RADIOSYNTHESIS, EVALUATION AND PRECLINICAL STUDIES OF A NEW $5HT_{2A}$ RADIOLIGAND

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Abstract

^{123}I-5-I-R91150, a radioiodinated analogue of R91150 (a ligand (antagonist) of Janssen Research Foundation), showing high affinity and selectivity for $5HT_{2A}$ receptors, was developped as a potential in vivo $5HT_{2A}$ receptor tracer for SPECT. The applied radiochemistry, whereby the radioiodine was substituted on the 5 - position of the benzamide ring, allowed to obtain the tracer with high specific activity and high purity.

In vitro and in vivo rat studies revealed that the new tracer bound reversibly with the required high affinity (Kd = 0.1 nM) and high selectivity (a factor ranging from 10000 to at least 50 vis à vis other receptors) to $5HT_{2A}$ receptors.

In young normal subjects the major part of the ^{123}I-5-I-R91150 radioactivity in the brain is present in cortical areas. Cortical area to cerebellum activity ratio reaches an equilibrium value of about 1.8 around 90 min. till 4 hours p.i.. This binding was specific and reversible. The cortical activity reflects a distribution in the brain simular to that of the mapping of $5HT_{2A}$ receptors from post mortem studies.

These findings suggested that ^{123}I-5-I-R91150 allows imaging and quantitative estimation with SPECT and could be used for further clinical studies.

The radiobromine analogue was synthetised as a potential PET tracer.

1. INTRODUCTION.

$5HT_{2A}$-Receptors seem to play an important role in psychiatric disorders. In order to investigate the role of these receptors in pathology there is an increasing interest in obtaining a selective and high affinity radiolabelled ligand suitable for in vivo receptor binding studies. For in vitro and rodent studies ^{125}I labelled tracers are preferred. Labelled with ^{123}I or ^{75}Br a suitable ligand offers the opportunity to perform SPE(C)T or PET.

Recently 4-amino-N-[1-[3-(4-fluorophenoxy)propyl]-4-methyl-4-piperidinyl]-2-methoxybenzamide (R91150, a Janssen Research Foundation compound) derivatives have been discovered as a new class of $5HT_{2A}$ antagonists. The parent compound shows very high affinity and selectivity for $5HT_{2A}$-receptors (Ki for inhibition of [^{3}H]ketanserin binding to rat frontal cortex membranes is 0.2 nM ; selectivity with regard to other neurotransmitter receptor sites such as $5HT_{1A}$, $5HT_{1B}$, $5HT_{1D}$, $5HT_{2C}$, $5HT_3$, α_1 and α_2 adrenergic, histamine-H_1 and dopamine-D_2 is at least a factor of 50). The non - radioactive 5-bromo-benzamide and 5-iodo-benzamide analogues showed an affinity and selectivity similar to the parent compound mentioned above [1]. So it was decided to label this compound with radioiodine and radiobromine in order to develop a new radiolabelled ligand for SPECT and PET with high affinity for $5HT_{2A}$ receptors. In view of the lower increase in lipophilicity (increased lipophilicity causes increased non-specific binding) we have chosen the 5-position in the benzamide group i.e. ortho to the polar amine function.

2. EXPERIMENTAL.

2.1. Radiosynthesis of [^{123}I]- or [^{125}I]-4-amino-N-[1-[3-(4-fluorophenoxy)propyl]-4-methyl-4-piperidinyl]-5-iodo-2-methoxybenzamide .

0.7 mg 4-amino-[1-[3-(4-fluorophenoxy)propyl]-4-methyl-4-piperidinyl]-2-methoxybenzamide.$2H_2O$ was dissolved in 500 µl of glacial acetic acid. The

radioiodide (N.C.A. for ^{125}I and a specific activity brought to 370 TBq / mmol for ^{123}I) was added while stirring followed by the addition of 100 µl of 30% H_2O_2. The reaction was allowed to proceed for 15 minutes at room temperature. The reaction vial was transferred to a small ice-bath. To the reaction mixture 2 ml of ice-cold H_2O and 1.8 ml of 1M Na_2SO_3 were added while stirring and the pH was brought to 7.4 by addition of 2N NaOH. This solution was passed through a Baker Bond Octadecyl 100 mg column. The column was consecutively rinsed with 10 ml of a NaOH solution of pH 10 and 10 ml of H_20. The starting compound and radioactive tracer were recovered in 600 µl of MeOH. 300 µl acetonitrile and 500 ?l of H_2O were added and the complete mixture was filtered through a 0.45 µ filter. The filtrate was injected for semi-preparative HPLC separation. The pure radioactive tracer was recovered in 8 ml eluent, approximately 20 ml of H_20 was added and the solution brought to pH 9 with 2N NaOH. Preconcentration was performed on a Baker Bond Octadecyl column as described above.

After blowing the column apparently dry, the radioactive tracer was recovered in 500 µl EtOH .For human application 5ml of isotonic saline was added and the solution sterilised by means of a 0.22 µm Millex GV filter.The mean labelling yield reached 98% . The overall radiochemical yield is about 80%. Analytical and semi-preparative HPLC control of the final product revealed a purity of at least 99%.

2.2.Radiosynthesis of N.C.A. ^{77}Br-4-amino-N-[1-[3-(4-fluorophenoxy)propyl]-4-methyl-4-piperidi-nyl]-5-bromo-2-methoxybenzamide .

The longer lived ^{77}Br was used as isotope for development work.

0.7 Mg 4-amino-[1-[3-(4-fluorophenoxy)propyl]-4-methyl-4-piperidinyl]-2-methoxybenzamide.$2H_2O$ was dissolved in 500µl of glacial acetic acid. 50µl of the radiobromide solution was added while stirring followed by the addition of 100µl of 30 % hydrogen peroxide solution. The reaction was allowed to proceed for 15

minutes at room temperature. The reaction vial was transferred to a small ice-bath. To the reaction mixture 1.8 ml of 1M Na_2SO_3 was added while stirring and the pH was brought to 7.4 by addition of 2N NaOH. The obtained solution was passed through a Baker Bond Octadecyl 100 mg column. Purification and recovery was performed as described for the radioiodinated compound.

3.RESULTS AND DISCUSSION.

3.1.Radiochemistry.

As in theory the 3-position, ortho to both an amino and methoxy group of the benzamide group, is more activated for electrophilic substitution than the 5-position a mixture of 3 and 5 iodinated compounds could be expected. Nevertheless the electrophilic substitution occurs nearly entirely at the 5-position. This is due to steric hindrance by the freely rotating methoxy group on the 2-position.Less than 0.5% of the compound labelled at the 3-position is observed by HPLC.

The overall yield of N.C.A. radioiodinated R91150 amounts to 80% and quality control revealed a purity of at least 99%.

In case of the radiobromination reaction, after 15 minutes the radiobromide is quantitatively consumed for substitution, 80 - 85 % is found in the 5-position and 20 - 15 % in the 3-position.

The overall radiochemical yield of ^{77}Br-5-Br-R91150 obtained with the proposed method varied from 75 to 80 %. Analytical and semi-preparative HPLC control of the final radiobrominated product revealed a purity of at least 99 % .

3.2.In vitro and in vivo binding properties to the rat brain.

The binding of the radioiodinated compound to rat frontal cortex membranes is saturable . A linear Scatchard plot yields a Kd value of 0.11 ± 0.01 nM and a Bmax

value of 38.0 ± 0.7 fmoles/mg. Inhibition of the binding by ketanserin and ritanserin shows that the binding is reversible.

In vivo in rat brain the frontal cortex (FC) to cerebellum (CER) and FC to Blood ratio increases to a steady value of about 10 for FC / CER and 6 for FC / Blood up from 60 minutes to 3 hours [2]. Displacement by ketanserin, which was i.v. injected 60 minutes after the administration of the radioactive compound, showed that also in the in vivo brain the binding was reversible.

^{123}I-5- I- R91150 was also demonstrated to be suitable for imaging $5HT_{2A}$ receptors in different cortical areas and the striatum of a living baboon with a dedicated SPECT [3].

3.3. Preclinical studies.

Preliminary studies of the distribution of ^{123}I-5-I-R91150 (with a specific activity of 37 10^7 MBq/mmol) in young normal subjects showed that an average of 2% of a bolus dose is present in the brain between 30 and 240 minutes p.i..

The major part of the radioactivity in the brain is present in cortical areas. Cortical area to cerebellum activity ratio reaches an equilibrium around 90 min. p.i. which is maintained up to 4 hours. The cortico - cerebellar ratios (relative binding indices with a mean value of 1.8) reflects a distribution in the brain (cerebral cortex > striatum >> cerebellum) simular to that expected for $5HT_{2A}$ receptors from post mortem studies [4]. This binding was specific and reversible as shown by inhibition of the uptake by risperidone, an antipsychotic which is known to have high affinity for $5HT_{2A}$ receptors [5].

As ex vivo studies and limited PET studies pointed at a decline of $5HT_{2A}$ receptors with age , we decided to carry out this type of study in a large group of male and female volunteers with the new tracer and SPECT. This is an important factor in understanding $5HT_{2A}$ receptor related pathologies compared to matched

controls in clinical studies as well as for the follow - up of pharmacological dose - drug actions for antipsychotics used for treatment of those pathologies.

For semi quantitative analysis, ratios of ligand binding in different regions of interest to the binding in the cerebellum were calculated.. 5-HT_{2A} binding was shown to decline with age but no gender difference was demonstrated. These results are in agreement with in vitro and PET findings of a decline in 5-HT_{2A} receptor binding with age. These findings also highlight the necessity of age matched controls in clinical studies.

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REFERENCES

[1] Mertens J, Terriere D, eds. Radiosynthesis of a New Radioiodinated Ligand for Serotonin - 5 HT_2 receptors, a Promising Tracer for γ - Emission Tomography. Journal of Labelled Compounds and Radiopharmaceuticals. 1994; 34: 795 - 806.

[2] Terriere D , Janssen PMF, eds. Evaluation of Radioiodo - 4 - amino - N - [1 - [3 - (4 - fluorophenoxy) - propyl] - 4 - methyl - 4 - piperidinyl] - 5 – iodo - 2 - methoxybenzamide as a potential 5-HT_2 receptor tracer for SPE(C)T. Nucl Med Biol. 1995; 22 (8): 1005 - 1010.

[3] Abi-Dargham A, Zea - Ponce Y, Terriere D, eds. Preclinical evaluation of [^{123}I] R93274 as a SPECT radiotracer for imaging serotonin 5-HT_{2A} receptors. Eur J Pharmacol 1997; 321: 285 - 293.

[4] Schotte A, Maloteaux M and Laduron PM. Characterization and Regional Distribution of Serotonin S2 - Receptors in Human Brain. Brain Research. 1983; 276: 231 - 235.

[5] Travis MJ, Busatto GF, 5HT2A receptor blockade in vivo in Schizophrenic patients treated with Clozapine or Risperidone : S P E T Findings. Eur. J. Nucl. Med., 1997, 244(8) : 891.