University of Groningen

Development of new muscarinic and ß-adrenergic receptor radiopharmaceuticals for Positron Emission Tomography

Visser, Thomas Jakob

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
1997

[Link to publication in University of Groningen/UMCG research database](#)

CHAPTER 8

GENERAL DISCUSSION AND FUTURE PERSPECTIVES

8.1 Introduction

Muscarinic and β-adrenergic receptors are responsible for the contraction and relaxation of the smooth muscle cells in the airways. Changes in the properties of these receptors may play a role in asthma and/or COPD. Medicamentous treatment may also lead to changed characteristics of these binding sites. For example patients suffering from asthma and COPD are often treated with β-mimetics (β-agonists) and parasympathicolytics (muscarinic antagonists). Chronic exposure of tissues to these drugs can lead to a phenomenon known as tolerance, that is a reduction in the sensitivity of the tissue to the drug, accomplished by uncoupling, internalization or down regulation of the receptors and to rebound effects when medication is stopped.

Provided suitable radiopharmaceuticals are available, positron emission tomography (PET) may offer a unique possibility to study the muscarinic and β-adrenergic receptors *in vivo*. Consequently it may become clear whether there are differences in receptor densities between the lungs of patients suffering from asthma or COPD in comparison to healthy volunteers. Also the effects at the receptor level after chronic treatment of these patients with long and-short acting β-sympathicomimetics, can be studied.

The aim of this thesis was the development of new PET radiopharmaceuticals and procedures for quantification of muscarinic and β-adrenergic receptors in human lungs to detect changes induced by disease, age, or pharmacological manipulations.

8.2 General discussion and future perspectives

Muscarinic receptors in the human lungs are of the M_1, M_2 and M_3 subtype. Because M_3 receptors are responsible for the regulation and contraction of the smooth muscle cells in the airways, quantification of this subtype could be of great clinical interest. Although in literature it has been put forward that the prejunctional M_2 muscarinic receptor subtype may be defective in asthma, it is currently impossible to detect this particular subtype with PET, because of the very low M_2 receptor density. From *in vitro* studies it is clear that even the total muscarinic receptor population in the airways is small. Because of the low receptor density and the fact that the human lungs consist for approximately 85% of air (introduction,

119

page 12), the affinity of a radioligand for muscarinic receptors must be very high in order to observe specific binding with PET.

The most potent M_3-selective ligand known until now, is 4-DAMP. Although in pilot studies, performed by our group, 4-[^{3}H]-DAMP showed specific uptake in rat organs containing M_3 receptors (bladder, pancreas, stomach, submandibular glands, and trachea), no specific binding in rat lungs was observed, probably because pulmonary M_3 receptor densities and the affinity of the radioligand for this particular subtype were too low. Because no M_3 selective ligands more potent than 4-DAMP are known, we decided to synthesize and label two highly potent, non-subtype selective muscarinic antagonists (**chapter 2**). The different muscarinic receptor subtypes may then be labelled selectively by co-injection of a non-radioactive subtype selective cholinoceptor antagonist.

In biodistribution studies performed in male Wistar rats, both radioligands demonstrated high specific binding in tissues known to contain muscarinic receptors. One of the radioligands ([^{11}C]-VC-002) was used in a pilot study in three healthy volunteers to assess its suitability for non-invasive measurement of human pulmonary muscarinic receptor densities (**chapter 3**). With [^{11}C]-VC-002, human pulmonary muscarinic receptors were visualized for the first time. The favourable *in vivo* properties of this radioligand in combination with its relatively simple and reliable synthesis indicate that [^{11}C]-VC-002 has great potential for determination of pulmonary muscarinic receptor densities in humans by means of a multiple injection protocol and mathematical models. Additional experiments should be performed to determine the fraction of radioligand bound to bronchial (rather than alveolar) cholinoceptors. If non-labelled agonists and antagonists are administered by means of inhalation prior to administration of the radioligand, these non-labelled ligands will only block binding to airway cholinoceptors. Such experiments can therefore give additional information about the fraction of radioligand binding which takes place to bronchial cholinoceptors. If a large fraction of the binding occurs in the airways, comparative studies between healthy volunteers and patients suffering from asthma or COPD will become possible.

Chapter 4 describes the synthesis of the non-selective β-adrenoceptor antagonist [^{11}C]-CGP-12177 and a study with this radioligand in 8 healthy volunteers, using PET. The pulmonary receptor density estimated in human volunteers (1.93 ± 0.45 pmol/ml) was comparable with literature. Thorough examination of the data demonstrated a correlation (r=0.78, p<0.0001) between the calculated receptor density and the injected mass of the radioligand. The estimated receptor density increased with increasing mass. Therefore in

future studies it is very important to keep the injected mass constant. Then differences between patients and healthy volunteers are based on alterations in binding capacity and not on differences in the amount of radioligand. The double injection protocol in combination with the graphical analysis method used for the estimation of the B_{max}, was also sensitive to other experimental details such as the rate of injection of the radioligand. It is necessary to administer the ligand with a remote controlled pump which ensures reproducible injections. Because it is now generally accepted that [^{11}C]-CGP 12177 is hardly metabolized within the time period of a PET scan, kinetic analysis of the data based on a fitting procedure may also be possible.

Although [^{11}C]-CGP 12177 is easily synthesized in quantities sufficient for human studies, the specific activity of the radioligand showed large variations and was often too low for injection in humans. For medical/ethical and model technical reasons, this was not acceptable. Despite protracted efforts towards optimization, the specific activity remained low and unreliable; it was therefore not possible to study patients with asthma or COPD.

An alternative for [^{11}C]-CGP 12177 was found in the β-adrenoceptor antagonist fluorocarazolol which is an analogue of the β-blocker carazolol. [^{18}F]-fluorocarazolol could be reliably prepared and the specific activity was always high. This radioligand has been extensively evaluated in animals by our group and demonstrated to be suitable for *in vivo* imaging.

Chapter 5 of this thesis describes the evaluation of [^{18}F]-fluorocarazolol in six healthy volunteers in order to establish whether this radioligand is suitable for the *in vivo* imaging of pulmonary and myocardial β-adrenoceptor densities. From these experiments, it became clear that in the lungs, i.v. administered [^{18}F]-fluorocarazolol binds mainly to alveolar receptors (for more than 90%) and only a minor fraction of the binding occurs to receptors on the smooth muscle cells in the airways (less than 10%). Since alveolar receptors are not directly involved in the pathophysiology of asthma, it did not seem worthwhile to perform a comparative PET study of normals and asthmatic patients, using intravenous [^{18}F]-fluorocarazolol. When other β-adrenoceptor ligands are administered intravenously, binding will also occur mainly to alveolar receptors. In a PET study with [^{11}C]-CGP 12177 which was very recently performed by Qing *et al*, no differences were observed in pulmonary receptor densities between healthy volunteers and mild asthmatics. These results may be due to the fact that intravenous [^{11}C]-CGP 12177 also binds to alveolar rather than airway β-adrenoceptors. Consequently future research will only be successful when airway β-adrenoceptors can be labelled selectively. Such labelling may be possible by administering

121

the radioligand per inhalation. However, in that case probably only qualitative conclusions about receptor densities can be drawn, since the input of the radioligand to the tissues cannot be determined.

Very recently another useful alternative for [^{11}C]-CGP 12177 was developed in our laboratory: [^{11}C]-CGP 12388, an analogue of CGP 12177 in which the t-butyl group is replaced by an isopropyl group.

Figure 8.1   *The radiosynthesis of [^{11}C]-CGP 12388.*
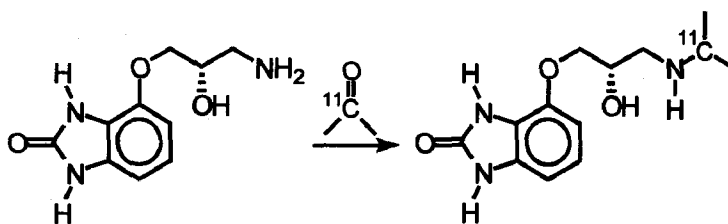
The affinity of CGP 12388 for to β_1 and β_2-adrenoceptors is comparable to CGP 12177 and both compounds are equally hydrophilic. [^{11}C]-CGP 12388 is prepared by reductive alkylation of the ethanolamine precursor with [^{11}C]-acetone (figure 8.1). The synthesis of [^{11}C]-acetone and subsequent reaction with the ethanolamine precursor is much easier to accomplish than the synthesis of [^{11}C]-CGP 12177 via [^{11}C]-phosgene. [^{11}C]-CGP 12388 has the advantage over [^{11}C]-CGP 12177 that it is reliably and easily prepared, in good radiochemical yields and high specific activities and the radiosynthesis is much easier to automate. In rats, [^{11}C]-CGP 12388 displayed the same properties regarding binding profile, clearance, metabolism and imaging quality as [^{11}C]-CGP 12177. Therefore, [^{11}C]-CGP 12388 may become the ligand of choice for imaging/quantification of β-adrenoceptors in human heart and lungs.

In **chapter 6** a PET study with [^{18}F]-fluorocarazolol in the brain of healthy volunteers is described. The uptake of [^{18}F]-fluorocarazolol in different brain areas corresponded to the localisation of β-adrenoceptors known from post-mortem autoradiography. Cerebral β-adrenoceptors have been reported to be affected in a variety of disorders like depression, schizophrenia, alcoholism, Alzheimer's disease and Huntington's chorea. These observations have been made in autopsy studies, and in animal experiments. Cerebral β-adrenoceptors have not been detected previously by PET, simply because no suitable radioligands were

available which could cross the blood brain barrier. Using fluorocarazolol the distribution of β-adrenoceptors in the human brain has now been imaged for the first time. It may thus become possible to assess the role of cerebral β-adrenoceptors in the action of antidepressant drugs and in disorders such as depression and schizophrenia.

The previously mentioned β-adrenoceptor ligands are all antagonists. *In vitro* studies have demonstrated that antagonists only bind with high affinity (without initiating a physiological response), whereas agonist binding occurs to a high and a low affinity state. The high affinity state is the functional state of the receptor which transduces the signal that evokes the biologic effect. The fraction of β-adrenoceptors in the high affinity state may be altered in pathology (for instance asthma or COPD), or after pharmacological manipulations while the total amount of β-adrenoceptors may not show any change. The total amount of receptors may then be similar, although the physiological response after an adrenergic stimulus is different. It is thus very interesting to examine if the high affinity state of the β_2-adrenoceptor can be selectively visualized, using a radioactive *agonist*.

To test whether it is possible to visualize pulmonary β_2-adrenoceptors, *in vivo* with a radiolabelled agonist, [^{11}C]-formoterol was synthesized. Formoterol is a long-acting β_2-sympathicomimetic drug with a high affinity and selectivity for β_2-adrenoceptors. *In vitro* binding studies with a non-hydrolysable GTP analogue suggested that [^{3}H]-formoterol preferentially labels the high affinity state of the β_2-adrenoceptor. In **chapter 7** the synthesis of [^{11}C]-formoterol and the evaluation of this novel radioligand in male Wistar rats is described.

In PET-images of rats which were injected with [^{11}C]-formoterol, the lungs were clearly visible but when the rats were pretreated with propranolol (a non-selective β-adrenoceptor antagonist) pulmonary uptake of [^{11}C]-formoterol was negligible. Biodistribution studies in rats (controls as well as propranolol pretreated) showed specific uptake in β_2-adrenoceptor containing organs. Pulmonary binding of the radioligand was blocked by propranolol, ICI 118,551 (a β_2-selective adrenoceptor antagonist) and isoprenaline (a non-selective β-adrenoceptor agonist), but not affected by the β_1-selective antagonist CGP 20712A. These results are in accordance with the β_2-selectivity of the radioligand.

This is the first time that specific binding to β_2-adrenoceptors was demonstrated *in vivo*, using a [^{11}C]-labelled β_2-adrenoceptor agonist. Whether this radioligand visualizes the high affinity state of these receptors remains to be elucidated. Quantification of bronchial β_2-adrenoceptors will be hampered by binding of the radioligand to alveolar instead of bronchial

β-adrenoceptors after i.v. administration. Thus, in future studies the possibility should be explored to administer formoterol by inhalation and to selectively label airway β_2-adrenoceptors. By labelling formoterol and other sympathicomimetic drugs with a positron emitter, the concentration and retention of these ligands in the lungs can be determined. In this way the pharmacokinetics of long- and short-acting β-mimetics can be compared which may give some additional clues about the mechanisms underlying the long duration of action.