SYNTHESIS OF [METHOXY-^{3}H]- and [METHOXY-^{11}C]- LABELLED RACLOPRIDE. SPECIFIC DOPAMINE-D_2 RECEPTOR LIGANDS.

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SUMMARY

Radioactive labelled raclopride and its (R)-isomer were prepared by O-alkylation of the corresponding phenols with labelled methyl iodide. The syntheses of the two phenolic precursors from (R)- and (S)-2-aminomethyl-1-ethylpyrrolidine are described. Resolution of the racemic amine into the enantiomers was accomplished by fractional crystallization of the ditartrates. Coupling of the amines with 3,5-dichloro-2,6-dimethoxybenzoyl chloride, followed by dealkylation, led to the diphenolic precursors. Mono-alkylation of the corresponding precursor with [^{3}H]methyl iodide furnished the potent dopamine-D_2 ligand [^{3}H]raclopride and its (R)-isomer. [^{11}C]Methyl iodide was prepared from [^{11}C]carbon dioxide. Subsequent reaction with phenolic (S)-precursor furnished [^{11}C]raclopride which has been used in positron emission tomography (PET) studies of the human brain.

Key-words: Raclopride, Tritium labelling, Carbon-11 labelling, Dopamine-D_2 ligands

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INTRODUCTION

A possible mode of action for antipsychotic agents relates to their ability to block dopamine-D_2 receptors, which can be uncoupled to or negatively coupled to adenylate cyclase (1,2). Most antipsychotics of butyrophenone and phenothiazine type interact with other receptors in the brain as well (3,4). Recently, the interest has been focused on a new class of atypical antipsychotic compounds, the substituted benzamides, which preferentially block the dopamine-D_2 receptors (5-8).

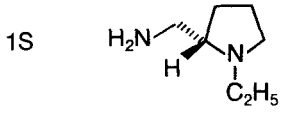
The studies of interactions of antipsychotics with dopamine-D_2 receptors rely, to a large extent, on the availability of radiolabelled ligands. Several such compounds have been used in the characterization of the dopamine-D_2 receptors (9-11). However, many of these ligands have inherent problems associated with insufficient selectivity (e.g. [^{3}H]spiperone) or poor penetration into the brain (e.g. [^{3}H]sulpiride and [^{3}H]domperidone)(10,12,13). Two recently developed ligands of the substituted benzamide class, [^{3}H]eticlopride and [^{3}H]raclopride, overcome these drawbacks. Both ligands are highly selective and potent and they enter the brain readily. [^{3}H]Eticlopride and [^{3}H]raclopride have thus proved to be useful tools in both _in vitro_ and _in vivo_ investigations of central dopamine-D_2 receptors (14-18). Furthermore, [^{11}C]raclopride has been used in visualization and quantitative analysis of dopamine-D_2 receptors in the living human brain by positron emission tomography (PET)(19,20).

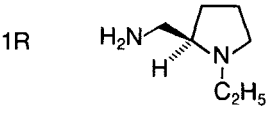
In the present paper the syntheses of the (S)- and (R)-dihydroxy precursors 4S and 4R and their reactions with [^{3}H]methyl iodide and [^{11}C]methyl iodide to produce labelled raclopride and its inactive (R)-isomer are described.

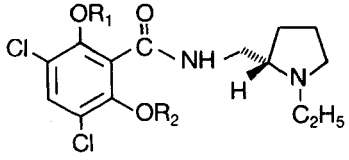
RESULTS AND DISCUSSION

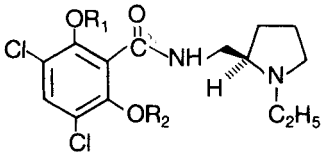
[^{3}H]Raclopride has been made with high specific activity by tritiation of the pyrrolidine moiety followed by coupling with the corresponding acid chloride (21). The advantage in this method lies in the option to alter the benzamide moiety readily. However, the reaction is hampered by a rather cumbersome synthesis of the pyrrolidine precursor. For the present purpose, this obstacle makes it desirable to have a more direct synthesis via an already coupled precursor. Such a technique has been utilized in the syntesis of [^{11}C]raclopride. Because of the short half-life (20.4 min) of carbon-11, the syntheses of ^{11}C-compounds

require a facile incorporation of carbon-11 at a late stage in the synthesis. Accordingly, [^{11}C]raclopride has been obtained either by N-alkylation with [1-^{11}C]ethyl iodide or by O-alkylation with [^{11}C]methyl iodide (19,22). Here, we detail the latter O-alkylation procedure. This reaction is obviously also suitable for the synthesis of ^{3}H-labelled raclopride. Furthermore, this approach leads to a flexible synthesis which enables us to make the corresponding ^{3}H- or ^{11}C-labelled (R)-isomers from the corresponding (R)-precursor.









(S)	R_1	R_2	(R)
2S	CH_3	CH_3	2R
raclopride, 3S	H	CH_3	3R
4S	H	H	4R
[^{3}H]raclopride	H	$C^{3}H_3$	(R)-[^{3}H]raclopride
[^{11}C]raclopride	H	$^{11}CH_3$	(R)-[^{11}C]raclopride

In the receptor binding experiments it is of utmost importance to have enantiomerically pure compounds. This is especially important for work involving the inactive (R)-isomer in order to exclude any interference from the potent (S)-isomer, i.e. raclopride. We resolved the racemic amine 1 by fractional crystallization of the ditartrates. The enantiomeric purity was found to be >99.9% by gas chromatographic analysis of diastereomeric O-methylmandelamides. Direct coupling of the resolved amines 1 with various activated derivatives of the diphenolic acid gave no satisfactory yields of the precursors 4. Thus, these diphenolic amides 4 were obtained by demethylation of the dimethoxybenzamides 2. The chiral amides 2 were obtained by reaction of 3,5-dichloro-2,6-dimethoxybenzoyl chloride with the resolved amines 1.

The demethylation can be made directly by the reaction of 2 with hydrobromic acid in acetic acid in a yield of 45%. Alternatively, the

diphenol 4 can be obtained in two steps, i.e. first monodemethylation of 2 with boron tribromide in dichloromethane followed by the reaction of the salicylamide 3 with hydrobromic acid in an overall yield of over 80%.

The conditions for the methylation of diphenol 4 with [^{3}H]methyl iodide were elucidated from experiments with unlabelled methyl iodide. When the hydrobromide 4 in dimethyl sulfoxide was treated with solid potassium hydroxide or 5 M aqueous sodium hydroxide, varying between 2-9 equivalents, and reacted with 1.2 or 2.4 equivalents of methyl iodide, product 3 was formed in yields up to 40%. An excess of the base was found to be necessary since the anion formed by the reaction between hydrobromide 4 and two equivalents of potassium hydride did not produce any alkylation product 3 when treated with methyl iodide. The alkylations were run at temperatures between 0° and 80°C. Radiosynthesis under optimum conditions with [^{3}H]methyl iodide gave labelled 3 in radiochemical yields of 10-20%, i.e. lower than the yields obtained in the cold runs. This discrepancy might be due to the fact that the syntheses with unlabelled material were run in dimethyl sulfoxide under homogenous conditions, while the radiosyntheses were performed in a mixture containing the toluene from the supplied [^{3}H]methyl iodide solution.

Concentration of the reaction mixture and isolation by HPLC furnished the radiochemically pure [methoxy-^{3}H]-labelled derivatives [^{3}H]raclopride and (R)-[^{3}H]raclopride, in radiochemical yields of 14% and 20%, respectively. The specific activity of the isomers was 74 Ci/mmol.

The previously reported synthesis of [^{11}C]raclopride used [1-^{11}C]ethyl iodide as the labelling reagent (22). The Grignard reagent used in the preparation of [1-^{11}C]ethyl iodide had to be prepared once a week in order to ascertain high, reproducible yields of the labelling reagent. On the other hand, the synthesis of the alternative reagent [^{11}C]methyl iodide is more suitable for a frequent production of labelled material (24). Accordingly, [^{11}C]methyl iodide was used in the O-alkylation of the hydrobromide 4 under similar conditions as the tritium labelling above. The O-alkylation procedure has further advantages in comparison to the N-alkylation with [1-^{11}C]ethyl iodide, i.e. a 5 to 10 minutes shorter time of preparation from the end of bombardment and a circa 10% higher yield. The specific activity was in the range of 200-500 Ci/mmol.

EXPERIMENTAL

Melting points were obtained on a Mettler FP 61 apparatus and are uncorrected. Optical rotations were measured on an Optical Activity AA-100 polarimeter. ^1H NMR spectra were obtained on a Varian EM 360A or a JEOL FX 200 spectrometer using Me_4Si as internal standard. Mass spectra (EI, 70eV) were recorded on an LKB 2091 mass spectrometer. GLC's were run on an SE 30 capillary column and the amounts determined by a Hewlett-Packard 3390 A integrator assuming identical response factors. Elemental analysis were performed by Analytische Laboratorien, Elbach, W.Germany. [^3H]Methyl iodide (specific activity 85 Ci/mmol) was purchased from Amersham International plc, Amersham, Bucks, England. Radiochemical purity was determined from TLC using a Berthold LB 283 TLC Linear Analyzer. TLC analyses were done on silica gel 60 F_{254} (Merck) glass plates developed in $CHCl_3/CH_3OH$/conc. NH_3 (9:1:0.05). Radioactivity was determined in a Packard Tri-Carb 460 C liquid scintillation spectrometer using Biofluor (New England Nuclear) as the counting medium.

Resolution of 2-aminomethyl-1-ethylpyrrolidine (1)

The (S)-isomer 1S was obtained by fractional crystallization of the di-(-)-tartaric acid salt of racemic amine from aqueous ethanol and 1R analogously as the di-(+)-tartrate. The recrystallization process was continued until the optical purity was >99.9%. The optical purity of the isomers was determined by derivatization of ca 1 mg amine ditartrate in 1 ml 1 M NaOH and 1 ml CH_2Cl_2 by addition of 0.7 ml 12 mM (-)-(R)-O-methylmandeloyl chloride in CH_2Cl_2. After vigorous shaking the organic layer was separated, washed with water and dried over Na_2SO_4. The diastereomeric amides were analyzed on a capillary GLC (SE 30, 20 m, 175°C). The retention times were 7.90 (R) and 7.44 (S) minutes, respectively.

(R)-3,5-Dichloro-2,6-dimethoxy-N-[(1-ethyl-2-pyrrolidinyl)methyl]-benzamide (2R)

A mixture of 3,5-dichloro-2,6-dimethoxybenzoic acid (23) (3.0 g, 12 mmol), thionyl chloride (2 ml) and dimethylformamide (3 drops) in 20 ml toluene was heated at 60°C for 1 h. The solvent was removed in vacuo and toluene was added again and removed. This procedure was repeated once with CH_2Cl_2 and finally the residue of acid chloride was dissolved in 10 ml CH_2Cl_2. The amine base of (R)-2-(aminomethyl)-1-ethylpyrrolidine (+)-ditartrate (5.1 g, 12 mmol) was liberated by partitioning between CH_2Cl_2 and 2 M NaOH. The organic layer containing the amine was dried over Na_2SO_4 and added to the solution of acid chloride. The

reaction mixture was stirred overnight at room temperature. After basifying with 50 ml 1 M NaOH the mixture was extracted twice with CH_2Cl_2. Drying (Na_2SO_4) and evaporation afforded 3.91 g (91%) of the title compound. An analytical sample was recrystallized from iPr_2O, mp 146-147°C. $[\alpha]_D^{22}$= +71° (c=1.3, $CHCl_3$). Anal. Calcd for $C_{16}H_{22}Cl_2N_2O_3$: C, 53.19; H, 6.14; Cl, 19.67; N, 7.75. Found: C, 53.18; H, 6.10; Cl, 19.51; N, 7.73.

(R)-3,5-Dichloro-2,6-dihydroxy-N-[(1-ethyl-2-pyrrolidinyl)methyl]-benzamide hydrobromide (4R)

The benzamide 2R (2.8 g, 8 mmol) was heated at 60°C in 10 ml 33% HBr in HOAc for 4 h. After cooling toluene was added and the solvent was evaporated in vacuo. The residue was recrystallized twice from EtOH/iPr_2O to give 1.5 g (45%) of the title compound, mp 209-210°C. $[\alpha]_D^{22}$ = -10.8° (c=1.5, EtOH). Anal. Calcd for $C_{14}H_{18}Cl_2N_2O_3 \cdot HBr$: C, 40.60; H, 4.62; N, 6.76. Found: C, 40.56; H, 4.71; N, 6.78.

(S)-3,5-Dichloro-N-[(1-ethyl-2-pyrrolidinyl)methyl]-6-methoxy-salicylamide (raclopride, 3S)

Following the recently described procedure (8), 3,5-dichloro-2,6-dimethoxybenzoyl chloride was reacted with (S)-2-(aminomethyl)-1-ethylpyrrolidine (1S) in CH_2Cl_2 to give the benzamide 2S, which was monodemethylated with BBr_3 in the presence of HCl in CH_2Cl_2 to give raclopride (3S). $[\alpha]_D^{25}$ = -64° (c=1.3, $CHCl_3$).

(S)-3,5-Dichloro-2,6-dihydroxy-N-[(1-ethyl-2-pyrrolidinyl)methyl]-benzamide hydrobromide (4S)

Raclopride (3S, 11.3 g, 0.032 mol) was heated at 60°C in 18 ml 33% HBr in HOAc. A solid precipitated after 10 min and the reaction mixture was diluted with 50 ml HOAc and the heating was continued for 4 h. The solvent was removed in vacuo and the residue was recrystallized from EtOH/iPr_2O to give 11.3 g (84%) of the title compound, mp 212-213°C. $[\alpha]_D^{22}$ = +11.4° (c=1.5, EtOH). Anal. Calcd for $C_{14}H_{18}Cl_2N_2O_3 \cdot HBr$: C, 40.60; H, 4.62; N, 6.76; O, 11.59. Found: C, 40.57; H, 4.66; N, 6.78; O, 11.75.

(S)-3,5-Dichloro-N-[(1-ethyl-2-pyrrolidinyl)methyl]-6-[^{3}H]methoxy-salicylamide, [^{3}H]raclopride

To a solution of 4S (8.7 mg, 21 μmol) in 400 μl DMSO was added 30 μl of 5 M NaOH at room temperature. After stirring for 20 min, 200 μl of the solution was added to 10 mCi of C^3H_3I in 1 ml of toluene. The reaction

mixture was vigorously stirred at 65-70°C for 12 min. After cooling, the toluene was evaporated in a stream of nitrogen gas and the residue was acidified with HCl-methanol. Evaporation of the methanol left a residue from which 1.4 mCi of [^{3}H]raclopride was isolated by HPLC on a Nucleosil C-18, 5μ column eluted with 25% CH_3CN in NaH_2PO_4-buffer (pH2) containing 1 mM dimethylnonylamine.

(R)-3,5-Dichloro-N-[(1-ethyl-2-pyrrolidinyl)methyl]-6-[^{3}H]methoxy-salicylamide, (R)-[^{3}H]raclopride

Following the method described above, 4R (8.5 mg, 20 μmol) was reacted with 10 mCi of C^3H_3I in toluene and isolated by HPLC to furnish 2.0 mCi of (R)-[^{3}H]raclopride.

The specific activity of the two tritiated isomers was 74 Ci/mmol as measured by quantitative HPLC analysis. Radioactivity scanning of thin layer chromatograms of the compounds showed only one peak of radioactivity co-chromatographing with an unlabelled reference.

(S)-3,5-Dichloro-N-[(1-ethyl-2-pyrrolidinyl)methyl]-6-[^{11}C]methoxy-salicylamide, [^{11}C]raclopride

Radionuclide production. [^{11}C]carbon dioxide was produced by the $^{14}N(p, \alpha)^{11}C$ nuclear reaction at Karolinska Hospital using a Scanditronix RNP 16 cyclotron, and trapped at a high flow rate (ca 150 ml/min) in a stainless steel coil cooled in liquid nitrogen.

Preparation of [^{11}C]methyl iodide (24). The stainless steel coil containing the [^{11}C]carbon dioxide was brought to room temperature. The $^{11}CO_2$ was transferred by a slow flow of nitrogen (ca 2 ml/min) to a cooled reaction vessel (-78°C) containing $LiAlH_4$ (10 μmol) in 500 μl tetrahydrofuran. The solvent was evaporated under vacuum (oil bath, 130°C) and the reaction vessel was cooled again (-78°C) and hydriodic acid (1 ml, 54%) was added. The reaction vessel was again heated in the oil bath (130°C) and the generated [^{11}C]methyl iodide was distilled with a slow flow of nitrogen (ca 2 ml/min) through traps of soda lime and phosphorus pentoxide to eliminate all traces of hydriodic acid and water.

Preparation of [^{11}C]raclopride. The [^{11}C]methyl iodide produced was trapped in a solution of (S)-3,5-dichloro-2,6-dihydroxy-N-[(1-ethyl-2--pyrrolidinyl)methyl]benzamide hydrobromide (4S, 1 mg, 2.4 μmol) and 10 μl 5 M NaOH in 500 μl DMSO in a test tube at ambient temperature. The tube was then sealed and heated at 85°C for 15 min. The solution was magnetically stirred throughout the reaction. After the reaction

the solution was diluted with 400 µl of mobile phase (acetonitrile : 0.01 M phosphoric acid, 27:73) and injected into a µBondapak C-18 column (300x7.8 mm, Waters) which was eluted at 5 ml/min with the same mobile phase. UV-absorbance (251 nm) and radioactivity were monitored simultaneously and [^{11}C]raclopride, which separated from other labelled and non-labelled compounds, was collected between 13.5-15 min. This solution was evaporated to dryness in a rotary evaporator and the residue dissolved in 10 ml sterile saline and sterilized by filtration through a Millipore filter, 0.22 µm. The time of synthesis was 50 min with an overall radiochemical yield of 40-50% (decay-corrected from [^{11}C]carbon dioxide). The specific activity varied between 200-500 Ci/mmol.

The [^{11}C]raclopride had the same capacity factor in the HPLC as a reference standard of authentic material. For further analysis of the obtained product, the synthesis was performed under the same conditions as described above using non-labelled methyl iodide. The IR-spectrum of the obtained product was identical to that of the reference standard.

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