A COMPARATIVE STUDY OF THE PREPARATION OF RADIOACTIVE 4-BROMOANTIPYRINE AND 4-IODOANTIPYRINE FOR THE MEASUREMENT OF CEREBRAL BLOOD FLOW

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SUMMARY

4-[^{82}Br]Bromoantipyrine has been synthesized by three methods: the melt method, the isotopic exchange in acidic medium method and the silica gel catalyzed method. The melt method is the simplest one and gives the highest radiochemical yield (~ 90%). 4-[^{131}I]-Iodoantipyrine has also been synthesized by these three methods with higher radiochemical yield than the bromo analog. The radiochemical yield of 4-[^{131}I]iodoantipyrine synthesized by the silica gel catalyzed method depends on the reaction time of antipyrine with [^{131}I]NaI solution, but does not depend largely on the contact time between antipyrine-[^{131}I]NaI solution with silica gel. I_2 or I^+ was proposed as an intermediate for this reaction.

The mean octanol/pH 7 buffer solution partition coefficient of 4-[^{82}Br]bromoantipyrine is 9.78.

Keywords: 4-[^{82}Br]Bromoantipyrine, 4-[^{131}I]Iodoantipyrine, melt method labelling, silica gel catalyzed labelling

INTRODUCTION

The advent of positron emission tomography (PET) has made it possible for the measurement of cerebral glucose metabolism under different pathological states in humans non-invasively (1,2). Recently, considerable effort has also been devoted to the application of PET for the measurement of regional cerebral blood flow (rCBF) in patients under different pathological states (2). Several compounds, such as [^{13}N]NH_3 (3), N_2O (4), [^{13}N]N_2O (5), [^{15}O]N_2O (6), ^{77}Kr (7), [^{131}I]CF_3I (8), [^{18}F]CH_3F (9),^{133}Xe (10), [^{15}O]H_2O (11-13) and ^{11}C-alcohols (14) have been used for the measurement of rCBF. However, these are all volatile compounds and there are a number of technical disadvantages associated with them. For example, the assay of volatile tracers in blood and tissues, particularly by the autoradiographic procedure, is far from convenient. A nonvolatile tracer to substitute for these tracers is therefore sought.

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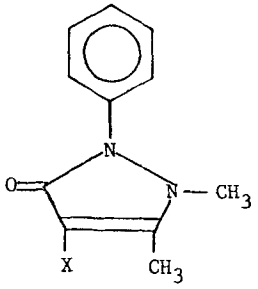
Recently, ^{123}I-labeled N-isopropyl-p-iodoamphetamine and ^{123}I-N,N,N'-trimethyl-N'-(2-hydroxy-3-methyl-5-iodobenzyl)-1,3-propanediamine have been used as rCBF tracers (15-20). The other lipophilic compound, radioisotopically labeled antipyrines, labeled with ^{14}C, ^{131}I, ^{125}I, ^{123}I or ^{11}C have also been used for the measurement of rCBF using autoradiography (21), gamma camera (22), single photon tomography (23) and positron emission tomography (24). However, there are several problems associated with radioiodinated 4-iodoantipyrine. It is unstable in vivo and the half-life for radioactive iodine is relatively long ($t_{1/2}$ = 13.3 hrs for ^{123}I; $t_{1/2}$ = 8 days for ^{131}I). In order to overcome these problems, we have synthesized 4-[^{18}F]fluoroantipyrine ([^{18}F]-4-FAP) (1) (Fig. 1) and used it successfully as a rCBF tracer (25-29). In extending the availability of radiopharmaceuticals such as [^{18}F]-4-FAP, we have attempted to synthesize other analogs which will have similar biological activities. We report here the synthesis of 4-[^{82}Br]bromoantipyrine ([^{82}Br]-4-BrAP) (2) (30) and compare its radiochemical yield with the iodo analog (3) synthesized by the same methods. Although in this study, ^{82}Br was used for convenience, because of its commercial availability, the same methods can be used for the synthesis of 4-bromoantipyrine labeled with the most useful positron emitter isotope of bromine, i.e. ^{75}Br.



1 X = F
2 X = Br
3 X = I

Fig. 1. Structure of 4-haloantipyrine

MATERIALS AND METHODS

Antipyrine and 4-bromoantipyrine were purchased from Aldrich Chemical Co. and used without further purification. 4-Iodoantipyrine was synthesized by the method of diMattio et al (31). [^{131}I]NaI in 0.1 N NaOH (reductant free) solution and [^{82}Br]NH_4Br in 0.1 M NH_4OH solution were obtained from New England Nuclear, Boston, MA. Thin layer chromatography was performed on plastic-back TLC plates coated with silica gel (Eastman Chromatogram 13181, 100μ thick). Silica gel (40-140 mesh) used for column chromatography was obtained from J. T. Baker Chem. Co. The solvent used for the development of antipyrine analogs was toluene-ethyl acetate (1:1 by volume).

Synthesis of 4-[^{82}Br]Bromoantipyrine (2)

Compound 2 was synthesized by three methods.

A. Melt Method: In a typical reaction, a solution of 4-bromoantipyrine (14.3 mg, 54 μmol) and [^{82}Br]NH_4Br (33.7 μCi) in 1 mL of methanol was placed in a V-shaped flask and was heated briefly under a gentle stream of nitrogen to dryness. The residue was allowed to melt (140°C) and remain as melt for 5 min and then cooled to room temperature. The mixture was dissolved in 1 mL of water and extracted with chloroform (3 x 1 mL). The organic layer was separated, passed through an anhydrous sodium sulfate column (1 x 4 cm) and evaporated to dryness to give 29.7 μCi (88.13%) of compound 2. The synthesis time was ~ 20 min. Radiochemical purity of 2 was determined to be > 99% by thin layer chromatography on silica gel, R_f = 0.61.

B. Isotopic Exchange in Acidic Medium: The synthesis is similar to that used to synthesize 4-[^{131}I]iodoantipyrine (32). In a typical experiment, a solution of 4-bromoantipyrine (12 mg, 45 μmol) in 1 mL of H_2O, [^{82}Br]NH_4Br (45.7 μCi) and 1 mL of 0.1 M H_3PO_4 in a multi-injection vial was kept in a water bath (100°C) for 5 min. The solution was cooled to room temperature and then 0.5 mL of 0.5 N NaOH was added. The solution was passed through a Dowex 1-X8 column (Cl^- form, 50-100 mesh, 3 mL), eluted with 1 mL of H_2O to give 5.7 μCi (12.5% radiochemical yield) of compound 2.

Table 1

Comparison of the Radiochemical Yields of 4-[^{131}I]Iodoantipyrine and 4-[^{82}Br]Bromoantipyrine Synthesized by Surface Catalysis

Contact Time (Hr) of Antipyrine-Halide Solution with Silica Gel	Yield (%)	
	[^{131}I]-4-IAP	[^{82}Br]-4-BrAP
2	50	< 1
24	90	~ 10

C. Silica Gel Catalyzed Reaction: An aliquot of antipyrine and [^{82}Br]NH_4Br in pH 2 buffer solution (KCl-HCl) was spotted on a silica gel plate, allowed to stand at room temperature at different time intervals and then developed with toluene-ethyl acetate (1:1). The tlc plate was cut into small sections and were assayed on a packard counter. The yield was determined as a ratio of ^{82}Br activity in the [^{82}Br]-4-BrAP and the total activity on the silica gel plates. The results are shown in Table 1.

Synthesis of 4-[^{131}I]Iodoantipyrine (3)

A. Melt Method: In a typical experiment, a solution of 4-iodoantipyrine (14.95 mg, 48 μmol) and [^{131}I]NaI (18.5 μCi) in 1 mL of methanol was placed in a V-shaped flask and was heated briefly under a gentle stream of nitrogen to dryness. The residue was allowed to melt (165°C) and remain as melt for 5 min and then cooled to room temperature. The mixture was dissolved in 1 mL of water and extracted with chloroform (3 x 1 mL). The organic layer was separated, dried over anhydrous sodium sulfate column and evaporated to dryness to give 14.2 μCi (76.8% radiochemical yield) of compound 3. Radiochemical purity of compound 3 was determined to be > 97% by thin layer chromatography on silica gel, R_f = 0.58.

B. Silica Gel Catalyzed Method: The synthesis of compound 3 by this method was similar to that described for the synthesis of compound 2 and can be subdivided into two methods.

1) **Silica gel plates:** Antipyrine (12.17 mg, 65 μmol) was dissolved in 0.1 mL of pH 2 buffer solution and then [^{131}I]NaI (0.433 mCi) was added. The solution was allowed to stand at room temperature at different time intervals. An aliquot of the solution was spotted on tlc plate, dried, allowed to stand at room temperature for 2 hrs and then developed. The tlc plate was cut into small sections and were assayed on a Packard counter. In a separated experiment, the antipyrine-[^{131}I]NaI solution was allowed to stand at room temperature at different time intervals, spotted on tlc plate, developed immediately and assayed as before. The yield of [^{131}I]-4-IAP was determined as a ratio of ^{131}I activity in [^{131}I]-4-IAP and the total activity on the silica gel plates. The results are shown in Table 2.

2) **Silica gel column:** The synthesis of [^{131}I]-4-IAP by this method is similar to that using silica gel plate except the solution of antipyrine and [^{131}I]NaI was applied on silica gel column instead of silica gel plate. The results are shown in Table 2. In a typical experiment, a solution of antipyrine and [^{131}I]NaI (250 μCi) was allowed to stand at room temperature for 24 hrs, loaded on silica gel column (5 x 0.5 cm), dried and then eluted with ethyl acetate-toluene (v/v 1:1, 8 ml) to give 189 μCi (75.6%) of [^{131}I]-4-IAP.

Determination of Partition Coefficients

Lipid/water partition coefficients were determined by adding 10 μL of 4-[^{82}Br]bromoantipyrine into a 4 mL vial containing 1 mL each of 1-octanol and pH 7.0 phosphate buffer. The vial was capped and mechanically shaken vigorously for 20 minutes at room temperature. After reaching equilibrium, each phase was counted in an automated packard NaI well counter. The partition coefficient was calculated as follows:

$$\frac{\text{cpm in 1-octanol}}{\text{cpm in pH 7.0 phosphate buffer}}$$

Table 2

Effect of Silica Gel on the Radiochemical Yield of 4-[^{131}I]Iodoantipyrine

Reaction Time (Hr) of Antipyrine with [^{131}I]NaI Solution Before Applying to Silica Gel	Yield (%)*			
	Without Silica Gel		With Silica Gel for 2 Hrs	
	From TLC	From Column	From TLC	From Column
0	9	11	17	6
2	50	--	--	--
22	--	--	69	60
24	91	76	--	--
47	--	--	87	98
50	96	90	--	--
70	98	90	96	97

* Percent yield was determined as a ratio of ^{131}I activity in [^{131}I]-4-IAP and the total activity applied on the silica gel.

RESULTS AND DISCUSSION

Radioiodinated antipyrines have been used as tracers for the measurement of regional cerebral blood flow (21-24). However, compared with radioiodine, bromine-75 has several attractive properties for use in nuclear medicine (33): (i) it is a positron emitter (β^+ = 1.7 MeV, 76%) and has a useful half-life (98 min), (ii) the C-Br bond is stronger than C-I bond (70 kcal/mole vs. 56 kcal/mole), resulting in relative stability of the label, (iii) radioiodide, which can be released by in vivo deiodination, is concentrated by the thyroid, delivering a high radiation dose to that gland. Free bromide, on the other hand, is not concentrated by any organ (34). Therefore, it is of interest to synthesize radioactive 4-bromoantipyrine for the measurement of cerebral blood flow.

Radioiodinated 4-iodoantipyrine has been synthesized by two methods: (a) the halogen exchange of radioactive iodide with 4-iodoantipyrine or 4-bromoantipyrine in acidic medium (32) and (b) iodination of antipyrine with radioactive iodide by surface catalysis (35). We have attempted to synthesize radioactive 4-bromoantipyrine by these two methods. However, the radiochemical yield of the radiobrominated compound synthesized by these two methods was much lower than the iodo analog especially by the latter method (Table 1). The melt method, on the other hand, gave both 4-[^{82}Br]bromoantipyrine (2) and 4-[^{131}I]iodoantipyrine (3) in high yield (80-90%).

The mechanism of the halogenation of antipyrine with radioactive halide catalyzed by silica gel is of interest. The radiochemical yield of 4-iodoantipyrine synthesized by surface catalysis depends on the reaction time of antipyrine with $^{131}I^-$ in acidic solution, but does not depend largely on the contact time of antipyrine-NaI solution with silica gel as depicted in Table 2. This is somewhat different from a recent report (36). The discrepancy between these two studies is probably due to different silica gel used as suggested by Diksic et al. However, our results would suggest that the formation of compound 3 synthesized by surface catalysis is probably due to

iodination of antipyrine by I_2 or I^+, generated by the oxidation of iodide by air in the presence of silica gel. Bromide ion has a higher oxidation potential than iodide ion (-1.065 volts vs. -0.5356 volts) and therefore is more difficult to be oxidized by air in the presence of silica gel to give Br_2 or Br^+. Hence, the reaction of antipyrine with [^{82}Br]NH_4Br at pH 2 on a silica gel surface gave 4-[^{82}Br]bromoantipyrine in very poor yield.

Although the reaction of antipyrine with [^{131}I]NaI in acidic conditions on a silica gel column can produce "no carrier added" 4-[^{131}I]iodoantipyrine, the "no carrier added" compound is usually not necessary for the measurement of rCBF. The melt method is therefore the simplest method to synthesize radioactive 4-iodoantipyrine and 4-bromoantipyrine, especially radioactive 4-bromoantipyrine.

The mean octanol/pH 7 buffer solution partition coefficient of [^{82}Br]-4-BrAP was 9.78 which was between [^{131}I]-4-IAP (11.34) and [^{18}F]-4-FAP (5.18) (37).

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