4-Iodophenylcholine: a potential myocardial imaging agent

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This paper is dedicated to Professor Paul de Mayo on the occasion of his 60th birthday

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The compound O-(4-iodophenyl)choline iodide has been synthesized using iodine-125, iodine-127, and iodine-131 by diazonium salt and melt exchange procedures. Radiochemical yields of approximately 2% and 56%, respectively, were obtained. The biodistribution of this compound in mice was analyzed in order to determine its potential as a myocardial imaging agent. Selective myocardial concentration was observed at 5 min post-injection, with a heart-to-blood ratio of 18:1.

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On a synthétisé l'iodure de la O-(iodo-4 phényl) choline marquée à l'iode-125, à l'iode-127 et à l'iode-131 en faisant appel soit à un processus impliquant un sel de diazonium soit à des échanges à l'état fondu. Pour chacune de ces méthodes, les rendements radiochimiques sont respectivement de 2% et 56%. Afin de déterminer le potentiel de ce composé comme agent pouvant créer des images du myocarde, on a analysé la biodistribution de ce composé dans les souris. On a déterminé les concentrations sélectives au niveau du myocarde 5 min après les injections et avec un rapport coeur/sang de 18:1.
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Introduction

Variations in regional myocardial blood flow can be detected by external imaging of radiotracer distributions in the myocardium, after intravenous injection. Thallous-201 ion is the current radionuclide of choice (1), but it has the disadvantages of being costly, of having suboptimal energy emmissions, and of requiring long study times during which the distribution may change. In tracer studies the initial distribution is blood flow limited and therefore a number of compounds may be considered as myocardial perfusion agents regardless of the mechanism of their concentration in the tissue.

Since iodine-123 is less expensive than thallous-201 ion, the development of an iodine-labelled myocardial imaging agent seemed attractive. Bioactive quaternary ammonium compounds are taken up by heart tissue by mechanisms which may or may not be receptor specific (2) and, when radiolabelled, are potentially useful as heart imaging agents. In pursuit of such agents, we were attracted to certain choline derivatives.

Benzoyl choline derivatives have been recently investigated (3) as myocardial imaging agents and have proven somewhat successful. While the ester **1** hydrolyzes too readily to permit accumulation, this is not the case for the amide **2**. An ether analog **3** should also be resistant to hydrolysis.

The 4-iodophenyl derivative **3** of choline can be labelled by a diazonium salt procedure. Alternatively, a direct exchange of iodide ion for aromatic iodide is another route to the labelled compound, provided that the specific activity is not critical.

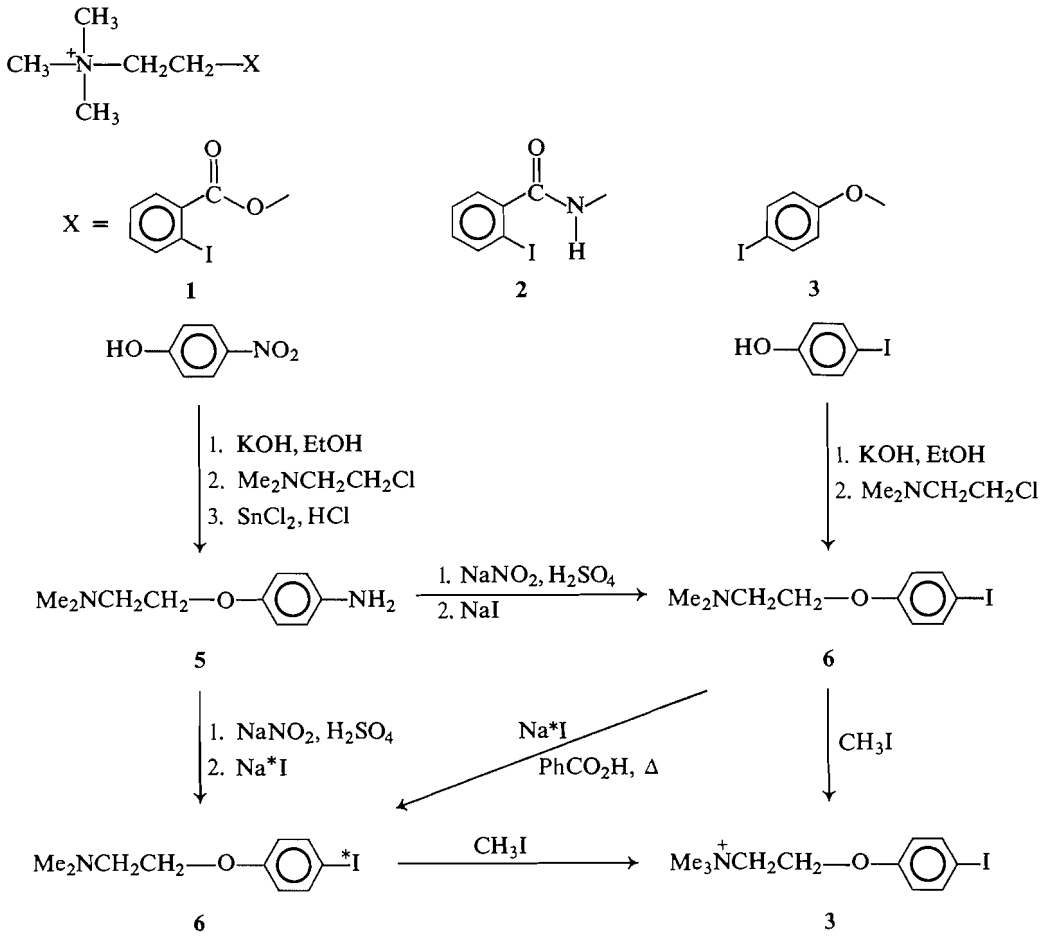
The position of the radioiodide within the molecule may affect both the labelling procedure and the biological distribution. From considerations of receptor specificity, an *ortho* halogen may produce undesirable steric effects. Further, it has been noted that nicotinic activity is more pronounced with *meta* halogen substitution than with the *para* analog (4). With the hope of minimizing such activity, the 4-iodo compound was prepared.

Results and discussion

As illustrated in the preparative scheme, several independent routes to the iodine-labelled quaternary ammonium salt **3** were investigated. The final step in each case involved reaction of the amine **6** with excess methyl iodide. Even with radioiodine-labelled **6**, this methylation requires only a few minutes and yields directly a saline solution of **3**. Using [^{125}I]-**6**, a sample of [^{125}I]-**3** was obtained in about 95% radiochemical yield and showed only one radioactive spot on tlc with an R_f corresponding to **3**.

As shown, the preparation of the radioiodine-labelled amine **6** was attempted both by a diazonium salt procedure (5) and by the direct exchange of **6**. The diazonium salt procedure using [^{125}I]-NaI gave very low radiochemical yields even with added carrier ([^{127}I]-NaI) to increase the overall iodide ion concentration. For example, with a 10,000-fold excess of [^{127}I]-NaI, a radiochemical yield of 2% was obtained. Consequently, the direct exchange of **6** with radioiodide in molten benzoic acid (6) was investigated. Treatment of 1 mg of **6** with [^{131}I]-NaI at 165°C for 1 h resulted in a 56% radiochemical yield. The **6** prepared in both these ways showed the same chromatographic behaviour as **6** that had been characterized by spectroscopic techniques.

This surprisingly facile and efficient exchange between iodide ion and aryl iodide seems to be fairly general and does not require a ring activated to nucleophilic substitution. A variety of solvents and conditions have been reported for iodide ion – aryl iodide exchange; neat liquid at 100–185°C (3, 7); acetamide at 140–180°C (8); benzoic acid at 170°C (6); and water at 100–150°C (9). We have found that, for **6**, thorough re-



moval of water is essential for efficient exchange using benzoic acid. While the mechanism of this process is in doubt ($S_{RN}1$?) (10), it is worth noting that there seems to be no production of the *ortho* or *meta* iodo analogues during the exchange process. A simple equilibration does not seem to be involved, since the exchange stops short of the anticipated equilibrium value and further heating does not lead to further exchange.

The time needed to convert the amino compound **5** into the ammonium salt **3** or to exchange **6** and convert it to **3** is about 2 h, which should be short enough to extend its use to iodine-123, which has a half-life of 13.3 h.

Biodistribution studies (Table 1) in mice showed rapid uptake in the myocardial tissue, with a maximum value of 20% of the injected dose per gram of tissue at 5 min post-injection. This percentage decreased continually from 5 min onward, dropping substantially in the heart between 15 and 30 min post injection. The only other organs showing significant amounts of radioactivity were the liver (21%) and the lungs (13%). Liver radioactivity decreased more slowly, as did the radioactivity in the other organs sampled. Blood radioactivity was always very low and increased somwhat as the study progressed, contrasting with the drop of radioactivity in the other organs.

From Table 2 it can be seen that the optimal time for imaging in mice as judged by heart/lung and heart/liver ratios would be within 5 to 15 min post-injection. In contrast, the amide **2** showed optimal ratios nearer to 30 min post-injection (3).

In considering the application of this to human imaging, interspecies variation must be borne in mind together with the shorter time scale often seen for physiological processes in smaller animals (11).

TABLE 1. Biodistribution of [^{125}I]-O-(4-iodophenyl)choline in mice

	% Uptake per gram of tissue: average of 4 mice ± S.D.			
Organ	5 min	15 min	30 min	60 min
Blood	1.1 ± 0.3	1.2 ± 0.2	1.4 ± 0.3	1.5 ± 0.2
Heart	19.9 ± 2.1	17.9 ± 1.6	6.0 ± 3.0	4.6 ± 1.0
Lung	13.1 ± 3.7	10.0 ± 0.4	4.1 ± 0.5	3.8 ± 1.0
Liver	20.6 ± 1.9	13.9 ± 1.4	11.1 ± 1.5	9.6 ± 3.0
Spleen	6.6 ± 1.9	6.4 ± 0.2	4.6 ± 0.6	5.8 ± 0.9
Muscle	1.9 ± 1.0	1.8 ± 0.3	1.2 ± 0.1	1.2 ± 0.4
Bone	3.0 ± 0.9	2.5 ± 0.3	1.6 ± 0.1	1.6 ± 0.5

Although in vitro protein binding experiments showed relatively high plasma protein binding (65%), very little of the compound was present in the blood at any time during the study. Since significant amounts of radioactivity resided in the heart, it would appear that the affinity of [^{125}I]-**3** for the heart tissue was much greater than that for the plasma protein component of the blood.

The present work involving radiolabelling with [^{125}I]-NaI and [^{131}I]-NaI has led to **3**, with specific activities of about 7.4 GBq/mmol (0.2 Ci/mmol) from the diazonium salt procedure and 148 MBq/mmol (4 mCi/mmol) for the melt exchange technique. Work is in progress to augment the specific activities from both these procedures in order to obtain material for experiments in receptor localization.

Biodistribution studies using the 4-iodo compound have shown significant uptake in the myocardium of mice at 5–15

TABLE 2. Selected organ ratios

Time (min)	Heart/blood ratio	Heart/lung ratio	Heart/liver ratio
5	18	1.5	1.0
15	15	1.8	1.3
30	4.3	1.5	0.5
60	3	1.2	0.5

min post-injection, with favourable heart/lung and heart/blood ratios at these times. At present the *meta* and *ortho* iodinated phenyl analogs are being investigated to improve labelling yield, specific activity, and biodistribution.

Experimental

Materials and methods

Melting points reported are uncorrected, elemental analyses were performed commercially (Guelph Chem. Labs. Ltd., Guelph, Ontario), and gas chromatography separations were accomplished with a 6-ft stainless steel column packed with 10% Carbowax-20M on Chromosorb P. The iodide-125 and iodide-131 were obtained commercially (C.E. Frosst and Co., Kirkland, P.Q.) as aqueous solutions of sodium iodide with concentrations of about 1.85 GBq/mL (50 mCi/mL). Radioactivity was assayed in a dose calibrator and in an automatic γ counter.

*1-Dimethylamino-2-(4-nitrophenoxy)ethane (**4**)*

A solution of potassium hydroxide (5.16 g, 0.1 mol) in 30 mL absolute ethanol was added to a solution of 4-nitrophenol (14 g, 0.1 mol) in 20 mL absolute ethanol. The orange-yellow potassium 4-nitrophenoxide salt was filtered, washed with a few milliliters of cold absolute ethanol, and air dried. Yield, 14.3 g (81%).

To a cooled solution of 2-dimethylamino ethylchloride hydrochloride (12.6 g, 0.09 mol) in 40 mL water, a solution of sodium hydroxide (4.2 g, 0.1 mol) in 20 mL water was added. The reaction mixture was saturated with solid sodium chloride and extracted with five 30-mL portions of toluene. The toluene fractions were combined and dried over potassium hydroxide pellets. The dried toluene solution was added to a suspension of potassium 4-nitrophenoxide (14 g, 0.08 mol) in 80 mL toluene and refluxed overnight.

After cooling, the reaction mixture was filtered and the filtrate concentrated on a rotary evaporator. A brown oily residue was obtained (13.1 g, 78%). Proton nmr (chloroform-*d*) δ: 2.25 (s, 6H, CH_3), 2.65 (t, 2H, CH_2), 4.10 (t, 2H, CH_2), 6.9 (d, 2H, aromatic H), 8.10 (d, 2H, aromatic H).

About 1 g of the crude product was purified as its hydrochloride salt by recrystallization from 2-propanol (mp 166–167°C). Infrared (ir) (Nujol): 1510 cm^{-1} (s, NO_2), 1330 cm^{-1} (s, NO_2), 1300 cm^{-1} (m, C—O—C). The proton nmr spectrum (DMSO-d_6) was consistent with its structure; δ: 2.80 ppm (s, 6H, CH_3), 3.50 (t, 2H, CH_2), 4.50 (t, 2H, CH_2), 7.10 (d, 2H, aromatic H), 8.10 (d, 2H, aromatic H).

*1-Dimethylamino-2-(4-aminophenoxy)ethane (**5**)*

Compound **4** (11 g, 0.052 mol) was added slowly, with stirring, to a solution of concentrated hydrochloric acid (80 mL) and stannous chloride dihydrate (47.2 g, 0.2 mol). After stirring at 0°C for 30 min, and then at room temperature overnight, the reaction mixture was made basic by addition of a very concentrated sodium hydroxide solution until all stannous hydroxide redissolved.

The resulting mixture was extracted with dichloromethane (4 × 100 mL). After washing with water (100 mL), drying, and solvent evaporation, a dark brown oil was left as residue. Recrystallization from petroleum ether (30–55° bp) gave pale yellowish needles, mp 51–52°C; yield: 7.72 g, 82%; ^{1}Hmr (chloroform-*d*) δ: 2.25 (s, 6H, CH_3), 2.60 (t, 2H, CH_2), 3.37 (s, 2H, NH_2), 3.90 (t, 2H, CH_2), 6.50 (m, 4H, aromatic H). *Exact Mass* determination calcd. for $C_{10}H_{16}N_2O$: 180.126; found (ms): 180.126.

*1-Dimethylamino-2-(4-iodophenoxy)ethane (**6**)*

(a) From 4-iodophenol

Reaction of potassium hydroxide (13 g, 22.7 mmol) with 4-iodophenol (5 g, 22.7 mmol) in 20 mL of absolute ethanol at room temperature left the 4-iodophenoxide salt as a yellowish oil after solvent evaporation. This salt was refluxed overnight with a 50-mL toluene solution of 2-dimethylaminoethylchloride prepared from the hydrochloride (3.3 g, 22.7 mmol) as described above.

After cooling and filtration, solvent removal on a rotary evaporator yielded 6.4 g of a crude oil which was converted to its hydrochloride by bubbling dry hydrochloric acid into an ether solution. Yield: 5.88 g, 79%; mp 186–187°C; ^{1}Hmr (dimethylsulfoxide-d_6) δ: 2.8 (s, 6H, CH_3), 3.42 (t, 2H, CH_2), 4.34 (t, 2H, CH_2), 6.76 (d, 2H, aromatic H), 7.52 (d, 2H, aromatic H).

(b) *From **5** using the diazonium salt method*

*(i) [^{127}I]-**6***

A 1.02-g (5.7 mmol) sample of **5** in 25 mL of 50% sulfuric acid at 0°C was reacted with a 1.17-g (17 mmol) solution of sodium nitrite in 3 mL of water. After about 10 min at 0°C, urea (0.7 g, 12 mmol) was added to destroy any excess nitrite. After bubbling had ceased (~10 min), a solution of sodium iodide (8.5 g, 57 mmol) and sodium sulfite (2.9 g, 23 mmol) in 30 mL of water was added. After 10 min at 0°C, 10 min at ambient temperature, and about 6 min heating at 80°C, the reaction was worked up by first making basic with sodium hydroxide.

Extraction with methylene chloride followed by a water wash, drying with sodium sulfate, and solvent evaporation left a dark solid residue. Filtration of an ether solution of this residue through a short column of neutral alumina yielded 0.21 g of a red oily product. This was further purified by tlc on alumina using ether as the eluent to yield 0.18 g (11%) of a material showing identical tlc and gc behaviour, as well as identical ^{1}Hmr and ir spectra, to the material from *p*-iodophenol. *Anal.* calcd. for $C_{10}H_{14}NOI$: C 41.25, H 4.85, N 4.81, I 43.59; found: C 41.29, H 4.85, N 5.0, I 43.01. *Exact Mass* determination: calcd. for $C_{10}H_{14}NOI$: 291.011; found (ms): 291.011. A mp of the hydrochloride of this material and a mixture mp with the hydrochloride of **6** from *p*-iodophenol were identical.

*(ii) [^{125}I]-**6***

Following the procedure described above, 2 mg (11 μmol) of **5** in 100 μL of 50% sulfuric acid was reacted with sodium nitrite (2.3 mg in 40 μL, 33 μmol). Urea (1.5 mg, 25 μmol) was added after 10 min at 0°C and, when bubbling had ceased, sodium iodide-125, 92.5 MBq (2.5 mCi in 50 μL) was added, followed by 40 μL of a solution of sodium iodide-127 (3.0 mg, 20 μmol) and sodium sulfite (1.0 mg, 8 μmol). After the usual extractive work-up, a material showing the same R_f on tlc as the authentic sample prepared above, with an activity of 50 μCi (2% yield), was isolated. This material was used without further purification for the synthesis of O-(4-iodophenyl)choline iodide.

(c) *[^{131}I]-**6** from **6** using a melt exchange method*

In a typical experiment, 1 mg of [^{127}I]-**6** and 3 mg of benzoic acid were dissolved in 0.2 mL of absolute ethanol in a 1-mL multi-dose vial. Approximately 80 μL (192 μCi) of [^{131}I]-NaI solution, 92.5 MBq (2.5 mCi/mL); was added and, after cooling in a Dry Ice – acetone mixture, the solvents were removed on a rotary evaporator.

The vial was then sealed with a rubber septum and aluminum cap and the solid mixture heated in an oil bath at 165°C for 60 min. After alkalinization and extraction with portions of methylene chloride, and washing and drying of the combined extracts, the radioactivity in the CH_2Cl_2 layer was 5.18 MBq (140 μCi) (73%). The CH_2Cl_2 was reduced in volume and used for tlc purification on alumina/ether against [^{127}I]-**6**.

The [^{131}I]-**6** was recovered from the tlc plate by extraction with methanol, filtered, and the solvent evaporated to yield purified[^{131}I]-**6**, 3.96 MBq, (107 μCi; 56% incorporation).

*O-(4-iodophenyl)choline iodide (**3**)*

(a) *From [^{127}I]-**6***

To 44 mg (0.15 mmol) of **6** dissolved in 3 mL chloroform, at room temperature, was added a solution of 440 mg (3.1 mmol) methyl

iodide in 2 mL chloroform. The reaction mixture was stirred for 30 min at room temperature. The excess methyl iodide and chloroform were evaporated and the white crystals left were washed with three portions (3–5 mL each) of chloroform and air dried.

The crystals were redissolved in methanol, filtered, and the solvent evaporated, yielding 62 mg of **3** as white crystals (95%), mp (dec.) 246°C; ^{1}Hmr (dimethylsulfoxide-d_6) δ: 3.2 (s, 9H, $^+$N $(CH_3)_3$), 3.8 (t (br), 2H, CH_2), 4.45 (t (br), 2H, CH_2), 6.84 (d, 2H, aromatic H), 7.60 (d, 2H, aromatic H). *Anal.* calcd. for $C_{11}H_{17}NOI_2$: C 30.51, H 3.96, N 3.23, I 58.61; found: C 30.66, H 3.98, N 3.25, I 58.32.

(b) *From [^{125}I]-6 or [^{131}I]-6*

The 1.85-MBq (50 μCi) sample of [^{125}I]-**6** prepared as previously described was dissolved in 2 mL chloroform and excess methyl iodide (20–30 mg) was added. The mixture was refluxed for 30 min (65–70°C). After cooling, the chloroform layer was transferred to a 12-mL centrifuge tube and the reaction flask washed twice with 0.5 mL saline solution. The saline portions were added to the chloroform and thoroughly mixed. After separation of the two layers had occurred, the saline was carefully recovered and transferred to a 3-mL screw cap vial and its radioactivity assayed: 1.81 MBq (about 49 μCi).

The saline solution of [^{125}I]-**3** was later diluted, filtered through a millipore filter, and used for the biodistribution studies.

This material showed tlc (alumina; CH_3CN/H_2O 9:1) behaviour identical to the [^{127}I]-**3** material characterized above.

Biodistribution studies

White Swiss mice were initially anaesthetized with an intraperitoneal injection of sodium pentobarbital (80 mg/kg body weight) and then received a 0.1-mL intravenous tail injection of 93 MBq (2.5 μCi) of [^{125}I]-**3**. Four mice were sacrificed by cardiac puncture/exsanguination at each post-injection period of 5, 15, 30, and 60 min. Selected organs were washed, patted dry, and weighed, along with the blood samples. Tissues and injection standards were subsequently assayed for radioactivity. Results were expressed as percent uptake per gram of wet tissue.

Protein binding

The in vitro binding of **3** to human serum proteins was determined by adding 100-μL aliquots of [^{125}I]-**3** to 1 mL of plasma in each of four centrifuge tubes. At 5, 15, 30, and 60 min, 100 μL of the plasma – [^{125}I]-**3** mixture was added to duplicate tubes containing 1 mL of 10% trichloroacetic acid, followed by centrifugation.

The supernatant was removed and, after washing the pellet with normal saline, the combined supernatant washings and the pellets were assayed for radioactivity. 66% of [^{125}I]-**3** was found bound at 5 and 15 min, 65% at 30 and 60 min.

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