Synthesis of 5-[^{123}I]Iodonicotinamide and Biodistribution in Rat

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SUMMARY

5-[^{123}I]iodonicotinamide (NAM) was prepared by halogen exchange iodination from 5-bromonicotinamide. In order to study biodistribution and brain accumulation ^{123}I-NAM was injected into rats. Dynamic gamma camera images were collected. The rats were sacrificed and tissue specific activities were measured. From dynamic images the maximum brain (head) activity after 30 minutes was 8.3 % of the injected dose and after 110 minutes, 4.5 %. ^{123}I-NAM was cleared by the kidneys. Radioactivity in the tissue samples of brain, kidney, blood, liver, spleen, lung, muscle, heart, bone and thyroid decreased over the course of 110-160 minutes, indicating rapid washout of the tracer. In conclusion the uptake in the brain and washout of ^{123}I-NAM was rapid. ^{123}I-NAM may be useful as a brain imaging agent.

Keywords: 5-[^{123}I]iodonicotinamide, Niacin, Brain, Iodination.

INTRODUCTION

Nicotinamide is a potential marker for studying nervous disorders caused by niacin deficiencies. The cerebral uptake of ^{11}C-nicotinamide has been studied in a pilot study in humans

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(1). Compounds of structural types like nicotinamide may also be etiological factors in some human neurological disorders

(2). We have developed a labelling method of 5-[^{123}I]iodonicotinamide (NAM) and have studied the biodistribution and brain accumulation of ^{123}I-NAM in rats.

RESULTS AND DISCUSSION

We chose to label the pyridine ring in the 5-position where the iodine is less susceptible to *in vivo* deiodination (3). The synthesis of ^{123}I-NAM is illustrated in Figure 1.

5-Bromonicotinamide was synthesized from 5-bromo-nicotinic acid (NAC). Briefly Br-NAC was acylated with thionyl chloride and the product was reacted with ammonia to produce Br-NAM. ^{1}H-NMR spectra were obtained from the Br-NAC and Br-NAM in order to examine the purity of the precursor and the product. The purity of Br-NAM after purification was over 99 % and the yield was 51 %.

Br-NAM was labelled with ^{123}I in 5-position by a halogen exchange reaction. Na^{123}I was reacted with Br-NAM in the presence of catalyst at 150°C. After the exchange reaction the mean (n=8) radiochemical purity of ^{123}I-NAM was 88 % (range: 78-97). The product was purified by HPLC or with minicolumn. After purification the radiochemical purity was > 95 %. After HPLC purification the specific activity was approximately 4 TBq/mmol.

The *in vivo* distribution of ^{123}I-NAM in rats was determined from dynamic gamma camera images after injection. ^{123}I-NAM showed rapid accumulation in the kidneys and liver. The maximum brain (head) activity after 30 minutes was 8.3 ± 0.9 % of the injected dose. The washout of the radioactivity

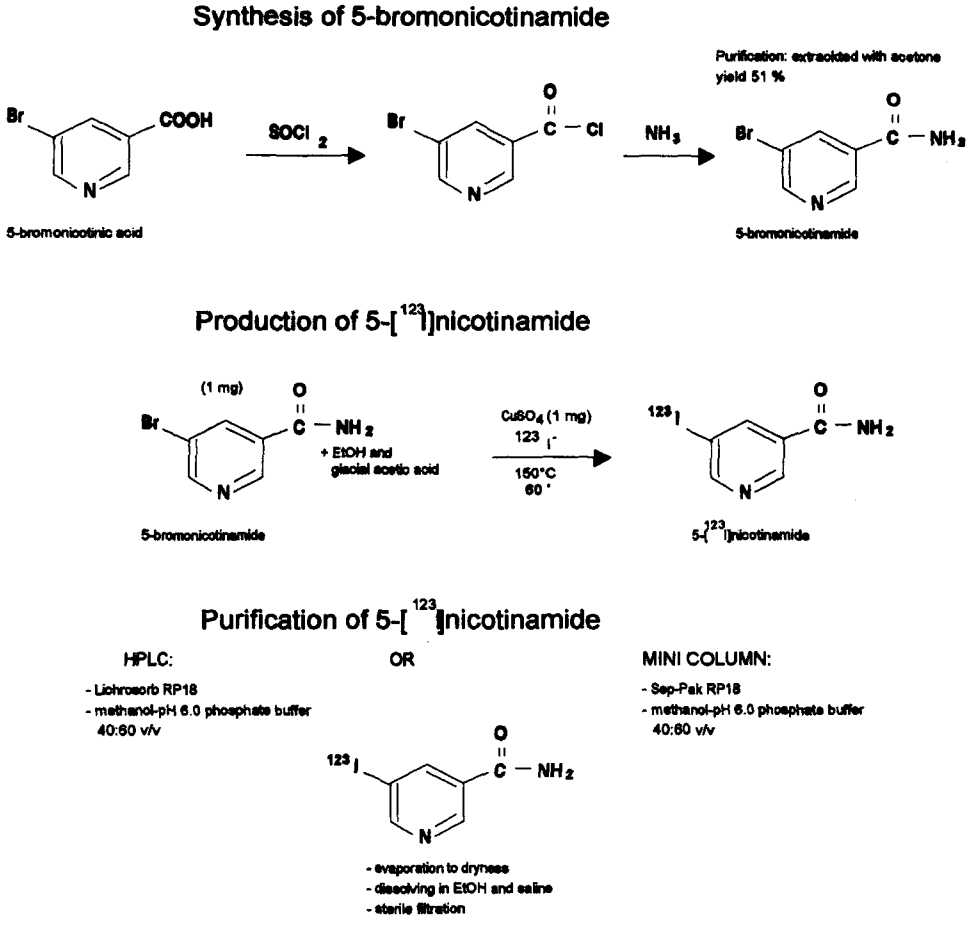

Figure 1.

from the brain and other tissues were similar. The percentage of the injected dose for the brain (head) and for the blood (heart) is plotted in Figure 2.

In vitro biodistribution was studied after gamma camera imaging. The rats were sacrificed after 110 and 160 minutes of injection and tissue activities of the brain, kidney, blood, liver, spleen, lung, muscle, heart, bone and thyroid were measured. Tissue activities in the brain and other tissues decreased during a 110-160 minutes period. The blood activity was high, indicating rapid washout. The results of

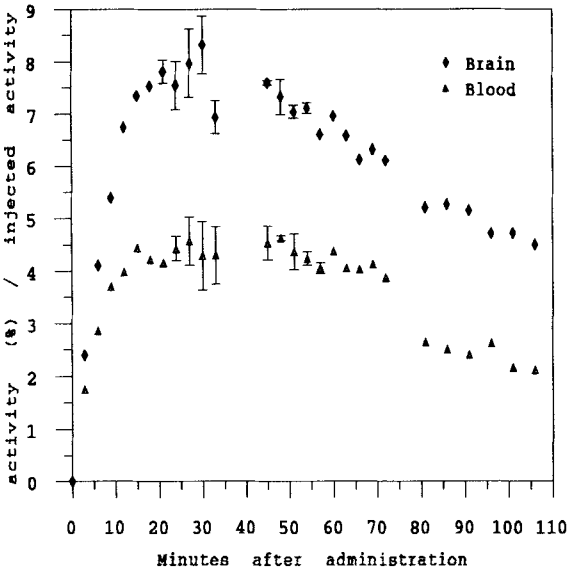

Figure 2.
Percentage of injected dose of 5-[^{123}I]iodonicotinamide in the brain (head) and blood (heart). Data points were calculated from regions of interest in dynamic pictures.

biodistribution experiments from tissue samples are seen in Table 1.

Table 1. Tissue distribution of 5-[^{123}I]iodonicotinamide in rat*

Tissue	Minutes after administration of I-123-NAM 110	160
Brain	0.20±0.03	0.11±0.00
Blood	0.71±0.00	0.55±0.14
Bone	0.24±0.02	0.17±0.01
Spleen	0.31±0.01	0.17±0.00
Liver	0.62±0.00	0.41±0.06
Kidney	2.94±0.18	1.55±0.11
Lung	0.38±0.02	0.24±0.02
Muscle	0.28±0.00	0.20±0.05
Heart	0.35±0.01	0.22±0.03
Thyroid	0.32±0.02	0.19±0.01

*Values represent mean dose (%) /g tissue for 2 rats per time interval with ± SEM.

The uptake of ^{123}I-NAM in the brain and other tissues was high with rapid washout after 30 minutes. The uptake in human

whole brain was 2.6 % of the injected C-11-NAM (1). The higher uptake in rat brain of injected ^{123}I-NAM might depend on nonspecific binding because the molecule is more liphophilic than nicotinamide. The 5-position of iodine in the pyridine ring suggests that the label is stable *in vivo* (3). The radioactivity in the thyroid declined during 110-160 minutes after injection indicating that no free iodine-123 was present. The rapid washout from the brain may have resulted from the rapid metabolism of the tracer into metabolites which have a lower affinity for the brain. Such metabolites of niacin in Wistar rats are N-methylnicotinamide, N-methyl-2-pyridone-5-carboxamide and N-methyl-4-pyridone-3-carboxamide (4). In conclusion the uptake in the brain and washout of ^{123}I-NAM was rapid. ^{123}I-NAM may be useful as a brain imaging agent.

EXPERIMENTAL

MATERIALS AND METHODS

Chemicals were purchased from Merck, Darmstadt if not otherwise mentioned. HPLC reagents were from Rathburn, UK. Gallenkamp, UK, melting point apparatus was used. After injection dynamic gamma camera (GE 400 A/T gamma camera with high resolution collimator) pictures were obtained. *In vivo* distribution was calculated from regions of interest of dynamic images. Radioactivity concentrations of tissue samples were determined by counting in a well NaI detector equipped with multichannel analyzer (EG&G Ortec ACEMate and model 916A MCB, USA). ^{1}H-NMR spectra were measured using a Bruker AM 400 WB instrument at 303 K. The purity of 5-bromo-NAM was estimated from the relative intensities from the signals in the ^{1}H-NMR spectrum. The HPLC system (Waters) used consisted of NaI gamma detector and UV detector with computer data acquisition. Analysis and purification was done using a Lichrosorb RP18 250 mm column and methanol-pH 6.0 phosphate buffer 40:60 v/v.

SYNTHESIS OF 5-BROMO-NICOTINAMIDE

5-Bromonicotinic acid (Aldrich, 3 g, 15 mmol) was dissolved in 10 ml of thionyl chloride and the mixture heated under reflux. After 2 hours the mixture was cooled and the remaining thionyl chloride was distilled off under reduced pressure. Cold NH_3 was added and the mixture filtered and washed with water. Acetone was added and the mixture filtered and the solution dried over anhydrous magnesium sulfate, filtered and evaporated. The ^{1}H-NMR spectrum obtained from the purified product showed no impurities.

Yield 1.5 g (51 %), mp 215 - 218°C. Literature melting point (acetone) 217°C. ^{1}H-NMR (acetone-d_6): δ 8.42 (1H, dd, $^4J_{H4,H2}$ = 1.9 Hz, $^4J_{H4,H6}$ = 2.3 Hz, C_4-H), δ 8.82 (1H, dd, $^4J_{H6,H2}$ = - 0.5 Hz, C_6-H), δ 9.29 (1H, d, C_2-H)

LABELLING OF 5-[^{123}I]IODONICOTINAMIDE

Purified 5-bromonicotinamide (1 mg, 5 μmol) was dissolved in 0.2 ml ethanol and added to a reaction vial containing the radioactive iodine is (2 mCi I-123 in 0.1 N NaOH, PSI, Switzerland). 20 μl Glacial acetic acid was added followed by 100 μl $CuSO_4$ (1mg, mol, anhydrous). The same volume of 0.01 N HCl was added as the volume of radioactive iodine (about 20 μ l). The reaction vial was heated at 150°C for 60 minutes. On cooling the mixture was dissolved in ethanol/0.01 M phosphate buffer pH 6.0. The solution was injected into the HPLC and the product peak collected and evaporated to dryness. The product was dissolved in 200 μl ethanol and 3 ml 0.9 % NaCl was added and the solution was steril filtered.

BIODISTRIBUTION STUDIES OF 5-[^{123}I]IODONICOTINAMIDE

Gamma camera experiments were performed with rats under anesthesia. 5-[^{123}I]nicotinamide was in normal saline with 5 % ethanol. The concentration was 30 μCi/200 μl. Five male Wistar rats (weight 100-150 g) were given a dose of 9-29 μCi

per rat via tail vein injection. After the gamma camera experiments the rats were sacrificed. Tissue activities were measured against a known standard. The amount of radioactivity in each tissue was expressed as a percentage of the injected dose per gram of tissue (dose (%) /g tissue).

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