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Radiochemical Labelling of the Dopamine D_3 Receptor Ligand RGH-1756

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Summary

The dopamine D_3 receptor is expressed in low density in limbic brain areas. The receptor subtype has been proposed as a target for novel antipsychotic drugs, however no selective positron emission tomography (PET) ligand is to date available to study the receptor distribution *in vivo*. 1-(2-Methoxyphenyl)-4-{4-[4-(6-imidazo[2,1-b]-thiazolyl)phenoxy]butyl}piperazine (RGH-1756) is a new methoxyphenylpiperazine derivative that possesses high affinity (K_i: 0.119 nM) and good selectivity for the human dopamine D_3 receptor. In an attempt to develop a PET radioligand for the visualisation of the dopamine D_3 receptor in human brain we synthesised carbon-11-labelled RGH-1756. The radiolabelling was performed by reaction of the corresponding desmethyl precursor 1-(2-hydroxyphenyl)-4-{4-[4-(6-imidazo[2,1-b]-thiazolyl)phenoxy]butyl}piperazine (04512626) with [^{11}C]methyl triflate in acetone and aqueous NaOH. The HPLC-determined incorporation yield was >90%. The total synthesis time was 35 min and the specific radioactivity at end of synthesis ranged from 66 to 132 GBq/μmol.

Key words: [^{11}C]RGH-1756, dopamine D_3 receptor, positron emission tomography

Abbreviated title: Radiochemical labelling of RGH-1756

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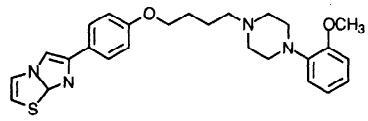
Introduction

The dopamine D_3 receptor (D_3R) has been shown to be localised primarily in limbic regions, such as the nucleus accumbens and the islands of Calleja, in rodent and human brain (1-4). The D_3R has been proposed as a target for novel and atypical antipsychotic drugs. The hypothesis is based on the common affinity of antipsychotics to the D_3R, the limbic distribution of the D_3R in brain and its proposed functional role as an autoreceptor regulating the synthesis and release of dopamine (for a recent review see: 5).

The aminotetralin derivatives [^{3}H](±)7-OH-DPAT and [^{125}I]S(-)5-OH-PIPAT as well as (+)-*S*-[^{3}H]PD 128907 have been used as radioligands in autoradiographic studies of the D_3R in animal and human brain (2-4,6-7). However, the D_3R coexists with the D_2R in various brain areas and these agonists also have affinity for the D_2R. An acceptable D_3/D_2 selectivity can be only achieved at *in vitro* assay conditions that disfavour agonist binding to the D_2R by forcing the D_2R into its agonist low-affinity state, such as addition of guanine nucleotides and exclusion of Mg^{2+}. Moreover, the D_3R concentration is comparatively low in the human brain (1-2 orders of magnitude lower than the D_2R) (4). A highly selective and potent radioligand is therefore required for *in vivo* imaging with single photon emission computed tomography (SPECT) or positron emission tomography (PET). Although some attempts have been made to develop such a D_3R ligand (8-10; for a review see: 11), an ideal tracer remains to be identified.

Recently, a series of methoxyphenylpiperazine derivatives has been synthesised at Gedeon Richter Ltd., Budapest (12,13) with the aim to develop new atypical antipsychotics. From this series emerged 1-(2-methoxyphenyl)-4-{4-[4-(6-imidazo-[2,1b]thiazolyl)phenoxy]butyl}piperazine (RGH-1756, Fig.1) as a promising candidate with high affinity for the cloned human D_3R (hD_3R) (K_i: 0.119 nM, determined against [^{3}H]spiperone) and about 100-fold selectivity for the hD_3R *versus* the hD_2R (K_i: 12.2 nM for the human $D_{2L}R$) (14). Given the high affinity to the hD_3R and the excellent D_3/D_2 selectivity RGH-1756 was selected for development as a PET radioligand for visualisation of the D_3R in the human brain.



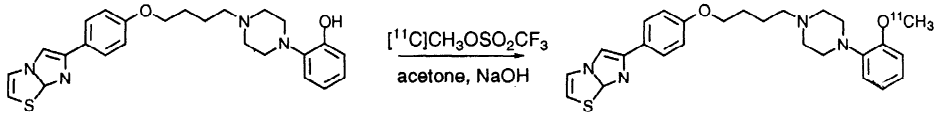
RGH-1756

Figure 1: Chemical structure of 1-(2-methoxyphenyl)-4-{4-[4-(6-imidazo[2,1-b]thiazolyl)phenoxy]butyl}piperazine (RGH-1756).

In this work, we report the carbon-11 radiolabelling of RGH-1756 by *O*-methylation of the corresponding desmethyl precursor 1-(2-hydroxyphenyl)-4-{4-[4-(6-imidazo-[2,1-b]thiazolyl)phenoxy]butyl}piperazine (04512626) using [^{11}C]methyl triflate.

Results and Discussion

The arylpiperazine derivative RGH-1756 (Fig. 1) possesses an aromatic methoxy substituent, which gives easy access to ^{11}C-labelling by *O*-methylation of the corresponding desmethyl compound with [^{11}C]methyl iodide ([^{11}C]MI) or [^{11}C]methyl triflate ([^{11}C]MT). We have previously used [^{11}C]MT as an efficient alternative to [^{11}C]MI in the preparation of various already established PET tracers (15-16). The radiolabelling of [^{11}C]RGH-1756 was performed employing the optimised reaction conditions for methylation of phenols with [^{11}C]MT, as recently described for the high-affinity D_2R ligand [^{11}C]FLB 457 (15). Reaction of the desmethyl precursor 04512626 with [^{11}C]MT in acetone and aqueous NaOH (1.8 eq) (Fig. 2) afforded [^{11}C]RGH-1756 in an incorporation yield of >90% (based on the HPLC chromatogram, Fig. 3).



04512626 [^{11}C]RGH-1756

Figure 2: Preparation of [^{11}C]RGH-1756 using [^{11}C]methyl triflate.

No radioactive by-products were observed (Fig. 3). Purification by semipreparative reverse-phase HPLC gave radiochemically and chemically pure (>99%) [^{11}C]RGH-1756, coeluting with unlabelled RGH-1756. The retention time of [^{11}C]RGH-1756 on the analytical reverse-phase HPLC system was 3.5-4.0 min. The entire synthetic procedure was automated in a recently developed methylation system (17) and the synthesis was completed within 35 min including HPLC purification and formulation of the radiotracer.

The automated system employed in the synthesis of [^{11}C]RGH-1756 relies upon the production of [^{11}C]MI by catalytic gas-phase iodination of [^{11}C]CH_4 (18). [^{11}C]MI was subsequently converted on-line into [^{11}C]MT by a gas-solid-phase reaction (19). This novel approach results, as previously discussed, in an improved specific radioactivity (SR) of the produced radioligand as compared to standard methods (17). This improvement is attributed to the avoidance of reagents considered as sources of carrier carbon, such as lithium aluminium hydride in tetrahydrofuran (17,19). A sufficiently high SR is a prerequisite for high-affinity radioligands used for

examination of low-density receptor populations. Using the automated methylation system the SR of [^{11}C]RGH-1756 at end of synthesis was about 66-132 GBq/μmol which is comparable to the values achieved in our laboratory with [^{11}C]FLB 457 (17), a radioligand suitable for the visualisation of low-density extrastriatal D_2R proteins (20).

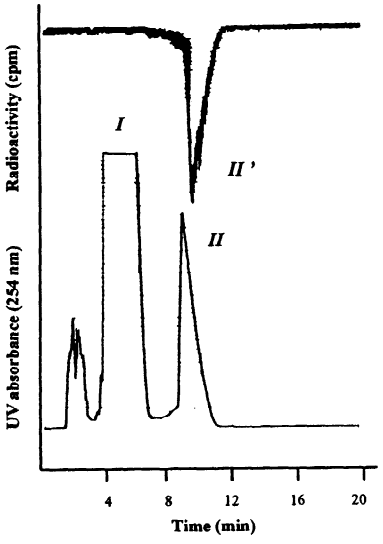


Figure 3: Semipreparative HPLC chromatogram from the purification of [^{11}C]RGH-1756. *I*: desmethyl precursor (04512626); *II*: RGH-1756; *II'*: [^{11}C]RGH-1756.

Experimental

General

Derivative RGH-1756 and the corresponding desmethyl precursor for carbon-11 labelling 04512626 were synthesised at Gedeon Richter Ltd., Budapest. Silver trifluoromethanesulfonate (silver triflate) and Graphpac GC (80–100 mesh) were obtained from Sigma-Aldrich Sweden AB and Alltech, respectively. Other chemicals were obtained from standard commercial sources and were of analytical grade. Silver-triflate-impregnated graphitised carbon was prepared according to a previously described method (19). [^{11}C]CO_2 was produced using a Scanditronix MC 16 cyclotron at the Karolinska Hospital/Institute by bombardment of a nitrogen gas target with 16 MeV protons in the ^{14}N(p,α)^{11}C reaction. The target gas was irradiated for 40 minutes with a beam intensity of 40 μA. The synthesis and purification of [^{11}C]RGH-1756 was performed in a fully automated methylation system that has been described in detail elsewhere (17). The system contains a GEMS MeI PETtrace MicroLab as an integrated part. [^{11}C]MI was prepared from [^{11}C]CO_2 *via* [^{11}C]CH_4 by

catalytic gas-phase iodination. Sweeping the [^{11}C]MI vapour through a glass column, heated at 150-200°C and containing silver-triflate-impregnated graphitised carbon, produced [^{11}C]MT (19). Purification of [^{11}C]RGH-1756 was performed in a built-in HPLC system. This system consists of a Gilson 234 Autoinjector, a Gilson 304 piston pump, a Waters μBondapak C18 column (300 x 7.8 mm, 10 μm), and a GILSON 118 UV/VIS detector (wavelength: 254 nm) in series with a Geiger Müller (GM) tube for radiation detection. The column was eluted with a mixture of 0.01 M aqueous H_3PO_4 and CH_3CN (80/20) and a flow rate of 6 mL/min. The radiochemical purity of [^{11}C]RGH-1756 was analysed by reverse-phase HPLC using a Waters μBondapak C18 column (300 x 3.9 mm, 10 μm) and an UV detector (wavelength: 254 nm) in series with a Beckman β-flow radiodetector. The column was eluted with a mixture of 0.01 M aqueous H_3PO_4 and CH_3CN (75/25) at a flow rate of 3 mL/min.

Preparation of [^{11}C]RGH-1756

The [^{11}C]MT was trapped at room temperature in a reaction vessel containing the desmethyl precursor 04512626 (0.5 mg, 1.1 μmol), acetone (350 μL) and 0.5 M NaOH (4 μL, 1.8 eq). Mobile phase was added and [^{11}C]RGH-1756 was purified by semipreparative reverse-phase HPLC. The mobile phase was removed continuously on-line by a vaporiser and the product formulated in sterile phosphate-buffered saline (pH=7.4, 7.0 mL). Filtration through a Millipore filter (0.22 μm) gave a solution that was sterile and free of pyrogens.

Conclusion

The novel D_3R ligand RGH-1756 was radiolabelled with [^{11}C]MT. The incorporation yield was >90% and the specific radioactivity at end of synthesis ranged from 66 to 132 GBq/μmol. Given a high affinity to the human D_3R (K_i: 0.119 nM) in combination with a 100-fold D_3/D_2 selectivity, [^{11}C]RGH-1756 appears to be a promising radioligand for the visualisation of the D_3R with PET. A detailed PET characterisation of [^{11}C]RGH-1756 binding in primate brain is currently underway and the results will be presented elsewhere.

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