JOURNAL OF LABELLED COMPOUNDS AND RADIOPHARMACEUTICALS
J Label Compd Radiopharm 2003; **46**: 873–882.
Published online in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/jlcr.726

Research Article

Synthesis of the serotonin transporter ligand (±)-10-methyl 3-[6-nitro-(2-quinolinyl)]-3,10-diazabicyclo-[4.3.1]-decane ([^{11}C-methyl]NS 2495) and first *in vivo* results

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Summary

The serotonin transporter ligand (±)-10-[^{11}C]-methyl 3-[6-nitro-(2-quinolinyl)]-3,10-diazabicyclo-[4.3.1]-decane ([^{11}C-methyl]NS 2495) was synthesized *via* a methylation reaction with [^{11}C]methyl iodide. The radiochemical purity exceeded 99% and the specific radioactivity was found to be 1.8 GBq/μmol at 40 min after the end of bombardment. The uptake of the tracer in the brain of a living pig was recorded by positron emission tomography (PET), first in a baseline condition, and again after treatment with citalopram (1 mg/kg, i.v.) to displace the specific binding. The distribution volume relative to the metabolism-corrected arterial input was high in pig brain, ranging from 75–150 ml g^{-1}; treatment with citalopram uniformly reduced the distribution volume to 75 ml g^{-1}. Binding potential (*pB*) maps generated using the cerebellum as a reference tissue showed highest binding in the mesencephalon and cingulate cortex, where the magnitude of *pB* was close to 0.6. Thus, the pattern of binding *in vivo* agrees with the known pattern of serotonin

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Contract/grant sponsor: GlaxoSmithKline

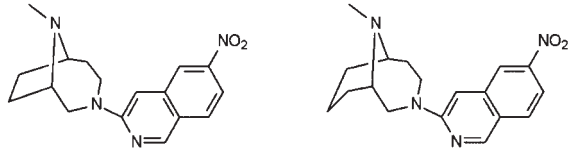
Received 14 March 2003
Accepted 28 April 2003

innervations in pig brain. However, the specific binding was incompletely displaced by pre-treatment with citalopram. Thus, [^{11}C-methyl]NS 2495 can label serotonin transporters in a PET study of the brain of a living pig, but full displacement by cold citalopram was not obtained *in vivo*, possibly reflecting binding sites which are inaccessible to citalopram.

Key Words: PET; radiolabelling; ^{11}C-methylation; selective serotonin reuptake inhibitor

Introduction

Serotoninergic mechanisms in human brain are implicated in impulse control and in the pathophysiology of diverse neuropsychiatric disorders.[1,2] The plasma membrane serotonin transporters are the site of action of the serotonin selective reuptake inhibitors (SSRIs) used in the treatment of anxiety, depression, obsessive-compulsive disorder and other disorders of impulse control.[3] However, little is known about alterations in serotoninergic innervations in human disease. Consequently, there is considerable impetus for obtaining optimal methods for the detection of serotonin transporters in positron emission tomography (PET) studies of living human brain. Diverse compounds have been tested as potential *in vivo* tracers for serotonin transporters.[4] As part of a search for improved agents for the detection of serotonin transporters by PET, we have reported on the binding properties in brain of living pigs of two azabicyclooctenes (Figure 1), a novel class of serotonin uptake inhibitors, and have recently radiolabelled the diazabicyclononane NS 4194.[5,6] We have identified the diazabicyclodecane NS 2495 as a serotonin uptake inhibitor with very high affinity and selectivity for serotonin transporters. From the uptake studies, it was found that NS 2495 had more than 3700-fold selectivity for blocking the



NS 4194 **NS 2495**

Figure 1. Structures of two potential selective serotonin reuptake inhibitors (NS 4194 and NS 2495)

synaptosomal uptake [^{3}H]serotonin (IC_{50} 3.2 ± 0.70 nM) than for [^{3}H]noradrenaline (IC_{50} 2.0 ± 0.25 μM) or [^{3}H]dopamine (IC_{50} 12.0 ± 3.1 μM). In the present study we report on the synthesis of [^{11}C-methyl]NS 2495, and describe the results of a PET study of the tracer's binding in living porcine brain.

Experimental

Biochemical procedures in vitro

The studies were performed in accordance with Danish Animal Experimentation Act on a licence granted by the Danish Ministry of Justice. Studies of the uptakes of tritiated serotonin, dopamine and noradrenaline were carried out *in vitro* using synaptosomes prepared from brain of male Wistar rats (150–200 g). Samples of cerebral corticies were used for serotonin uptake, samples of corpus striata were used for dopamine uptake, and samples of hippocampi were used for noradrenaline uptake. Preparation of synaptosomes and incubation of samples for ten minutes in the presence of 1 mM pargyline (Sigma Chemicals) and [^{3}H]serotonin (1 nM), [^{3}H]dopamine (1 nM) or [^{3}H]noradrenaline (1 nM) were carried out according to conventional procedures.[7,8]

All tritiated radiopharmaceuticals were from Amersham Pharmacia (Little Chalfont, UK). Samples were co-incubated with NS 4194 at concentrations ranging from 0.01 to 30 μM for noradrenaline and dopamine uptake, and 0.0001 to 1 μM for serotonin uptake. After completion of the incubations, samples were poured directly onto Whatman GF/C glass fibre filters under reduced pressure. The filters were washed three times with 5 ml of ice-cold 0.9% (w/v) NaCl solution, and the concentration of radioactivity retained on the filters was determined by conventional liquid scintillation counting. Specific uptake was calculated as the difference between total uptake and uptake measured in the presence of a selective uptake inhibitor (citalopram, Lundbeck Pharmaceuticals; benztropine, RBI; or desipramine, Sigma Chemicals for inhibition of serotonin, dopamine or noradrenaline uptake respectively) at a final concentration of 1 μM.

General methods

The NMR spectra were recorded on a Bruker AM 500 MHz spectrometer using $(CD_3)_2SO$ as the solvent and the chemical shifts were

referenced to tetramethylsilane. The mass spectra were obtained on a JEOL JMS AX-505W double focusing mass spectrometer. The melting points were determined with a Griffin melting point apparatus. [^{11}C]Carbon dioxide was produced by the $^{14}N(p,\alpha)^{11}C$ nuclear reaction using a nitrogen gas target pressurized to 150 psi and bombarded with 16 MeV protons produced by the General Electric Medical Systems PETtrace 200 cyclotron at Aarhus University Hospital. Irradiation for 60 min with a beam current of 40 μA was typically used.

The labelling procedure was performed using a fully automated system, including the preparation of [^{11}C]methyl iodide, the methylation step, the HPLC purification, the rotary evaporation and the formulation of the labelled product.[9]

Preparative HPLC was performed using an isocratic pump (Perkin Elmer model 200) equipped with 1 ml injection loop and connected in series with a Phenomenex Nucleosil 5CN column, 250 × 10 mm, a variable wavelength UV detector (Applied Biosystems model 759A, $\lambda = 232$ nm), and a photodiode radiodetector of in-house design. Analytical HPLC was performed using a Perkin Elmer model 250 pump with a 20 μl injection loop connected in series with a Phenomenex Luna 5uCN column (250 × 4.6 mm, 5 μm), a variable wavelength detector (Perkin Elmer model LC 295, $\lambda = 232$ nm), and a sodium iodide radiodetector of in-house design.

Materials

Solvents were HPLC grade and were purchased from Aldrich or Merck. Labelling reactions were performed using anhydrous Sure/Seal™ solvents from Aldrich or Fluka. Lithium aluminium hydride (LAH) was obtained from Fluka. 10-Methyl-3,10-diazabicyclo-[4.3.1]-decane was prepared according to published procedure from pseudo-pelletierine obtained from ABCR.[10] 2-Chloro-6-nitroquinoline was prepared according to published procedure.[11]

Production of [^{11}C]methyl iodide. [^{11}C]Carbon dioxide was purged from the target in a stream of nitrogen gas and trapped on 4 Å molecular sieves. The [^{11}C]CO_2 was released upon heating (ca.250°C) and was passed through a solution of LAH (300 μl) in a stream of nitrogen gas (25 ml/mn). On completion of [^{11}C]CO_2 transfer, the THF was evaporated and 1 ml of hydriodic acid was added. [^{11}C]Methyl

iodide was then transferred in a stream of nitrogen gas (20 ml/mn) to a solution of the desmethyl-precursor.

*(±)-10-Methyl 3-[6-nitro-(2-quinolinyl)]-3,10-diazabicyclo-[4.3.1]-decane (**NS 2495**).* A mixture of 2-chloro-6-nitroquinoline (9.0 g, 43 mmol), 10-methyl-3,10-diazabicyclo-[4.3.1]-decane (6.6 g, 43 mmol) and 1,2-diethoxyethane (150 ml) was heated at reflux for 5 h. Aqueous sodium hydroxide (100 ml, 1 M) was added. The aqueous phase was extracted with methylene chloride (2 × 200 ml). Chromatography on silica gel with dichloromethane, methanol and concentrated ammonia (89:10:1) gave the title compound. Yield 4.0 g (28%). m.p. 110–116°C. R_f = 0.41 (silica gel TLC using dichloromethane, methanol and concentrated ammonia (89:10:1) as eluent). ^{1}H-NMR: ($(CD_3)_2SO$) δ 8.70 (d, $J = 3, 1H$), 8.23 (d, $J = 9, 1H$), 8.20 (dd, $J = 9/3, 1H$), 7.55 (d, $J = 9, 1H$), 7.23 (d, broad, 1H), 4.55–4.45 (m, 1H), 3.97–3.72 (m, broad, 2H), 3.61(m, 1H), 3.34 (s, 3H), 3.05 (m, 1H), 2.97(m, 1H), 2.15 (m, 1H), 1.90 (m, 1H), 1.79 (m, 2H), 1.41(m, 2H), 1.27 (m, 1H), 1.15(m, 1H). MS (EI): $m/e = 326$.

*(±)-10-H-3-[6-nitro-(2-quinolinyl)]-3,10-diazabicyclo-[4.3.1]-decane fumaric acid salt (**NS 7113**).* 10-Methyl 3-[6-nitro-(2-quinolinyl)]-3,10-diazabicyclo-[4.3.1]-decane (2.6 g, 8.0 mmol), *N*-iodosuccinimide (5.4 g, 24 mmol) and acetonitrile (50 ml) was stirred at room temperature for 15 h. Aqueous sodium hydroxide (100 ml, 1 M) was added. The aqueous phase was extracted with methylene chloride (3 × 100 ml). Chromatography on silica gel with dichloromethane, methanol and concentrated ammonia (89:10:1) gave the title compound. Yield 0.25 g (8%). The corresponding salt was obtained by addition of a diethyl ether and methanol mixture (9:1) saturated with fumaric acid. M.p. 261 –263°C. R_f = 0.27 (silica gel TLC using dichloromethane, methanol and concentrated ammonia (89:10:1) as eluent). ^{1}H-NMR: ($(CD_3)_2SO$) δ 8.73 (d, $J = 3$, 1H), 8.27 (d, $J = 9, 1H$), 8.23 (dd, $J = 9/3, 1H$), 7.57 (d, $J = 9, 1H$), 7.32 (d, broad, $J = 9, 1H$), 6.45 (s, 2H, fumaric acid), 4.36 (m, 2H), 3.87 (s, broad, 1H), 3.70 (m, 1H), 3.40 (m, 2H), 2.52 (m, 2H), 2.07 (m, 1H), 1.98 (m, 1H), 1.66 (m, 2H), 1.43 (m, 2H), 1.31 (m, 1H). MS (EI): $m/e = 312$.

*(±)-10-[^{11}C]-Methyl 3-[6-nitro-(2-quinolinyl)]-3,10-diazabicyclo-[4.3.1]-decane (**[^{11}C-methyl]-NS 2495**).* [^{11}C]Methyl iodide was reacted with NS 7113 (1.0 mg, 2.33 μmol) dissolved in DMSO (300 μl) and in

2,6-di-*tert*-butylpyridine (10 µl, 50 µmol). After heating for 5 mn at 110°C, the crude product was purified on the preparative HPLC (acetonitrile: 70 mM NaH_2PO_4 ($pH = 3.5$), 17: 83; 8 ml/mn; λ = 232 nm). Once the fraction containing [^{11}C-methyl]-NS 2495 was isolated, the solvent was evaporated under reduced pressure until ca. 1 ml was left. A mixture of saline (9 ml) and 70% ethanol (1 ml) was finally added to the product. Analytical HPLC was run afterwards in order to determine the radiochemical purity and the product identity thanks to co-injection of a cold reference. (Radiochemical purity: > 99%; specific activity: 1.8 GBq/µmol)

PET Procedures

A study of *in vivo* brain uptake of [^{11}C]-NS 2495 was made in a single female pig (New Hampshire/Yorkshire/Duroc × Danish Landrace crossbreed, 40 kg) deprived of food, but not of water, for 24 h prior to the experiment. Anesthesia was induced with midazolam and ketamine (i.m., i.v.) prior to intubation and was maintained by ventilation with isoflurane (2%). Catheters were surgically installed in a femoral artery and vein. Physiological functions were monitored continuously, and disturbances in body fluid balance were corrected using appropriate procedures.[12] The pig was placed prone in the scanner (Siemens ECAT EXACT HR) with her head fixated with a custom-made head-holding device. After a brief attenuation scan, a dynamic emission recording lasting 90 minutes was made upon intravenous injection of [^{11}C-methyl]-NS 2495 (400 MBq). After the baseline scan, the pig received citalopram (1 mg/kg, i.v.), followed 30 min later by a second PET recording with [^{11}C-methyl]-NS 2495.

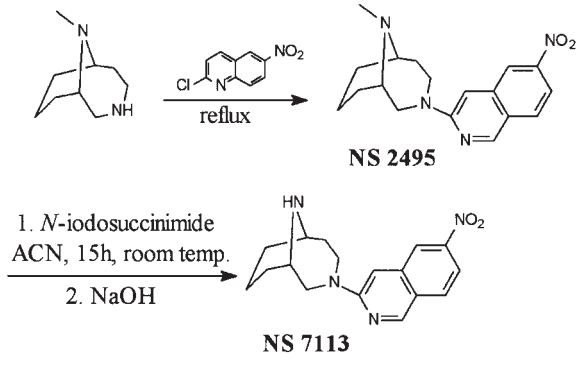
Arterial blood samples were drawn at intervals during the PET recordings, and the concentration of untransformed tracer in plasma was measured by HPLC and gamma-counting: acetonitrile was added to an equal volume of selected plasma samples, and protein was removed by centrifugation. The filtered supernatant (1 ml) was chromatographed using a Phenomenex Spherisorb 5 µ CN column (250 × 4.6 mm) with 50 mM NaH_2PO_4/acetonitrile (2/1 v/v) as the eluent at a flow rate of 1.5 ml/min. After integration of the radiochemical fractions, a bi-exponential function was fitted to the measured values of untransformed tracer fractions, and the concentrations of untransformed tracer were calculated as a function of time.[13]

Image analysis

The summed emission image of [^{11}C-methyl]-NS 2495 in the baseline condition was manually co-registered to the statistical MR atlas of porcine brain using nine degrees of freedom.[14] The calculated transformation matrix was used to resample the dynamic emission sequences into the common stereotaxic space. A statistically defined volume of interest for the cerebellum applied to the re-sampled emission sequences in order to extract dynamic time-radioactivity curves for the reference region. Parametric maps of distribution volume were calculated on a voxel-by-voxel basis using the metabolite-corrected arterial input and the linearization of Logan in the baseline condition and after the citalopram challenge.[15] Parametric maps of binding potential, pB, which is proportional to the ratio B_{max}/K_d, were calculated on a voxel-by-voxel basis using the cerebellum as a reference tissue in the baseline condition and after citalopram challenge.[15]

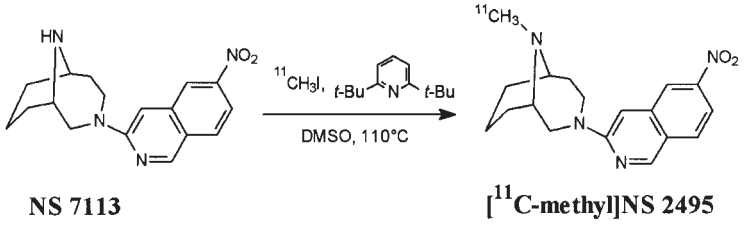
Results and discussion

The preparation of NS 2495 could be accomplished similarly to the synthesis of NS 4194 by a condensation reaction between 2-chloro-6-nitroquinoline and 10-methyl-3, 10-diazabicyclo-[4.3.1]-decane (Scheme 1).[6a] The demethylation step of NS 2495 with *N*-iodosuccinimide followed by a sodium hydroxide treatment gave rise to the precursor NS 7113 for the carbon-11 labelling methylation. [^{11}C-Methyl]-NS 2495 was obtained



Scheme 1. Synthesis of the precursor of NS 2495

after reaction with [^{11}C]methyl iodide in DMSO at 110°C for 5 minutes in the presence of 2,6-di-*tert*-butylpyridine in a total decay-corrected yield of 5% (counting from EOB) (Scheme 2). Analytical HPLC showed the product to be >99.5% radiochemically pure.



NS 7113 **[^{11}C-methyl]NS 2495**

Scheme 2. Synthesis of [^{11}C-methyl]NS 2495

In the baseline condition [^{11}C-methyl]-NS 2495 had a distribution volume ranging from 75 to 150 ml g^{-1} in porcine brain (Figure 2); treatment with citalopram uniformly reduced the distribution volume to less than 100 ml g^{-1}. In the baseline maps of *pB*, the highest binding was

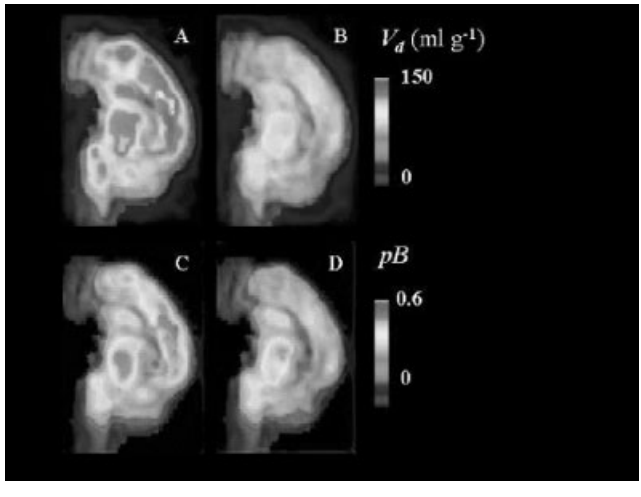


Figure 2. The binding of [^{11}C-methyl]-NS 2495 in brain of a living pig. The equilibrium distribution volume (V_d, ml g^{-1}) was calculated relative to the metabolism-corrected arterial input using the linearization of Logan in the baseline condition (A) and after treatment with citalopram (B). The binding potential (*pB*) was calculated using the cerebellum as a reference tissue in the baseline condition (C) and after treatment with citalopram. All images show a sagittal plane 4 mm lateral to the midline. The parametric images are co-registered to an MR image of the average pig brain (grey scale)

present in the midbrain, the cingulate cortex, and the basal ganglia, consistent with the known pattern of serotonin uptake sites in frozen sections of pig brain.[16] The magnitude of *pB*, which ranged from 0.2 to 0.6 in living pig brain (Figure 2), was comparable to earlier results obtained with the related compound, [^{11}C-methyl]-NS 4194.[6b] The displacement of [^{11}C-methyl]-NS 2495 binding after citalopram treatment was incomplete, as earlier reported for [^{11}C-methyl]-NS 4194, a close congener.[6b] In contrast, the same citalopram treatment quantitatively displaced the specific binding of [^{11}C]-DASB from serotonin transporters in pig brain.[6b] The lack of complete displacement of [^{11}C-methyl]-NS 2495 and [^{11}C-methyl]-NS 4194 suggests that the calculation of *pB* is uncertain due to the presence of very high non-specific binding. Alternately, the binding sites resistant to citalopram displacement may be present in an intracellular compartment specifically accessible to these very lipophilic PET tracers.

Conclusion

[^{11}C-Methyl]-NS 2495 can be readily prepared by [^{11}C]methylation of the precursor. The *in vivo* PET study shows that [^{11}C-methyl]-NS 2495 has high non-specific binding throughout the living porcine brain, with a distribution volume close to $75\,\mathrm{ml\,g^{-1}}$ in non-binding regions. Binding potentials, calculated by a cerebellum reference method, ranged from 0.2 to 0.6, and were consistent with the known pattern of serotonin innervation of the pig forebrain. However, complete displacement of specific binding in *pB* maps was not obtained after treatment with citalopram. Thus, the kinetics of the binding of [^{11}C]-NS 2495 are unfavorable for the quantitative assay of serotonin uptake sites in living brain.

Acknowledgements

The authors thank GlaxoSmithKline for providing financial support to Hélène Audrain.

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