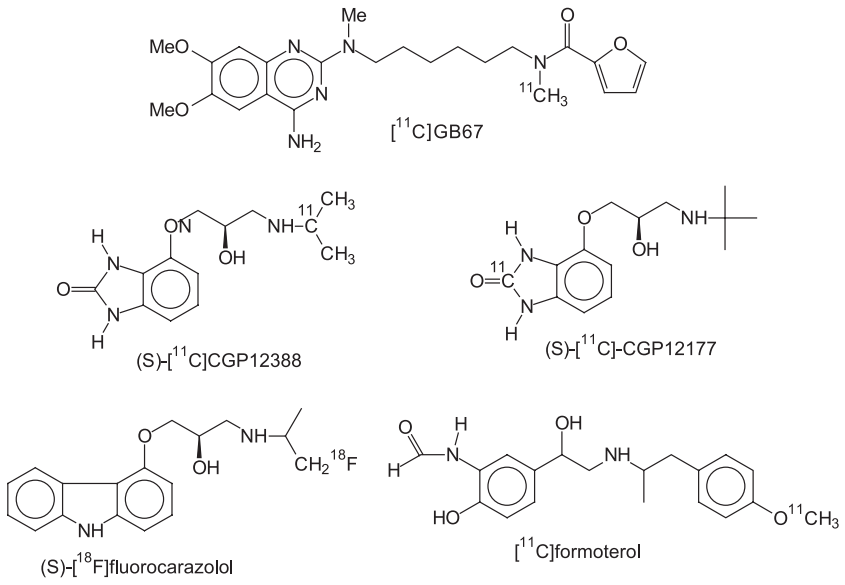

Fig. 1. Chemical structures of several PET-radioligands for adrenoceptor.

antagonist prazosin, shows high potency and selectivity for the α_1-subtype (Giardina et al., 1993) and has been labelled with carbon-11 ($t_{1/2}$=20.4 min) by ^{11}C-methylation of *N*-desmethylamido-GB67 (GB99; Law et al., 2000). During the preclinical evaluation, [^{11}C]GB67 was injected intravenously (i.v.) into conscious rats. Myocardial uptake was maximal at 1–2 min and decreased slowly during the subsequent 60 min. Predosing with adrenoceptor antagonists demonstrated selectivity of [^{11}C]GB67 for myocardial α_1-adrenoceptors. GB67 and prazosin blocked uptake of radioactivity; the nonselective α-adrenoceptor antagonist, phentolamine, partially blocked the uptake; the α_2-adrenoceptor antagonist RX 821002 (2-methoxy-idazoxan), only blocked uptake at high dose; and the β-adrenoceptor antagonist, CGP 12177 (*S*-(3′-*t*-butylamino-2′-hydroxypropoxy)-benzimidazolone), had no effect. Additionally, injection of prazosin at 20 min after radioligand displaced bound radioactivity. No specific binding could be demonstrated in the lung. In vivo competition curves obtained by co-injecting [^{11}C]GB67 with varying amounts of either unlabelled GB67 or its precursor GB99 were fitted to a competitive binding model to provide estimates of the maximum number of binding sites (B_{max}) and half saturation doses (K) for myocardium. Assuming a tissue protein content of 10%, the values of B_{max} (13 pmol/g tissue) were in the same order of magnitude than those reported for myocardial α_1-adrenoceptors assessed in vitro (50–170 fmol/mg protein). Both GB67 and its precursor GB99 had a high affinity for α_1-adrenoceptors (K_{GB67}=1.5 nM, K_{GB99}=4.8 nM). High-performance liquid chromatography (HPLC) demonstrated four radioactive metabolites in rat plasma. [^{11}C]GB67 was 80% of the radioactivity at 5 min and 50% at 45 min. No radioactive metabolites were detected in myocardium up to 60 min after injection.

[^{11}C]GB67 was assessed in two male human volunteers. PET demonstrated high myocardial uptake. The profile of radioactive metabolites in plasma was comparable to that in the rat, although metabolism was slower in humans. Thus, [^{11}C]GB67 is a promising radioligand for assessing α_1-adrenoceptors in human myocardium with PET.

Recently, another promising ligand 3-[2-[4-(2-[^{11}C]methoxyphenyl)piperazin-1-yl]ethyl]pyrimido[5,4-b]indole-2,4-dione ([^{11}C]RN5: K_i=0.21 nM) has been prepared by [^{11}C]methylation and has been evaluated in rats by biodistribution studies (Matarrese et al., 2002). Radioactivity uptake was high in heart, spleen and lung. Blocking studies revealed that more than 70% of the myocardial uptake of [^{11}C]RN5 was due to α_1-adrenoceptor-mediated uptake.

2.2. Radioligands for the α_2-adrenoceptor

A few radioligands for the α_2-subtype have been developed. Carbon-11-labelled RS-79948-197 ((8a*R*,12a-*S*,13a*S*)-5,8,8a,9,10,11,12,12a,13,13a-decahydro-3-methoxy-12-(ethylsulphonyl)-6H-*iso*-quino[2,1-g][1,6]naphthyridine (Hume et al., 1996)); RS-15385-197 (delequamine; Hume et al., 2000); and MK-912 ((2*S*,12b*S*)1′,3′-dimethylspiro(1,3,4,5′,6,6′,7,12b-octahydro-2H-benzo[b]furo[2,3-a]quinazoline)-2,4′-pyrimidin-2′-one) (Shiue et al., 1998) were evaluated for imaging cerebral α_2-adrenoceptors, but brain uptake was low and specific binding was not observed. In addition, no specific binding was detectable in heart or lung. The authors concluded that these radioligands are not suitable for PET imaging.

3. β-adrenoceptors

The β-adrenoceptor is G-protein coupled and can be subdivided into four classes: β_1- to β_4-subtypes (Brodde,

1991; Hieble, 2000). PET-imaging studies have focussed on the β_1 and β_2-subtypes (Elsinga et al., 1998; Riemann et al., 2003;Pike et al., 2000).

3.1. Cardiac β-adrenoceptors

β-Adrenoceptors play an important role in the regulation of heart rate and myocardial contractility. Agonists induce positive chronotropic and inotropic effects (Brodde, 1991). β-Adrenoceptors are present both in the atria and in the ventricles. The average β-adrenoceptor density (B_{max}) in atria and ventricles is normally 70–100 fmol/mg protein. In the human heart, the ratio of the β_1/β_2-subtypes is about 80:20 (Brodde, 1991).

β-Adrenergic receptor density in the human heart is altered in various pathophysiological conditions including hypertension, heart failure, ischemia, hypertrophic and dilated cardiomyopathy (Brodde, 1991;Michel et al., 1988). The β_1- and β_2-subtypes may be equally affected (Brodde, 1991) or the β_1-subpopulation can be selectively diminished while the density of β_2-adrenoceptors is relatively unchanged (Michel et al., 1988; Bristow et al., 1990). In the end stage of dilated cardiomyopathy, the ratio of β_1/β_2-adrenoceptors is shifted from 80:20 to 60:40 and B_{max} is reduced to 30–50 fmol/mg protein. In other forms of heart failure, such as ischemic cardiomyopathy and mitral valve disease, the ratio of the β_1- and β_2-adrenoceptor subpopulations remains unchanged, while B_{max} is lowered to 30–40 fmol/mg protein (Brodde, 1991). Little information is available about the in vivo time course of these alterations, the spatial distribution of β-adrenoceptors within the heart or the influence of therapy.

3.2. Pulmonary β-adrenoceptors

β_2-Adrenoceptors predominate in the human lung. Stimulation of pulmonary β_2-adrenoceptors by β_2-adrenoceptor agonists results in activation of adenylate cyclase via G_s protein followed by cAMP accumulation, which then leads to relaxation of smooth muscle cells in the walls of the airways. Such relaxation seems to be impaired in humans with pulmonary dysfunction such as patients suffering from asthma or chronic obstructive pulmonary disease. β-Adrenoceptor density in human peripheral lung is normally 100–130 fmol/mg protein and the ratio of the β_1/β_2 subtypes is 30:70 (Brodde, 1991).

Tests of pulmonary β-adrenoceptor function have indicated normal parameters in mild forms of asthma (Harvey and Tattersfield, 1982), but a reduced sensitivity to β-adrenoceptor agonists and a diminished β-adrenoceptor function in severe asthmatics (Barnes and Pride, 1983; Nijkamp and Henricks, 1990). In vitro examination of the underlying causes of this reduced sensitivity have produced conflicting results. Some authors have reported uncoupling of β-adrenoceptors from adenylate cyclase (Goldie, 1990), others detected a reduction of the number of β-adrenoceptors in the airway walls (Raaijmakers et al., 1987), normal numbers of β-adrenoceptors (Sharma and Jeffery, 1990) or an increase of β-adrenoceptor density in the bronchi (Bai et al., 1992).

Genetic variation in the β_2-adrenoceptor and its associated proteins is common and therefore potentially relevant to the clinician (Taylor and Kennedy, 2002). Several functional mutations have been described but in vitro studies have yielded inconsistent results. A variable presence of other polymorphic alleles may be the explanation for these inconsistent results. In vitro studies have concentrated on downregulation and desensitization as functional endpoints. However, the relevance of these phenomena is unclear and extrapolating in vitro data to the clinical setting may not be appropriate. To date, only the Arg(16) polymorphism appears to be important in determining β_2-adrenoceptor agonist drug responses, but the data, as well as their clinical application, are limited. The contribution of β_2-adrenoceptor polymorphisms to asthma appears to be a disease modifying rather than a disease causing nature. Most studies have shown weak or no association between β_2-adrenoceptor polymorphisms and the presence of asthma per se, or phenotypic markers such as bronchial responsiveness to methacholine, atopy and wheeze frequency (Joos and Sandford, 2002; Fenech and Hall, 2002). Knowledge of the β_2-adrenoceptor genotype of asthmatic patients may be useful in order to predict response to bronchodilator therapy, and therefore to provide prescribers with additional knowledge which will enable them to tailor disease management programs to individual patients.

3.3. Radioligands for positron emission tomography of β-adrenoceptors

At this moment, three radiolabelled antagonists have been used for the visualization of β-adrenoceptors in humans: [^{11}C]CGP12177 (*S*-(3′-*t*-butylamino-2′-hydroxypropoxy)-benzimidazol-2-[^{11}C]one), [^{11}C]CGP12388 ((*S*)-4-(3-(2′-[^{11}C]isopropylamino)-2-hydroxypropoxy)-2H-benzimidazol-2-one)) and (*S*)-[^{18}F]fluorocarazolol. Their chemical structures are shown in Fig. 1. All tracers lack selectivity for either the β_1- or the β_2-subtype. A few β_1-subtype selective radioligands, (*R*,*S*)-1-[2-((carbamoyl-4-hydroxy)phenoxy)-ethylamino]-3-[4-(1-[^{11}C]-methyl-4-trifluoromethyl-2-imidazolyl)phenoxy]-2-propanol ([^{11}C]CGP 20712A; Elsinga et al., 1994), 2-[4-[3-*tert*-butylamino)-2-hydroxypropoxy]phenyl]-3-methyl-6-methoxy-4(3H)-quinazolinone (HX-CH 44 BS; Valette et al., 1999) and (±)-1-[4-(2-isopropoxyethoxymethyl)-phenoxy]-3-isopropylamino-2-propanol ([^{11}C]bisoprolol; Soloviev et al., 2001) have been prepared and evaluated in rodents. To measure the β_2-adrenoceptor the antagonists (±)-*erythro*-5-(1-hydroxy-2-[^{11}C]isopropyl-aminobutyl)-8-hydroxy-carbostyril ([^{11}C]procaterol; Visser et al., 2000) and *erythro*-(±)-1-[2,3-(dihydro-7-methyl-1H-inden-4-yl)oxy]-

3-[isopropylamino]-2-butanol ([^{11}C]ICI 118551; Moresco et al., 2000) have been prepared. To examine whether it is possible to image β_2-adrenoceptors in vivo with a radiolabelled agonist, *N*-[2-hydroxy-5-[1-hydroxy-2-[[2-(4-[^{11}C]-methoxyphenyl)-1-methylethyl]amino]ethyl]phenyl]-formamide ([^{11}C]formoterol; Fig. 1) has been radiolabelled (Visser et al., 1998). An advantage of this agonist is the possibility of selective mapping receptors in the high-affinity state and to investigate the relationship of function and β_2-polymorphism.

3.3.1. (S)-[^{11}C]CGP 12177

(*S*)-[^{11}C]CGP 12177 produces good quality PET images of the human heart and lung. Because it is hydrophilic, (*S*)-[^{11}C]CGP 12177 binds only to functional receptors at the cell surface and not to internalized receptors. The radioligand is slowly metabolized both in rodents and humans (van Waarde et al., 1995a,b). (*S*)-[^{11}C]CGP 12177 has been used to determine cardiac and pulmonary β-adrenoceptor density in humans (Ueki et al., 1993; Qing et al., 1996). Using a two-injection protocol (high and low specific activity), the B_{max} can be calculated with a graphical method as shown in Fig. 2 (Ueki et al., 1993; Delforge et al., 2002).

Human studies with this radioligand have produced interesting results. β-Adrenoceptor density in the left ventricle of patients with heart failure related to an idiopathic dilated cardiomyopathy was found to be decreased compared to controls (3.12±0.51 vs. 6.60±1.18 pmol/ml, P<0.001; Merlet et al., 1993). The β-adrenoceptor concentration measured by PET agreed relatively well with receptor densities determined in left ventricular endomyocardial biopsy samples from the same subjects by in vitro assays (n=8, r=0.79, P<0.02). Myocardial β-adrenoceptor concentration (measured by PET) was related to the contractile responsiveness of the heart to intracoronary dobutamine infusion (r=0.83, P<0.005). Another group reported a similar reduction of myocardial β-adrenoceptor density in patients with a hypertrophic cardiomyopathy (7.70±1.86 pmol/g tissue vs. 11.50±2.18 pmol/g in healthy controls, P<0.001; Lefroy et al., 1993). The distribution of β-adrenoceptors was uniform throughout the left ventricle, both in healthy controls and in subjects with hypertrophic cardiomyopathy. Subjects with hypertrophic cardiomyopathy and heart failure showed lower cardiac β-adrenoceptor densities (5.61±0.88 pmol/g; Choudhury et al., 1996). The loss of myocardial contractility appeared to be related to the loss of β-adrenoceptors.

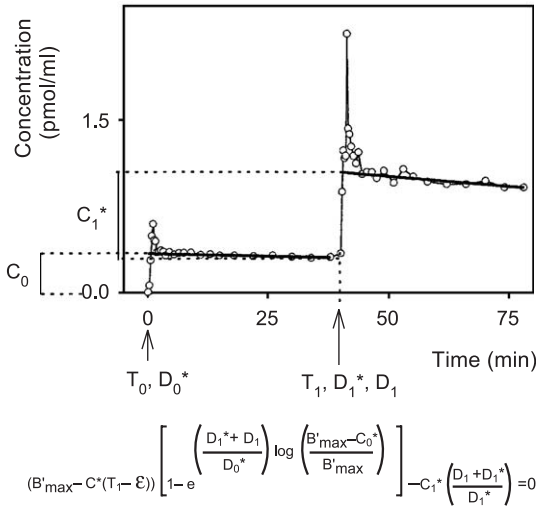

Fig. 2. A typical example of a pulmonary or myocardial time activity curve after a double injection of (*S*)-[^{11}C]CGP12177 and the equation of β-adrenoceptor concentration B_{max}. B_{max}=β-adrenoceptor concentration; $C^*(T_1-\varepsilon)$=concentration of labelled ligand before the second injection; D_0^*, D_1^*=dose of radioligand in the first and second injection; D_1=dose of nonlabelled ligand in the second injection; C_0^* and C_1^*=estimated concentration of bound radioligand after the first and second injection.

By the use of [^{11}C]CGP 12177 and [^{11}C]hydroxyephedrine, it is possible to gain insight into pre- and postsynaptic autonomic dysfunction of the human heart. In hypertrophic cardiomyopathy, a reduced density of β-adrenoceptors was found to be accompanied by a reduced capacity of sympathic neurons to accumulate noradrenaline. Increased levels of noradrenaline in the synaptic cleft may thus have contributed to the downregulation of β-adrenoceptors in hypertrophic cardiomyopathy (Wichter et al., 2000; Schafers et al., 2001). Similar observations have been made in patients suffering from arrhythmogenic right ventricular cardiomyopathy (Schafers et al., 1998; Langer and Halldin, 2002).

Pulmonary β-adrenoceptor density measured by PET after i.v. injection of [^{11}C]CGP 12177 correlated closely with in vitro binding assays (r=0.92, p<0.05; Qing et al., 1996). For these studies, patients with lung cancer were scanned on the day before thoracotomy so that PET data and lung biopsies could be acquired within a short time interval. In another study, the downregulation of pulmonary β-adrenoceptors during 2 weeks of treatment, with oral and inhaled salbutamol, was assessed. Pulmonary β-adrenoceptor density decreased with 22±14% (P<0.05), while the number of β-adrenoceptors on mononuclear leukocytes dropped by 42±19% (P<0.05; Hayes et al., 1996). Asthmatic subjects (36±8 year of age) showed similar pulmonary β-adrenoceptor densities (Qing et al., 1997) as aged-matched healthy controls (10.3±1.8 vs. 10.9±1.9 pmol/g tissue). These data were interpreted as indicating the absence of a primary β-adrenergic deficit in asthma. However, it should be noted that the employed imaging technique visualizes all pulmonary β-adrenoceptors and not only β-adrenoceptors in the airways. Changes in the relatively small population of airway β-adrenoceptors may not have been detected in these studies because of the very strong signal from the alveoli.

(*S*)-[^{11}C]CGP 12177 is produced by the reaction of [^{11}C]phosgene with the appropriate (*S*)-diamine precursor in high radiochemical yield (Boullais et al., 1986; Brady et al., 1991; Aigbirhio et al., 1992). Unfortunately, several PET centers reported variable and usually very low specific

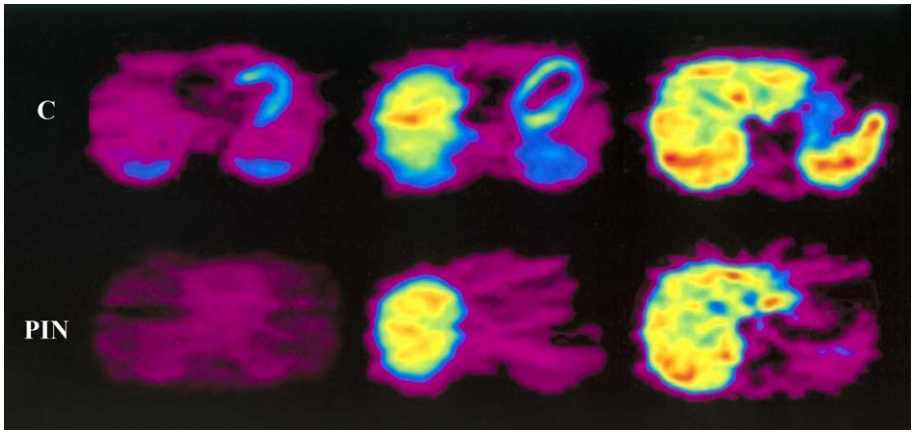

Fig. 3. PET images of a human volunteer acquired with (*S*)-[^{11}C]CGP 12388. Transaxial cross-sections in the time frame 14–60 min postinjection are displayed. The upper row is the control study; the bottom row is the pindolol-blocked study.

activities. The troublesome and laborious radiolabelling via [^{11}C]phosgene is an important drawback to apply [^{11}C]CGP 12177 for clinical studies and to prevent widespread use of this radioligand.

3.3.2. (S)-[^{11}C]CGP 12388

Because of the problems involved in the routine production of (*S*)-[^{11}C]CGP 12177, another hydrophilic β-adrenoceptor ligand, (*S*)-CGP 12388 was developed for clinical use (Fig. 1). CGP 12388 is the isopropyl analog of CGP 12177. In vitro experiments have indicated that racemic CGP 12388 is almost equally potent as racemic CGP 12177 (Elsinga et al., 1997).

(*S*)-[^{11}C]CGP 12388 is prepared via a one-pot procedure using 2-[^{11}C]acetone (Elsinga et al., 1997). This synthetic procedure is easily performed and therefore more suitable for clinical use than the multistep synthesis of (*S*)-[^{11}C]CGP 12177 from [^{11}C]phosgene. Sufficient amounts could be obtained for a multi-injection protocol in humans.

In vivo blocking experiments have indicated that (*S*)-[^{11}C]CGP 12388 binds to β_1-and β_2-adrenoceptors (van Waarde et al., 1998). The rate of metabolism of (*S*)-[^{11}C]CGP 12388 in male Wistar rats was similar to that of (*S*)-[^{11}C]CGP 12177. Radiochromatograms of tissue extracts made 60 min post injection indicated that heart and lungs contain mainly (>90%) native (*S*)-[^{11}C]CGP 12388 (van Waarde et al., 1998).

(*S*)-[^{11}C]CGP 12388 has been evaluated in healthy subjects and in patients with IDC. The myocardial left ventricle, peripheral lung, liver and spleen were clearly visible (Fig. 3). After administration of pindolol, the left ventricle, lung and spleen were no longer visible, whereas uptake in liver was unchanged. After ingestion of pindolol, the maximal tissue uptake was decreased and the washout rate was increased. Pindolol reduced the uptake of radioactivity in heart, lung and spleen by 76%, 68% and 77%, respectively, at 60 min postinjection.

In another study, the B_{max} and the ligand affinity (K_d) of (*S*)-[^{11}C]CGP 12388 for the β-adrenoceptor were determined using tracer kinetic modelling (Doze et al., 2002). Dynamic PET data were acquired in six healthy subjects during 60 min. A three-injection protocol was applied: high specific activity, low specific activity or unlabelled ligand only. Time–activity curves showed that unlabelled ligand could displace the radioligand from the receptor. This resulted in increased radioactivity levels in plasma. Modelling results yielded B_{max} values of 9.74±1.80 nM and a K_d of 0.58±0.22 nM, assuming a reaction volume of 0.15. Parametric polar images of B_{max} could be calculated. The kinetics was also investigated in an isolated rat heart according to the Langendorff method (Momose et al., 2004). B_{max} and distribution volume were estimated using compartmental modelling and were estimated using a two-injection protocol (control and propranolol blocking). The binding of [^{11}C]CGP12388 was flow independent, with low nonspecific binding.

Myocardial β-adrenoceptor density was investigated in six patients with idiopathic dilated cardiomyopathy and six age-matched healthy controls using (*S*)-[^{11}C]CGP 12388 PET and the same tracer kinetic model. Patients showed a β-adrenoceptor density of 5.4±1.3 pmol/g which was significantly lower than that of their healthy counterparts (8.4±1.5 pmol/g, $P<0.005$). The measured B_{max} of the idiopathic dilated cardiomyopathy patients decreased with 36% compared to the healthy controls. No significant regional differences in B_{max} were found in the heart after analysis of the regions of interest in the basal, mid-ventricular and apical short-axis slices. *K*-values for controls and idiopathic dilated cardiomyopathy patients were not significantly different.

The pulmonary uptake of radiolabelled β-adrenoceptor ligands reflects binding to alveolar sites rather than binding to receptors on smooth muscle cells in the airways (Carstairs et al., 1985). Alveolar β-adrenoceptors are not directly involved in the pathophysiology of asthma.

Therefore, it seems worthwhile to perform PET studies with inhaled β-adrenoceptor ligands. Inhalation may result in the selective labelling of airway rather than alveolar β-adrenoceptors. A nebulizer was used to administer (*S*)-[^{11}C]-CGP12388 in aerosol form (van Waarde et al., 2002). Volunteers inhaled the radioligand twice: at baseline and after pretreatment with β-adrenergic drug. Pretreatment consisted either of inhaled salbutamol or of orally administered pindolol. In both PET studies, a dynamic, 60-min scan of the lungs was followed by a whole body scan to assess the inhaled dose. Drug pretreatment did not affect the initial deposition of radioactivity within the airways. The agonist salbutamol caused a significant increase of the monoexponential washout kinetics of ^{11}C from human lung (baseline k, 0.0033±0.0009, pretreated k, 0.0052±0.0006 min^{-1}, p=0.015). A similar increase of the washout rate was induced by the antagonist pindolol (baseline k, 0.0037±0.0005, pretreated k, 0.0066±0.0013 min^{-1}). Whole body scans showed the oral cavity, trachea, main bronchi and lungs, stomach, duodenum, bladder and/or kidneys. The similar effects of an agonist and antagonist on pulmonary washout kinetics suggests that increased washout is due to blockade of β-adrenoceptors rather than increased perfusion or mucociliary clearance. However, the apparent rate of washout is slow ($t_{1/2}$=2 to 4 h) and the reduction in pulmonary radioactivity after drug pretreatment is minor but statistically significant (only 7% at 60 min, P=0.01).

3.3.3. (S)-[^{18}F]Fluorocarazolol

(*S*)-[^{18}F]Fluorocarazolol is a nonsubtype selective, lipophilic radioligand with high affinity for β_1- and β_2-adrenoceptors. (*S*)-[^{18}F]Fluorocarazolol was found to cross the blood–brain barrier in humans, so it may be possible to investigate myocardial, pulmonary and cerebral β-adrenoceptors with this radioligand (van Waarde et al., 1997). A disadvantage of the lipophilicity is that the radioligand probably binds to internalized receptors.

(*S*)-1′-[^{18}F]Fluorocarazolol was synthesized from [^{18}F]fluoroacetone and *S*-desisopropylcarazolol (Elsinga et al., 1996; Zheng et al., 1994). (*S*)-[^{18}F]Fluorocarazolol has been evaluated in several animal models and in humans. Specific binding to β-adrenoceptors of (*S*)-[^{18}F]fluorocarazolol was demonstrated:

(1) by measuring the uptake difference between the (*S*)-isomer and (*R*)-isomer (Zheng et al., 1994);
(2) by blocking experiments with various β-adrenoceptor agonists and antagonists (van Waarde et al., 1995b) and
(3) by saturation experiments (Doze et al., 1998).

Specific binding was in good agreement with β-adrenoceptor densities determined by in vitro assays. Metabolite analyses of [^{18}F]fluorocarazolol in rats showed a rapid (<5 min) appearance of polar metabolites in plasma, while at 60 min postinjection, 92% and 82% of the total radioactivity in lung and heart remained native (*S*)-[^{18}F]fluorocarazolol (van Waarde et al., 1995a).

PET scans in human volunteers clearly showed β-adrenoceptors in both lung and heart (Visser et al., 1997). The (metabolite-corrected) myocardium/plasma ratio of *S*-1′-[^{18}F]fluorocarazolol increased to a plateau value of 18, which was reached at 45–50 min postinjection. Lung/plasma ratio was 11.6. Cardiac and pulmonary uptake of radioactivity was strongly inhibited after ingestion of pindolol (tissue/plasma ratios declined to 2.0 for both organs). Metabolite analysis showed that 20% of plasma radioactivity at 60 min postinjection was intact [^{18}F]fluorocarazolol.

These pilot studies in humans were performed with non carrier-added (*S*)-[^{18}F]fluorocarazolol (~1 nmol), after it had been shown that fluorocarazolol is not acutely toxic in rodents at doses >10,000-fold higher than were administered to volunteers (Doze et al., 2000). For quantification of receptor densities with compartment models, a multi-injection protocol is required involving the administration of a pharmacological dose of the radioligand (~100 nmol). Such protocols can only be carried out after extensive toxicological screening of the experimental drug. Fluorocarazolol showed a positive Ames test (strong mutagenicity in bacterial strains) during such examination. Further research to elucidate if this radioligand is a risk to humans should be carried out in mammalian cells and in vivo animal experiments.

3.4. Radioligands for the β_1-adrenoceptor

A β_1-subtype selective radioligand is preferable over a nonsubtype selective radiolabelled antagonist for cardiac imaging. For this purpose, [^{11}C]CGP 20712A (Elsinga et al., 1994), [^{11}C]HX-CH 44 BS (Valette et al., 1999) and [^{11}C]bisoprolol (Soloviev et al., 2001) have been synthesized. In addition, derivatives of the β_1-selective antagonist ICI 89406 have been prepared and tested in vitro for their affinities. Two compounds that are amenable for [^{11}C]-methylation have high in vitro affinities and high β_1-selectivities (Kopka et al., 2003). The cardiac binding of [^{11}C]CGP 20712A could not be inhibited in the proper fashion by subtype-selective β-adrenoceptor antagonists and the radioligand showed very high nonspecific binding. Evaluation of [^{11}C]HX-CH 44 BS showed a heart-to-lung ratio of 3 between 5 and 30 min postinjection. Only 35% of the myocardial radioactivity could be displaced. Tissue uptake could not be blocked with appropriate compounds. The affinity constant of HX-CH 44 BS for binding to β_1-adrenoceptors is 10 nM (Daemmgen et al., 1985). PET images acquired with this radiopharmaceutical can therefore be expected to show poor signal-to-noise ratios. Evaluation of (*S*)-[^{11}C]bisoprolol in rats showed that about 30% of radioactivity uptake in heart is due to specific binding. The authors suggest that the high nonspecific uptake in lung

might mask the heart uptake and preclude the use of (*S*)-[^{11}C]bisoprolol for heart and lung studies by PET.

3.5. Radioligands for the β_2-adrenoceptor

[^{11}C]Formoterol was developed to examine whether it is possible to image pulmonary β_2-adrenoceptors with a radiolabelled agonist in vivo (Visser et al., 1998). [^{11}C]Formoterol (a mixture of (*S*,*S*)- and (*R*,*R*)-isomers) was labelled via reaction of a benzyl-protected precursor with [^{11}C]CH_3I, followed by deprotection with Pd/C and H_2. Only animal studies have been performed. Formoterol displays a high affinity (Mak et al., 1994) and selectivity (β_2/β_1=90:10) for the β_2-subtype (Roux et al., 1996). In [^{125}I]-iodocyanopindolol-labelled bronchial membranes of guinea pigs, it was demonstrated that formoterol induces high affinity states (pK_i 9.6±0.4) of the β_2-adrenoceptor (Roux et al., 1996). It may be of clinical interest to know the fraction of receptors in the high affinity state and its changes in disease or after treatment. The efficacy of agonist drugs may be changed by alterations of the fraction of receptors in the high affinity state.

In a PET study with [^{11}C]formoterol in male Wistar rats, lungs were clearly visible. After pretreatment of the rats with the nonselective β-adrenoceptor antagonist propranolol, the lungs could no longer be seen. Biodistribution studies in male Wistar rats, either untreated or predosed with propranolol, showed significant specific binding in tissues known to contain β_2-adrenoceptors (lungs, spleen and heart). Uptake in these target organs was blocked by the β_2-adrenoceptor antagonist ICI 118551 and the nonselective β-adrenoceptor agonist isoprenaline but not by the β_1-adrenoceptor antagonist CGP 20712A. These results are consistent with the β_2-selectivity of formoterol and they show that it is possible to image β-adrenoceptors with a radiolabelled agonist. Whether this radioligand visualizes the high affinity state of these receptors is difficult to prove and remains to be elucidated.

[^{11}C]ICI 118551, a potent, clinically used β_2-adrenoceptor antagonist, has been prepared as a potential radioligand for the noninvasive assessment of β_2-adrenoceptors in the lung (Moresco et al., 2000). The radioligand was prepared by reductive *N*-alkylation of the corresponding desisopropyl precursor with [2-^{11}C]acetone. The evaluation of racemic [^{11}C]ICI 118551 in rats, and *Macaca nemestrina* showed a high radioactivity uptake in lung and heart. However, in both animal models, no detectable displacement of lung radioactivity concentration was observed after pretreatment with propranolol or ICI 118551, which indicates that radioligand uptake mostly reflects nonspecific binding. It was concluded that [^{11}C]ICI 118551 is not a suitable radioligand for PET imaging.

The potent, subtype-selective radioligand [^{11}C]procaterol is labelled by reductive alkylation of the desisopropyl precursor with [^{11}C]acetone (Visser et al., 2000). Biodistribution studies in Wistar rats were performed which were either untreated or predosed with propranolol, ICI 118551, CGP 20712A or isoprenaline. Specific binding was observed in lungs, spleen and red blood cells, tissues known to contain β_2-adrenoceptors. Pulmonary binding was blocked by propranolol, ICI 118551 and isoprenaline, but not by CGP 20712A. This binding pattern is consistent with the β_2-selectivity of the radioligand. In a dynamic PET study, the lungs of untreated control rats could be barely detected and total/nonspecific binding ratios rose to only 1.2 at 20 min postinjection. Therefore, [^{11}C]procaterol seems unsuitable for β-adrenoceptor imaging.

4. Muscarinic acetylcholine receptors

Muscarinic acetylcholine receptors (MACHRs) are widely distributed throughout the heart and lung. In the lung, they are found on submucosal glands, airway ganglia, smooth muscle cells of large and small airways and alveolar walls (Mak and Barnes, 1990; Barnes, 1993). Acetylcholine M_1-receptors are located on submucosal glands and alveolar walls in the peripheral lung. Acetylcholine M_2-receptors act as autoreceptors on postganglionic cholinergic nerves and inhibit acetylcholine release. The acetylcholine M_3-subtype, which is present on the surface of the tracheal smooth muscle fibres, the bronchi and the bronchioles, mediates muscle contraction. Pulmonary mucus secretion is stimulated via the acetylcholine M_1- and M_3-subtypes in submucosal glands. In the rat lung, the acetylcholine M_2-subtype predominates (80–90%), whereas in humans, up to 70% of the receptors are of the acetylcholine M_1 subtype and the remaining of the acetylcholine M_3-subtype. Only a very small portion is of the acetylcholine M_2-subtype. In human lung, a receptor density of 21–165 fmol/mg protein has been reported (Mak and Barnes, 1990).

Muscarinic receptors play an important role in bronchoconstriction and, therefore, in the pathophysiology of asthma and chronic obstructive pulmonary disease. Increased muscarinic cholinergic activity may occur through an increase in receptor density or affinity or through an increase in the efficacy of receptor/signal transduction coupling.

In the human heart, only the acetylcholine M_2-subtype is present with reported densities of 243 fmol/mg protein. Congestive heart failure is associated with decreased stimulated myocardial adenylate cyclase activity, increased G_i-binding protein, attenuated parasympathetic tone and increased modulation of β-adrenergic inotropic left ventricular stimulation by parasympathetic agonists.

4.1. Radioligands for positron emission tomography of muscarinic acetylcholine receptors

Only a few PET studies of muscarinic acetylcholine receptors in the airways and heart have been reported.

Research on these receptors was mainly focussed on the brain. A major obstacle in the quantification of muscarinic receptors in the lungs is their relatively low density. For the M_1-subtype of the muscarinic acetylcholine receptors, [^{11}C]xanomeline ([3(3-hexyloxy-1,2,5-thiadiazol-4-yl)-1,2,5,6-tetrahydro-1-[^{11}C]methylpyridine and [^{11}C]butylthio-TZTP ([^{11}C]butylthio-thiadiazolyltetrahydro-1-methyl-pyridine; Farde et al., 1996) and (*R*,*R*)-quinuclidinyl-4-[^{18}F]-fluoromethyl-benzilate (Kiesewetter et al., 1997) have been developed, but these radioligands have only been evaluated for studies of the brain.

4.2. Radioligands for the acetylcholine M_2-subtype

For the acetylcholine M_2-subtype, one single radioligand has been investigated in man: [^{18}F]fluoropropyl–TZTP has shown potential to image M_2 receptors in human brain (Carson et al., 1998; Eckelman, 2001). In rats, specific binding of this radioligand was also observed to myocardial and pulmonary acetylcholine M_2-receptors. The compound showed a K_i of 7.4 nM for M_1 and 2.2 nM for M_2. There was no binding to the acetylcholine M_3-subtype (Kiesewetter et al., 1999). Uptake values in heart expressed as percentage (%) injected dose/g decreased from 0.171±0.010 to 0.121±0.004 after pretreatment with 50 nmol of fluoropropyl–TZTP. In lung tissue, control values were 0.372±0.036, whereas after fluoropropyl–TZTP pretreatment, these numbers were 0.206±0.013. [^{18}F]fluoropropyl–TZTP is rapidly metabolized. The amount of parent compound in rat plasma decreased to 5% at 15 min postinjection. No studies of human heart and lung with this radiotracer have been reported. [^{18}F]fluoropropyl–TZTP is prepared by direct fluorination of the corresponding mesylate (Kiesewetter et al., 2003).

4.3. Nonsubtype-selective radioligands

The radioligands [^{11}C]MQNB (methylquinuclidinyl benzilate; Syrota et al., 1984, 1985) and [^{11}C]-VC002 (*N*-[^{11}C]-methyl-piperidin-4-yl-2-cyclohexyl-2-hydroxy-2-phenylacetate; Visser et al., 1999) are hydrophilic compounds and only suitable for imaging of peripheral muscarinic receptors (Fig. 4) because they do not cross the intact blood–brain barrier.

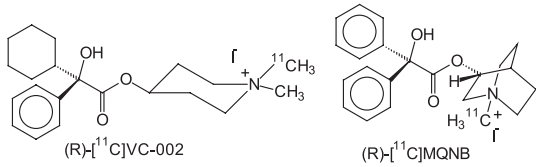

Fig. 4. Chemical structures of [^{11}C]VC002 and [^{11}C]MQNB as subtype nonselective PET-radioligands for the muscarinic receptor.

The density and affinity constants of myocardial muscarinic receptors were evaluated noninvasively with [^{11}C]MQNB in 20 patients with congestive heart failure due to idiopathic dilated cardiomyopathy (Le Guludec et al., 1997) and were compared to values in 12 normal subjects. The mean receptor concentration was significantly higher in patients than in control subjects (B_{max} 34.5±8.9 vs. 25±7.7 pmol/g tissue, $P<0.005$), with no changes in affinity constants (Delforge et al., 1993). From this study, it was concluded that congestive heart failure is associated with an upregulation of myocardial muscarinic receptors. This may be an adaptive mechanism to β-agonist stimulation and should increase the number of potential targets for pharmacological intervention. In contrast to these findings in patients with heart failure, a study comparing healthy subjects with patients with heart transplant found no difference in receptor densities (Le Guludec et al., 1994).

In another study, 21 patients with familial amyloid polyneuropathy (Delahaye et al., 2001) were investigated. This disease usually does not induce heart failure but is associated with sudden death, conduction disturbances and an increased risk of complications during anesthesia. Although cardiac sympathetic denervation has been clearly demonstrated, the postsynaptic status of the cardiac autonomic nervous system remains unelucidated. The mean

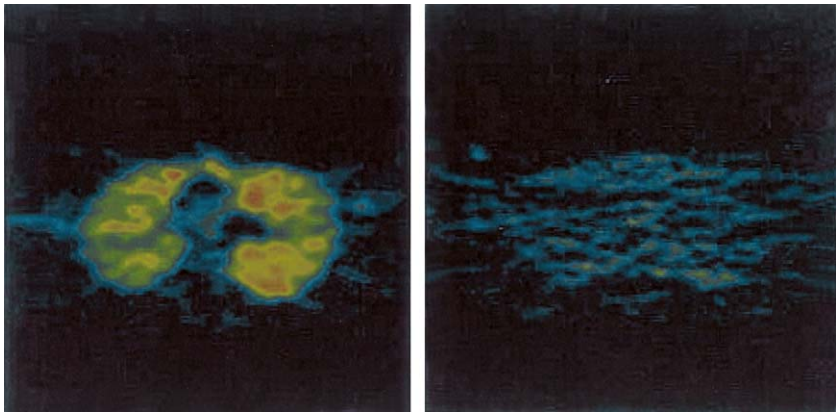

Fig. 5. PET images of the lungs of a healthy volunteer acquired with (*R*)-[^{11}C]VC002 before and after blockade with glycopyrronium bromide. Transaxial cross-sections in the time frames 24–60 min postinjection are shown.

muscarinic acetylcholine receptor density was higher in patients than in control subjects (B_{max} 35.5±8.9 vs. 26.1±6.7 pmol/g tissue, P=0.003), without any change in receptor affinity. Cardiac β-adrenoceptor functional efficiency was studied by the heart rate response to intravenous infusion of isoproterenol. Incremental infusion of isoproterenol induced a similar increase of HR in patients and control subjects. Cardiac autonomic denervation in familial amyloid polyneuropathy results in an upregulation of myocardial muscarinic receptors but without change in cardiac β-adrenoceptor responsiveness to catecholamines. Although [^{11}C]MQNB seems to be a useful radioligand for the quantification of muscarinic acetylcholine receptors in the heart, the lung uptake is very low. No explanation has been given by the authors.

(*R*)-[^{11}C]VC002 is another subtype nonselective antagonist (Visser et al., 1999). (*R*)-[^{11}C]VC002 was prepared by [^{11}C]methylation of the corresponding desmethyl precursor. In vitro binding assays showed that the ligand binds with very high affinity (<0.2 nM) to the muscarinic acetylcholine receptor subtypes M_1, M_2, and M_3. Biodistribution studies in control and atropine-treated rats demonstrated significant specific binding (90–99% of total tissue uptake) in heart and lung. In human PET studies, the radioligand was administered on two separate days. The first injection was without pretreatment; prior to the second injection, the anticholinergic compound glycopyrronium bromide was injected to block the receptors. In contrast to the findings with [^{11}C]MQNB, in the PET images obtained with [^{11}C]VC002 the lungs but not the heart were clearly visible (Fig. 5). Pulmonary radioactivity uptake was reduced to 30% at 60 min post injection after blocking with glycopyrronium bromide. [^{11}C]VC002 was rapidly cleared from plasma and metabolism was negligible during the PET investigation.

5. Conclusions

For both the β-adrenergic and the muscarinic acetylcholine systems, potent PET radioligands have been prepared and evaluated in patients. It has been possible to measure receptor densities both in human heart and in lung, although techniques should be developed to measure β-adrenoceptors in the upper airways. A disadvantage of the currently available radioligands is that they are subtype nonselective. Future research should be directed towards subtype-selective compounds resulting in more specific and sensitive PET measurements. To quantify receptor densities, multi-injection protocols have been applied. These protocols however, are patient unfriendly, time consuming and too complex for routine studies. Simplified protocols could be a breakthrough for using PET-receptor measurements in a routine clinical setting. In addition, some limitations of PET, such as low availability of PET centers and the need for a cyclotron and a radiochemistry laboratory, need to be taken into account when decisions are made on implementing PET-receptor measurements in a routine clinical setting. For the α-adrenoceptor, only [^{11}C]GB67 has been developed. Its potential for application in patients needs to be assessed.

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