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[C-11]N-METHYLHOMOEPIBATIDINE: RADIOLABELLING AND BIODISTRIBUTION STUDIES IN MICE.

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SUMMARY

The radiochemical synthesis of [C-11]N-methylhomoepibatidine (2) was accomplished by reacting homoepibatidine (1) and [C-11]iodomethane. The radiochemical yield was in the range of 5 to 10 % related to [C-11]iodomethane. A high specific activity of 150 to 370 GBq/μmol at the end of synthesis was achieved. The partition coefficient was log $P_{7.4} = 0.34$. Biological evaluation was performed with the racemate in mice. High brain uptake was found. Compared to [C-11]N-methylepibatidine (4) data, which were achieved by the same experimental setup, the values of [C-11]N-methylhomoepibatidine (2) uptake in the brain were slightly lower (approximately 15 %/g compared to 20 %/g for [C-11]N-methylepibatidine (4)). But in contrast to the homologous [C-11]N-methylepibatidine (4) the brain uptake curve decreased after approximately 15 minutes showing reversible binding to the receptor. Pretreatment of mice with (-)-epibatidine (3) resulted in a considerably lower brain uptake while uptake in other tissues remained unchanged.

Keywords: nAChR, nicotinic receptor, epibatidine, homoepibatidine

INTRODUCTION

Nicotinic receptors are involved in a broad variety of physiological and pathophysiological phenomena in the central nervous system (CNS). The development of new drugs acting as cholinergic channel modulators (ChCM) might be beneficial for the treatment of Alzheimer's disease (AD)(1,2), Parkinson's disease (PD) (3,4), attention deficit hyperactivity disorder (ADHD) (5,6), Tourette's syndrome (7,8), schizophrenia (9), anxiety (10), smoking cessation (11) and for the treatment of pain (12).

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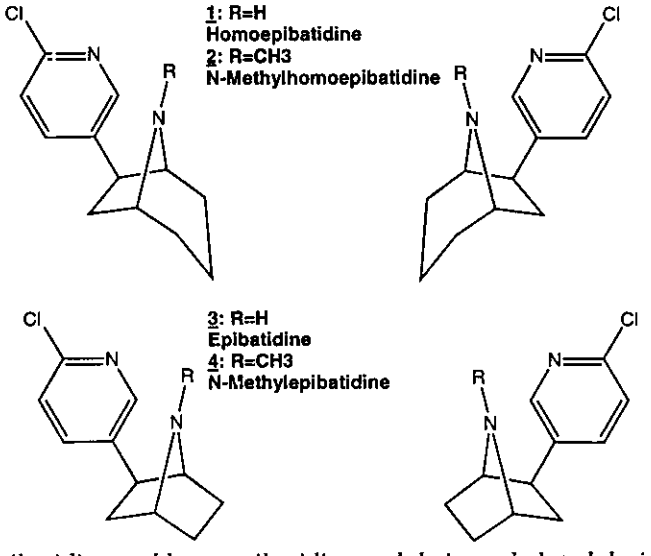
Furthermore nicotinic receptor subtypes are involved in learning, memory and cognitive performance (13-15). The broad variety of possible applications resulted in a high interest in ChCMs and led to an increasing number of new agents with different properties and selectivity's. Thus a number of parent structures for labelling are known today (16-20).
Epibatidine (3), a highly potent cholinergic channel agonist (ChCA) is a promising parent structure for several new PET and SPECT radiotracers for neuronal nAChR's. (21-25)]. But the high toxicity of this class of substances may cause problems for human application. The synthesis of a homologue of epibatidine was reported in 1996 (26,27). Homoepibatidine (1) was described to have similar pharmacological properties as epibatidine while the toxicity of homoepibatidine is lower (28). The structures of epibatidine (3) and homoepibatidine (1) are compared in figure 1.

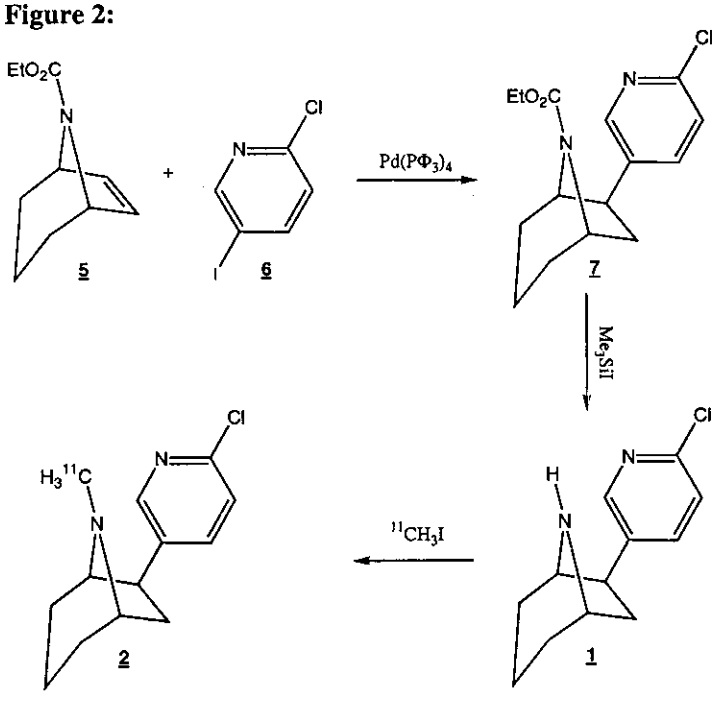
Figure 1



Structure of epibatidine and homoepibatidine and their methylated derivatives.

The key step of the homoepibatidine synthesis is the Heck reaction of the 8-carboethoxy 8-azabicyclo[3.2.1]octene (5) with 2-chloro-5-iodopyridine (6) followed by deprotection of the 8-aza position. The Heck reaction , a palladium catalysed coupling of olefins with organic halides, is very advantageous for several reasons:
It is simple to carry out, not requiring anhydrous conditions and tolerates a broad variety of functional groups. The main advantage is the possibility of variation of the pyridine moiety of the receptor ligand by variation of the last two steps of the preparation procedure. Thus, once the 8-azabicyclo[3.2.1]octene (5) is available, the biological activity of the ligand can be easily modulated by changes of the pyridine moiety.
In this paper we describe the radiosynthesis and some biological properties of [C-11]N-methylhomoepibatidine (2).

Figure 2:



Synthesis Scheme of [C-11]N-methylhomoepibatidine.

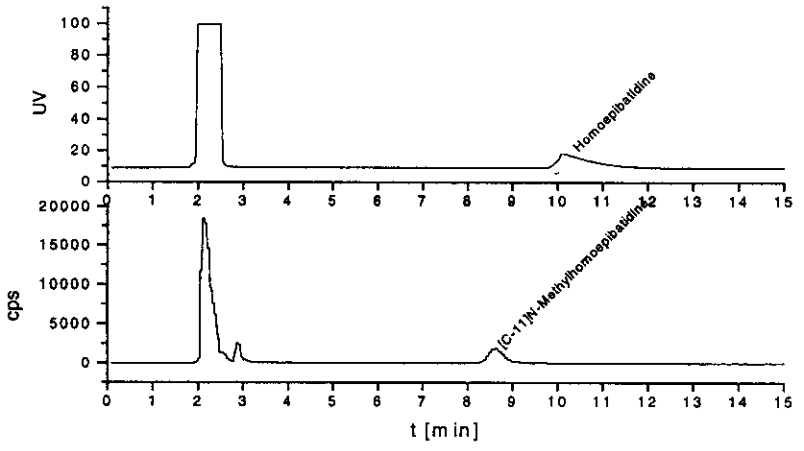
RESULTS and DISCUSSION

The key steps of the precursor preparation and the radiolabelling is shown in figure 2. Identity of the precursor and the cold methylated standard was checked by comparison of ^{1}H-NMR and mass spectra with published spectra (26,27).

[C-11]N-Methylhomeepibatidine (2) could be labelled with high radiochemical purity (>98 %) and high specific activity (150 to 370 GBq/μmol). The semipreparative chromatogram is shown in figure 3.
[C-11]N-Methylhomeepibatidine (2) elutes before the educt homoepibatidine (1), and thus precursor impurities were not found in the product solution. The radiochemical yield of [C-11]N-methylhomoepibatidine (2) were in the range of 5 to 10 % corrected for decay referred to [C-11]iodomethane. The yield was sufficient for the experiments performed and not further optimised.

The partition coefficients in different systems (i.e. 1-octanol/water, chloroform/water, cyclohexane/water) are models for penetration of the blood brain barrier (BBB) (29) which can easily be measured especially with labelled compounds. The most common 1-octanol/water partition coefficient is important because it is used in various calculation models describing BBB-passage of CNS agents.

Figure 3:



Purification of [C-11]N-methylhomoepibatidine by semipreparative HPLC. Column: Lichrosphere SI 100 - 7, (250 x 10 mm), eluent: CH_2Cl_2/MeOH/TEA; flow: 8 ml/min, [C-11]N-methylhomoepibatidine: 8.6 minutes, homoepibatidine: 10.3 minutes.

The log P value is only defined for the partitioning of identical species in lipophilic and aqueous phase. If protonation occurs as in the case of N-methylhomoepibatidine (2) at physiological pH and in acidic solution the log D, the distribution coefficient is measured (30). A better model for the penetration of the BBB is the Δlog P value (31) which can be interpreted as a measure of the hydrogen bonding capacity of a substance. Defined as the difference between the partition coefficient 1-octanol water and cyclohexane water ($\Delta\log P = \log P_{1\text{-octanol/water}} - \log P_{\text{cyclohexane/water}}$) it shows a good linear correlation with the log(BB) value. The brain to blood concentration ratio (BB) is a crude measure for the brain uptake of CNS agents. It was found that $\log P_{1\text{-octanol/water}}$ is poorly correlated with BB while Δlog P showed a good correlation (32,33).

The lipophilicity of [C-11]N-methylhomoepibatidine (2), determined by the shaken flask technique was $\log D_{7.4} = 0.34$ at physiological pH. The partition coefficient of the unprotonated form was measured as $\log P_{13} = 2.36$. It can be expected from the high polarity of the tracer at physiological pH, that non specific binding to brain membranes is low. Radiopharmaceuticals designed to measure blood flow should have a log P value greater than 0.9 (34). With a $\log P_{\text{cyclohexane/water}} = 1.5$ a $\Delta\log P = 0.9$ can be calculated. An excellent passage through the BBB can be expected for the deprotonated form of the tracer. From these results it can be deduced, that the tracer passes readily the blood brain barrier (BBB), but will not show a high non specific binding in the lipophilic brain membranes. The high polarity of the tracer is a positive feature leading to low levels of non specific binding due to lipophilic adsorbtion on

brain membranes. These results were in good agreement with the brain uptake in mice discussed below.

The data on biodistribution in female ICR-mice are presented in table 1. Considerable brain uptake was found during the whole experiment. Except for the 1 minute and some of the 5 minute experiments a high percentage of the injected C-11 activity was found in the urine (> 15 % of the injected dose, not shown in table 1).
The kidneys showed a very high level of C-11 activity. High levels of radioactivity were also found in the liver, in the stomach and in the spleen. The tracer showed a fast excretion via the kidneys.

Table 1: Time dependence of the biodistribution of [C-11]N-methylhomoepibatidine in female ICR-mice.

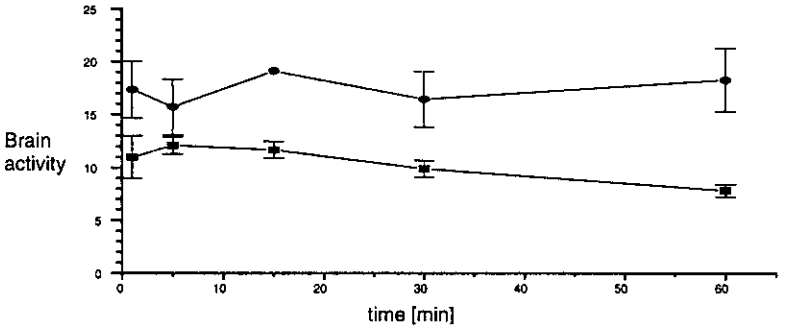
	C-11 activity in % of injected dose per g tissue				
Organ	1 min	5 min	15 min	30 min	60 min
blood	1.6 ± 0.2	1.6 ± 0.3	1.1 ± 0.2	1.0 ± 0.3	0.7 ± 0.1
heart	3.5 ± 1.0	3.3 ± 0.2	2.3 ± 0.2	1.9 ± 0.3	1.5 ± 0.5
lungs	7.6 ± 1.6	7.3 ± 1.8	4.5 ± 2.2	4.1 ± 0.1	3.0 ± 0.9
spleen	10.2 ± 2.4	10.9 ± 4.2	9.7 ± 3.0	7.2 ± 1.0	5.7 ± 2.8
kidneys	26.2 ± 4.8	17.7 ± 7.6	12.0 ± 3	10.0 ± 1.5	9.6 ± 3.6
stomach	2.7 ± 0.4	2.7 ± 1.0	6.3 ± 1.5	12.0 ± 8.3	9.8 ± 3.8
intestine	5.1 ± 0.5	4.9 ± 0.6	4.6 ± 0.4	3.6 ± 0.2	3.5 ± 1.0
liver	13.6 ± 2.8	14.2 ± 2.0	12.2 ± 1.5	9.5 ± 0.03	7.4 ± 1.0
muscle	2.7 ± 1.9	2.3 ± 0.7	1.3 ± 1.1	1.8 ± 0.4	1.6 ± 0.5
bone	1.9 ± 1.0	2.1 ± 0.5	1.8 ± 0.4	1.5 ± 0.1	1.4 ± 0.2
brain	11.0 ± 2.0	12.1 ± 0.8	11.7 ± 0.8	9.9 ± 0.8	7.8 ± 0.6
eyes	2.6 ± 0.5	3.0 ± 0.6	2.9 ± 0.5	3.0 ± 0.4	2.6 ± 0.7

The brain uptake of [C-11]N-methylhomoepibatidine (2) in comparison to [C-11]N-methylepibatidine (4) is shown in figure 2. [C-11]N-methylhomoepibatidine (2) showed a lower brain uptake in mice compared to [C-11]N-methylepibatidine (4). While [C-11]N-methylepibatidine (4) activity in the brain of female mice showed a slightly increasing tendency after a very rapid uptake, the [C-11]N-methyl-homoepibatidine (2) uptake curve passed through a maximum between 15 and 30 minutes and then decreased slowly till 60 minutes.
Pretreatment with epibatidine (3) resulted in a lower uptake of C-11 radioactivity in the brain. No significant influence of pretreatment with epibatidine on the C-11 activity in other tissues was found. The compound seems to compete with epibatidine at the nicotinic receptor in the brain. Because of the high toxicity of epibatidine the blocking effect was not further optimised.

Table 2: Effect of preblocking with 1µg/kg (-)-epibatidine on the biodistribution of [C-11]N-methylhomoepibatidine after 5 minutes.

	[C-11]N-methyl-homoepibatidine 0.001 - 0.01 µg/kg,	pretreatment with (-)epibatidine, 1 µg/kg, 5 minutes before injection of [C-11]N-methylhomoepibatidine 0.001 - 0.01 µg/kg,
blood	1.6 ± 0.3	1.6 ± 0.1
heart	3.3 ± 0.2	3.4 ± 0.1
lung	7.3 ± 1.8	7.2 ± 0.8
spleen	10.9 ± 4.2	11.4 ± 1
kidney	17.7 ± 7.6	13.4 ± 1.5
stomach	2.7 ± 1.0	3.5 ± 1.0
intestine	4.9 ± 0.6	5.1 ± 0.4
liver	14.2 ± 2.0	14.1 ± 1.7
muscle	2.3 ± 0.7	2.44 ± 0.5
bone	2.1 ± 0.5	2.6 ± 0.4
brain	12.1 ± 0.8	8.5 ± 0.6
eyes	3.0 ± 0.6	2.8 ± 0.1

Figure 4:



Brain uptake of [C-11]N-methylhomoepibatidine (squares) compared to [C-11]N-methylepibatidine (dots).

EXPERIMENTAL

Unless otherwise stated reagents were obtained in analytical grade from commercial sources (Fluka, Aldrich, Sigma, Merck).

Analytical methods

The quality control of the methylated derivatives as well as the labelled compounds was performed on a Merck-Hitachi LaChrom HPLC-system (L7100 pump, L7200 autosampler, L7400 UV-detector, D7000 interface). The radioactivity was measured

with a BIOSCAN Flow-Count and a NaI detector (1 inch) connected to the D7000 interface. A Supelcosil LC-ABZ column (25.0 cm x 4.6 mm) was used with acetonitrile/phosphate buffer pH 7 (0.0026 M KH_2PO_4, 0.0041 M Na_2HPO_4) 40/60 (v/v) as the mobile phase at a flow rate of 1.5 ml/min. UV absorption was measured at a wavelength of 270 nm. The analyses of the chromatograms were carried out using the Merck HPLC Manager software. C-11 tissue and blood samples were counted together with standards in a Packard Cobra II auto gamma counting system. The background was subtracted and the count rate was decay corrected. The ^{1}H-NMR and ^{13}C-NMR were recorded on VARIAN Gemini 300 spectrometer. Mass spectra were recorded on a Trio 2000 spectrometer (VG ORGANIC, UK) using electrospray in positive ion mode (ES+).

Precursor synthesis

Precursor synthesis of homoepibatidine was performed by coupling iodopyridine with the appropriate bicyclooctene (25). The Heck reaction was modified using tetrakis(triphenylphosphine)palladium instead of bis-(triphenylphosphine)-palladium-II-diacetate. The methylated standard was prepared by the reaction of homoepibatidine with iodomethane.

(+/-) Homoepibatidine (6-β-(2-Chloro-5-pyridinyl)-8-azabicyclo[3.2.1]octane (1))

The olefin (5) (200 mg, 1.1 mmol) and 2-chloro-5-iodopyridine (6) (670 mg, 2.8 mmol) were dissolved in 2 ml of DMF. Piperidine (332 mg, 3.9 mmol) and Tetrakis-(triphenylphosphine)-palladium (100 mg, 0.09 mmol) were added and the solution flushed with argon (6.0). HCOOH (112 µl) was added all at once and the reaction mixture was heated under argon pressure for 7 h at 70 °C. Ethylacetate (EtOAc) (8 ml) and water (3 ml) were added and the organic layer was separated. the aqueous layer was extracted several times with EtOAc. The organic extract was dried over Na_2SO_4 and the solvent evaporated. Chromatography on silica with petroleum ether/EtOAc 7:3 (v/v) resulted in a slightly yellow oil which was not further purified.
The crude carbamate was dissolved in dry $CHCl_3$ and flushed with argon (6.0). Iodotrimethylsilane (170 µl, 1.2 mmol) was added and the reaction mixture was refluxed under argon for 8 h. Methanol (1 ml) was added and the solvent evaporated under reduced pressure. The solid residue was dissolved in 1N NaOH and extracted with ether. The ether layer was dried with Na_2SO_4 and the solvent evaporated. The product was purified on PLC-plates silica gel 60 F_{254} (Merck) with ether/triethylamine 9/1 (v/v) as the mobile phase (R_f(homoepibatidine) = 0.2).
Yield: 132 mg, 0.6 mmol, 54 %
Homoepibatidine ^{1}H-NMR (300 MHz, $CDCl_3$):
8.3 (1H, d, 2.5), 7.7 (1H, dd, 8.4, 2.2), 7.2 (1H, d, 8.4), 3.7 (1H, m), 3.3 (1H, br,s), 3.1 (1H, dd, 9.2, 5.1), 2.2 (1H, 13.2, 9.2), 1.5-2.0 (7H, m). m/z (rel intensity) 223 $[M+1]^+$ (100), 225 $[M+1]^+$ (30).

(+/-)N-Methylhomoepibatidine (6β-(2-Chloro-5-pyridinyl)-8-methyl-8-azabicyclo [3.2.1]-octane (2))

Homoepibatidine (30 mg, 0.13 mmol) was dissolved in acetonitrile (5 ml) and iodomethane (25 mg, 0.18 mmol) was added. The reaction mixture was heated at 80 °C for 20 minutes. The solvent was evaporated and the product was purified by PLC-plates silica gel 60 F_{254} (Merck) using ether/triethylamine 9/1 (v/v) as mobile phase (R_f(methylhomoepibatidine) = 0.8).

Yield: 14 mg, 0.06 mmol, 46 %.
N-Methylhomoepibatidine ^{1}H-NMR (300 MHz, $CDCl_3$):
8.3 (1H, d, 2.4), 7.8 (1H, 8.2, 2.4), 7.2 (1H, d, 8.2), 3.3 (1H, m), 3.1 (1H; dd, 9.5, 5.2), 3.0 (1H, br s), 2.5 (3H, s), 2.2 (1H; dd, 12.5, 9.3), 1.5-2.0 (7H,m). m/z (rel intensity) 237 $[M+1]^+$ (100), 239 $[M+1]^+$ (29).

(+/-) [C-11]N-methylhomoepibatidine (2)

The radionuclide C-11 was produced with the GE-PETtrace system by irradiation of a nitrogen target (N_2 6.0 with 400 ppm O_2 6.0 purchased from AGA). The [C-11]carbondioxide was converted to n.c.a.-[C-11]iodomethane in the GE-MeI-Microlab according to a procedure described by Larsen et al.(35).
Homoepibatidine (1, free base, 0.3 mg) was dissolved in acetonitrile (200 µl). The solution was cooled to -30 °C in an automated methylation system. [C-11]iodomethane was introduced by bubbling through the solution at a flowrate of 60 ml/min. The reaction mixture was heated to 100 °C for 10 minutes. After cooling the reaction mixture was injected to a semipreparative HPLC system, the result is shown in figure 2. The product [C-11]N-methylhomoepibatidine (2) eluted at 8.6 minutes, while the precursor (1) eluted later at 10.3 minutes. The product peak was collected and the solvent evaporated. The product was dissolved in physiological saline and quality control was performed on the analytical HPLC system.

Lipophilicity

The lipophilicity of [C-11]N-methyl-homoepibatidine (2) was determined by the shake flask method. The log P was determined at pH 7.4 (NaH_2PO_4 / Na_2HPO_4 0.15 M) for physiological conditions and at pH 13 (KCl/NaOH 0.15 M) for the protonated and the deprotonated form. [C-11]N-methyl-homoepibatidine (2) and carrier (1 µmol) were added to the appropriate buffer. Carrier was added to avoid artefacts which might occur at concentrations < 10^{-6} M (28). The buffer solution (1 ml) and octanol (1 ml) were shaken gently for 10 minutes. After centrifugation (3000 g, 5 minutes) aliquots (100 µl) of 1-octanol and buffer phase were measured in a γ-counter. This procedure was repeated 6 times. The count rates were corrected for decay and the radioactivity ratio of organic and aqueous phase were calculated and the partition coefficient (log $P_{7.4}$) determined.
To determine the Δlog P according to Seiler the procedure as described above was repeated for the system cyclohexane/buffer pH 13 (KCl/NaOH 0.15 M). Seiler's Δlog P value was calculated according to the equation $\Delta\log P = \log P_{13}$ (1-octanol/water) - log P_{13} (cyclohexane/water).

Biodistribution in mice

Animal studies were performed according to the principles of laboratory animal care as well as the current version of the Swiss law on animal protection.
The biodistribution studies were carried out in female ICR-mice (25 to 30 g) originated from the "Institut fuer Labortierkunde" of the University of Zuerich. The injected dose was 0.001 to 0.01 µg/kg body weight containing 25 to 150 kBq of the C-11 compound. The mice were anaesthetised with Metofane™ and kept in narcosis during the incubation time. After i.v. injection of the radiopharmaceutical and incubation for designated times the heart was punctured and a blood sample was taken. After dislocation of the spinal cord the tissues were collected. The tissue

samples, the blood and the urine sample were measured in a γ-counter. A standard containing 20 % of the injected dose was counted together with the samples. The weight of the tissue was determined and uptake of C-11 was expressed as the percent of the injected dose per gram of tissue.

CONCLUSION

In summary, [C-11]N-methylhomoepibatidine (2) could be prepared in sufficient yield and high radiochemical purity and specific activity. Though a rather polar compound at physiological pH it passed the blood brain barrier and competed with epibatidine (3) at specific binding sites in the brain.

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