Synthesis of [2-^{11}C]-6,7-dichloro-2,3-dihydroxyquinoxaline and evaluation of its *in vivo* distribution in rat with PET

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Abstract

A method for labelling 6,7-dichloro-2,3-dihydroxyquinoxaline (DCQX) in position 2 with carbon-11 is presented. Diethyl [1-^{11}C]oxalate was synthesized in a two-step, microwave-assisted procedure from no-carrier-added [^{11}C]cyanide and was reacted with 4,5-dichloro-1,2-phenylenediamine in sulfuric acid at 150°C for 10 min. [2-^{11}C]DCQX, isolated by semi-preparative HPLC, was >99% radiochemically pure with a specific activity ranging between 19 - 26 TBq/mmol. The total time of synthesis was 45-55 min and the isolated, decay-corrected yields were on the order of 10%, based on the trapped [^{11}C]cyanide. A PET study of its biodistribution after intravenous injection in a male rat revealed that the extraction of [2-^{11}C]DCQX across the intact blood-brain barrier was ≤2%.

Key words: DCQX, excitatory amino acids, carbon-11, microwaves, PET

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Introduction

Abnormal excitation by neurotransmitters can eventually cause the death of neurons. Such a mechanism may be involved in the neuronal loss observed in neurodegenerative diseases such as ischemia, epilepsy, traumatic brain injury, Parkinson's and Alzheimer's disease. Drugs which act specifically to antagonize excitatory neurotransmission may therefore provide a means of treating acute and/or chronic degenerative disorders. Positron emission tomography (PET) scanning techniques provide a means for investigating *in vivo* the biodistribution and interaction of such drugs, properly labelled, with the excitatory amino acid (EAA) receptor systems.

Ligands for the EAA receptors which have previously been labelled with positron-emitting radionuclides have been non-competitive NMDA (N-methyl-D-aspartate) antagonists: [^{11}C]ketamine (1) as well as ^{11}C- and ^{18}F-analogues of MK-801 (2-5), phencyclidine (6) and thienylcyclohexylpiperidine (7-9). A number of substituted 2,3-dihydroxyquinoxalines (quinoxaline-2,3-dione) have been shown (10) to be antagonists of the EAA receptors: 6,7-dichloro-2,3-dihydroxyquinoxaline (DCQX), 6,7-dinitro-2,3-dihydroxyquinoxaline (DNQX), 6-cyano-7-nitro-2,3-dihydroxyquinoxaline (CNQX) and 6-nitro-7-sulfamoylbenzo[f]quinoxaline-2,3-dione (NBQX).These substances have moderately high affinities for non-NMDA receptors (IC_{50}s for

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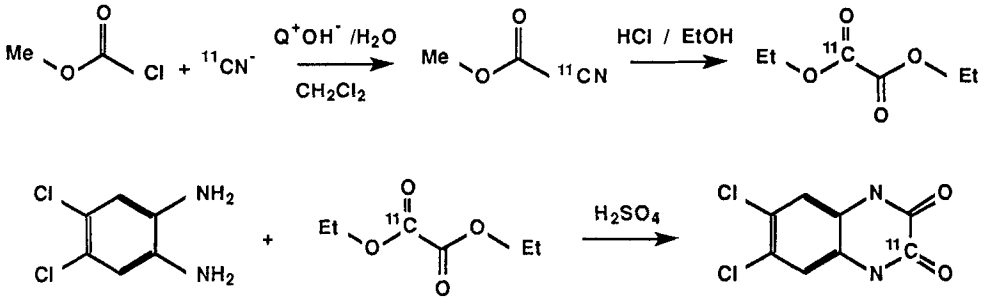
inhibition of [3H]AMPA binding are 0.15-8.2 μM (10)). Though affinities in this range are generally considered to be too low for use in quantifying receptor populations *in vivo* with positron emission tomography (PET), studies of the biodistribution of this family of substances may provide information about their relative bioavailabilities in healthy and threatened tissue. For this purpose, we developed (11) a general method for introducing a carbon-11 label in the quinoxaline ring which is a common structural feature of all these compounds. Here we report the application of this method in labelling DCQX with carbon-11 by the reaction of diethyl [1-^{11}C]oxalate derived from [^{11}C]cyanide with 4,5-dichloro-1,2-phenylenediamine (Scheme 1). This synthesis has been preliminarily reported elsewhere (12).

Scheme 1: Three-step synthetic method for labelling [2-^{11}C]DCQX from [^{11}C]CN$^-$



Experimental

General

Methyl chloroformate (MeOOCCl), 4,5-dichloro-1,2-phenylenediamine (DCPA) and diethyl oxalate were obtained from Aldrich, tetrabutylammonium hydroxide (Q^+OH^-) (0.8 M in methanol) from Fluka, sulfuric acid (H_2SO_4) from Merck and hydrogen chloride (HCl) gas from Aga Specialgas Sweden. Reference 6,7-dichloro-2,3-dihydroxyquinoxaline was purchased from Research Biochemicals Incorporated, USA. The solvents used were of analytical grade. The microwave cavity used in the second step in the synthesis of diethyl [1-^{11}C]oxalate was a prototype equipment previously described elsewhere (13 and 14) and now commercially available from Labwell AB (Uppsala, Sweden).

Analytical HPLC was performed using a Shimadzu LC 6A pump, a Valco injector (C6W with a 250 μL loop), a Shimadzu SPD 5A UV-detector (λ = 215 or 254 nm) in series with a Beckman 170 β-flow radiodetector. The detectors were connected to a Shimadzu C-R2AX data processor for integration of the peak areas. The columns used were μBondapak C-18 (Waters, 10 μm, 300 x 3.9 mm and 300 x 7.8 mm for the a) radioanalysis and b) isolation, respectively). The mobile phases were for a) CH_3CN : H_3PO_4 (0.01 M) 15:85 at 1.5 mL/min, (retention times ≈3.4, 4.1 and 6.2 min for the methyl [1-^{11}C]cyanoformate, diethyl [1-^{11}C]oxalate and [2-^{11}C]DCQX, respectively), and b) CH_3CN : H_3PO_4 (0.01 M) 20:80 at 4 mL/min.

Radionuclide production

[^{11}C]Carbon dioxide ([^{11}C]CO_2) was produced at the Karolinska Hospital/Institute with a Scanditronix MC 16 cyclotron by a batch irradiation of nitrogen gas with 17 MeV protons in the $^{14}N(p,\alpha)^{11}C$ reaction. [^{11}C]Cyanide ([^{11}C]CN^-) was produced in a two-step procedure from [^{11}C]CO_2 (15) and the ammonia added was removed by flowing the gas through heated H_2SO_4 (16).

Diethyl [1-^{11}C]oxalate

H[^{11}C]CN was trapped in an aqueous solution of Q^+OH^- (50 μL) in 0.5 mL of H_2O. After completion of trapping, MeOOCCl (50 μL) dissolved in 0.5 mL CH_2Cl_2 was added and the solution was vigorously stirred for 5 min at room temperature. The organic phase was removed and added to HCl (4-5 M in EtOH). The mixture was heated for 10 min at 80°C under stirring or treated with microwaves at 70 W for 0.5 min.

[2-^{11}C]-6,7-Dichloro-2,3-dihydroxyquinoxaline

The reaction vessel was evacuated and the alcoholic solution was evaporated with heat. DCPA (10 mg, 0.06 mmol) dissolved in H_2SO_4 (9 M, 0.5 mL) was added and heated at 150°C for 10 min in a 5 mL pear-shaped vessel sealed with a septum. The reaction vessel was cooled and H_2O (2 mL) was added. The resulting mixture was eluted through a SepPak C18 (Waters, pre-treated with 10 mL EtOH and 20 mL H_2O). After washing with H_2O (2 mL), the radioactive products including [2-^{11}C]DCQX were eluted with CH_3CN (1-1.5 mL). The volume was reduced by evaporation and heating and the residue was injected on the semi-preparative HPLC column. [2-^{11}C]DCQX eluted after 9.5-11 min. The mobile phase was evaporated on a rotary evaporator, the residue was dissolved in physiologically buffered saline (8 mL, 12.3 mM) and the solution was filtered through a Millipore filter (0.22 μm) into a 10 mL sterile injection vial.

PET evaluation

The *in vivo* distribution of [2-^{11}C]DCQX in the rat brain and whole body was examined using the rapid screening method of Ingvar et al. (17). The experimental procedure used was approved by the regional animal ethics committee of the Karolinska Institute. A male Sprague-Dawley rat was halothane anesthetized and provided with arterial and venous catheters. The rat's head was fixed on a horizontal plate and shifted to the desired position in the PET camera (GE-Scanditronix PC 2048-15B (18)). Following the injection of [2-^{11}C]DCQX (20 MBq) in the venous catheter, repeated arterial samples were drawn as rapidly as possible for the first 2 min followed by increased time intervals until the end of the investigation. Data were collected with the PET camera in a series of frames, divided into nine 20 sec scans, four 2 min scans and three 6 min scans, giving an investigation time span of 33 minutes. At the end of the PET investigation the rat was dissected and the radioactivity measured in weighed tissue samples to determine the relative organ distributions at the end of the scan.

Results and Discussion

Synthetic Procedure

The synthesis of [2-^{11}C]DCQX is a three-step reaction starting from [^{11}C]CN^-. The preparation of diethyl [1-^{11}C]oxalate has been described previously (11). Briefly, after removing excess ammonia

[^{11}C]CN$^-$ is reacted with MeOOCCl in a phase transfer reaction to generate methyl [1-^{11}C]cyanoformate after 5 min at room temperature (≈85-90% in the organic phase). The carbon-11 labelled nitrile is subsequently converted to diethyl [1-^{11}C]oxalate in an alcoholic solution of HCl by either thermal or microwave treatment (80°C for 10 min vs. 70 W for 0.5 min). The overall conversion of [^{11}C]CN$^-$ to diethyl [1-^{11}C]oxalate was *ca* 80%, according to analytical HPLC. The total time for the synthesis of diethyl [1-^{11}C]oxalate from [^{11}C]cyanide was 6 or 15 min for the microwave or thermal procedure, respectively.

After evaporation of the solvents the diethyl [1-^{11}C]oxalate was reacted with the dichloro-substituted phenylenediamine to produce [2-^{11}C]DCQX after 10 min under acidic conditions at high temperature. Both HCl (4M) and H_2SO_4 (9M) were tested in the cyclization step but, while HCl was previously shown to work very well with phenylenediamine, much better yields were obtained with the dichloro substrate using H_2SO_4. The reaction time was also dubbled to 10 min in order to obtain the cyclization conversions of 47±6% n=6, according to radioanalytical HPLC.

The final reaction step was also attempted using the microwave cavity, but, as found previously, microwave powers < 80W were not sufficient to achieve the cyclocondensation. With 100-120 W the vessel was unable to contain the high pressures which developed and the septum blew. Attempts to contain the pressure by reinforcing the septum with a metal liner caused the Pyrex vessel to shatter. The pressure generated during the thermal and microwave treatment of different reaction media with comparable head-space available was subsequently monitored by connecting a pressure gauge to the vessel by stainless steel HPLC tubing (i.d. = 0.5 mm, o.d. = 1.6 mm) which pierced the septum (Figure 1). The highly ionized acids are immediately heated by micromotion induced by the rapidly reversing electric field in the microwave cavity. The pressures resulting from the volatilization of the media are thus much higher than those observed by treatment of a corresponding volume of water alone with 60 W (diagram A). In a separate measurement (data not shown) it was observed that, at microwave powers needed for the cyclization (≥ 100 W), the pressure increased to 8 bar (maximum before the septum blew) within a few seconds (2-3 sec) even with reaction volumes that were only 5-10% of the vessel volume and the treatment had to be discontinued. These results are in sharp

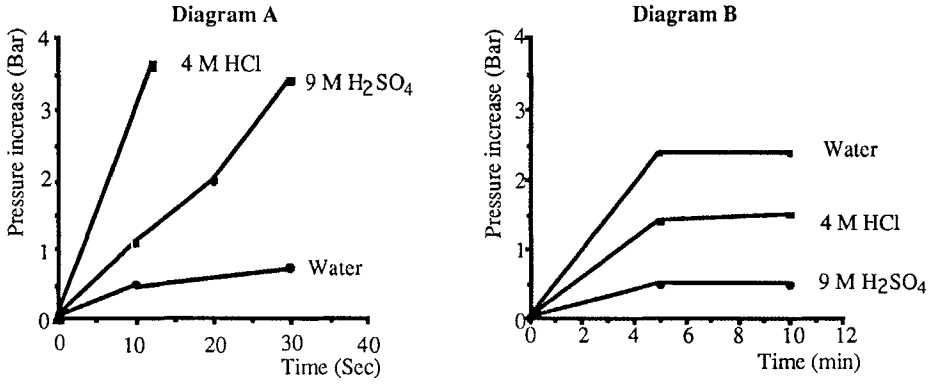


Figure 1: Pressures generated during the heating of HCl, H_2SO_4 and H_2O during microwave treatment with 60 W (A) or conventional heating at 150°C (B). The volumes treated were in all cases 10% of the total vessel volume (≈5 mL).

contrast to the pressures measured during the thermal treatment of the same media at 150°C for as much as 5-10 min (diagram B). Obviously the design of a vessel that can handle the high temperatures and pressures developing during the microwave treatment of these mineral acids could be a considerable asset in using microwaves to reduce reaction times for cyclizations of the type presented here.

The composition of the sample to be injected on the semi-preparative column was rapidly changed from concentrated acid to acetonitrile by solid phase extraction techniques using the SepPak C18 cartridge. Essentially no [2-^{11}C]DCQX was detected in the aqueous wash, which removed not only the H_2SO_4 but also a major portion of the diamine precursor. The radioactivity remaining on the SepPak (*ca* 40 %) was eluted with acetonitrile, evaporated and diluted with the mobile phase. [2-^{11}C]DCQX was isolated by HPLC with an overall decay-corrected yield of *ca* 10%, based on trapped [^{11}C]CN^-. The product obtained was radiochemically pure (>99%) by radioanalytical HPLC. The specific activity ranged between 18 - 26 TBq/mmol (500-700 Ci/mmol).

PET evaluation of the in vivo distribution of [2-^{11}C]DCQX

Following the bolus injection of [2-^{11}C]DCQX (20 MBq), the radioactivity in the plasma rapidly decreased after the peak and was followed by a much slower rate of decrease commencing at 2-3 min. The time course of the uptake in extracerebral tissue and in the whole brain of the rat (regions defined as described in (17)) is shown in Figure 2. Also plotted (whole lines) are the predicted results when applying a standard two-compartment model on the measured data, i.e. a model that describes an uptake and washout from the brain as well as an incorporation of the tracer in the tissue. Such a model gave an acceptable description of the kinetic events as seen by the reasonable fits that were obtained and, according to an Akaike analysis, was also preferred to a one-compartment analysis (deletion of parameters that describe an incorporation of the tracer in the tissue). The K_1 calculated for the whole brain (0.02 mL/100g,min) indicates that the extraction of [2-^{11}C]DCQX is as low as 2%, assuming a cerebral blood flow of 1 mL/min (17).

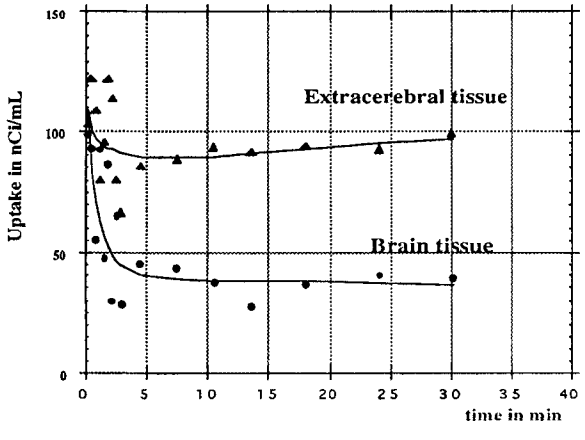


Figure 2:
The time course of the uptake in extracerebral tissue and the brain together with predictions from the two-compartmental model fits.

For whole brain:
K_1=0.02±0.01, k_2=0.69±0.20, k_3=0.02±0.01 (k_4 set to 0) and CBV=0.03±0.01

For extracerebral tissue:
K_1=0.04±0.01, k_2=0.34±0.02, k_3=0.03±0.01 and CBV=0.03±0.01

The tracer uptake in heart tissue, data not shown here, gave the best fit with only CBV as a parameter. In other words, the tissue retention of the tracer was insignificant compared to the content

of radioactivity in the blood. The relative uptake of the radiotracer at this late time was determined for weighed tissue samples obtained by dissection (Figure 3). The results obtained support the data from the *in vivo* PET investigation. Possible suggestions of differences in the regional uptake pattern in the brain should be regarded with some caution due to the small samples and very low amount of radioactivity. The largest portion of the radioactivity at this late time was found in the liver and kidneys.

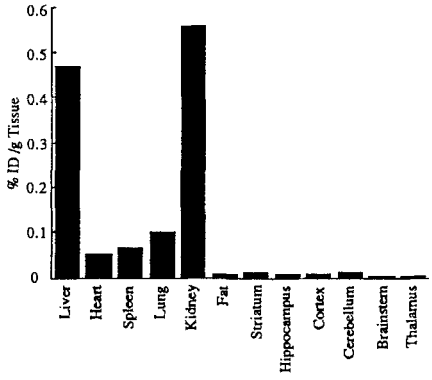


Figure 3:
Distribution of radioactivity in the rat at the end of the *in vivo* PET investigation (33 min). Data were obtained by tissue sampling and direct determination of radioactivity in preweighed vials.

Conclusions

DCQX was labelled in position 2 with ^{11}C by a three-step synthetic procedure. [^{11}C]CN^- was rapidly transformed to diethyl [1-^{11}C]oxalate by combined thermal and microwave procedures. Cyclization with DCPA could however only be accomplished using thermal treatment, due to the difficulty of containing the reaction mixture under the high pressures generated when sufficient microwave power was used to accomplish the ring closure reaction. The total time of synthesis, including isolation by semi-preparative HPLC, was ≈45 min. The isolated radiochemical yield was ≈10% (decay-corrected, based on [^{11}C]CN^- from end-of-trapping). The radiochemical purity was >99% and the specific activity ranged from 19 - 26 TBq/mmol.

The low cerebral bioavailability of this drug following i.v. injection makes it a poor candidate for investigating the cerebroprotective effects of blocking non-NMDA receptors in vivo. Derivatives with a higher cerebral extraction must therefore be developed. However, given the importance of the EAA system in the brain in both normal brain function and as part of the development of cerebral damage, it seems highly warranted to continue the search for compounds that are possible to use for imaging these systems in the human brain.

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