Potential Cerebral Perfusion Agents. Synthesis and Evaluation of Berberine Analogues

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The synthesis and tissue distribution studies in rats of tetra[^{3}H]-hydroberberine ([^{3}H]**2**) and 8-(*p*-[^{125}I]iodobenzyl)tetrahydroberberine ([^{125}I]**6**) are described. Compound **2** was synthesized by sodium borohydride reduction of berberine hydrochloride (**1**). Treatment of berberine hydrochloride with *p*-bromobenzylmagnesium bromide gave 8-(*p*-bromobenzyl)dihydroberberine (**4**) which after sodium borohydride reduction and iodine-125 bromine exchange gave [^{125}I]**6**. The unsubstituted tetrahydro compound [^{3}H]**2** showed significantly higher brain uptake (2.2% dose/gm after 5 minutes) as compared to the corresponding 8-substituted derivative [^{125}I]**6**. Both radiolabeled compounds washed out from the brain relatively quickly.

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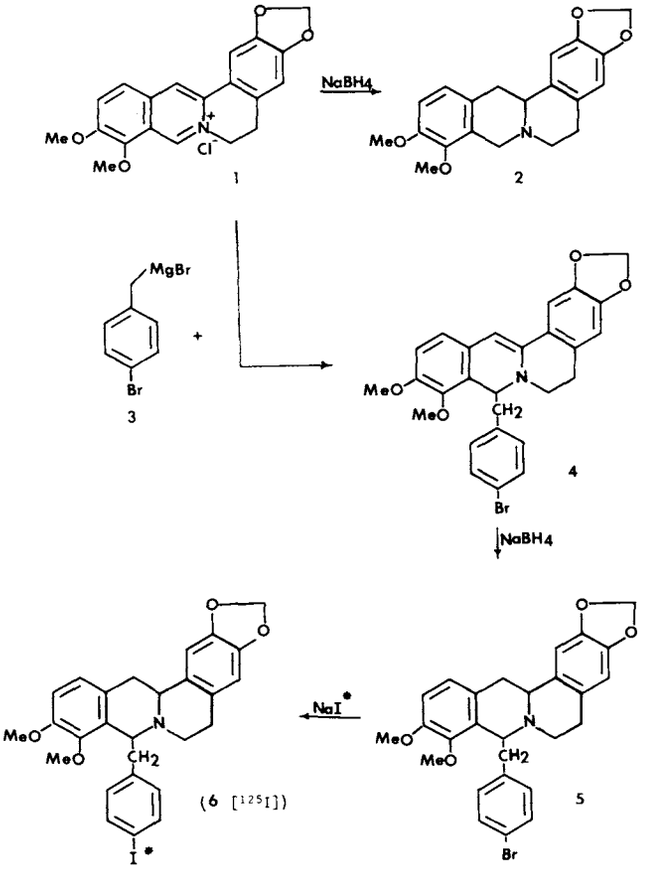
Many radiolabeled lipophilic agents cross the intact blood brain barrier and distribute in the brain as a function of regional cerebral blood flow [1]. These agents are potentially useful to measure regional cerebral blood perfusion for clinical detection of brain disease [2]. New studies [3,4] in this area have been stimulated as a result of the recent availability of high purity iodine-123 and the development of *p*-[^{123}I]iodo-*N*-isopropylamphetamine [5,6] which is being used for single photon emission computerized tomographic (SPECT) studies in humans. The development of new agents with high brain uptake, and prolonged retention with minimal redistribution are desired for SPECT studies which require long acquisition times.

Recently Bodor and Hassan [7] have reported a unique approach for brain specific delivery of redox agents such as berberine (**1**). According to this approach [7] a chemically reduced lipophilic agent is transported into the brain across the intact blood-brain barrier where the lipophylic agent is readily oxidized to a non-lipophilic quaternary form. The oxidized non-lipophilic form is not easily released from the brain resulting in prolonged intracerebral retention. We have evaluated this technique as a logical approach for the delivery of radiolabeled agents to the brain for potential SPECT studies using redox-type agents such as pyridines [8] and berberines.

Berberine (**1**) is a redox-type agent which shows a broad spectrum of biological effects including sedative and hypotensive activity [9-11]. The possibility of localization of berberine in the pancreas and an attempt to label berberine with iodine-131 by opening the exocyclic dioxole ring has been described in the literature [12]. In this report, we describe the synthesis and tissue distribution studies of radiolabeled berberine analogues to evaluate the redox approach for potential application in SPECT studies. Our obvious attempt was first to study, for brain uptake, a tritium labeled berberine which appeared to be relatively simple to synthesize and then, if the tissue distribution data justified, to proceed with an ^{125}I-labeled berberine analogue for similar studies.



Chemistry.

The preparation of dihydroberberine by sodium borohydride reduction of berberine hydrochloride (**1**) has been reported [7,13,14]. We have found, however, that sodium borohydride reduction of **1** in methanol gave pre-

dominantly tetrahydroberberine (**2**) as identified by elemental analysis and mass spectral data. In the ^{1}H nmr (DMSO-d_6) of **2**, the 8 and 13 protons were shifted upfield as compared to the C-8 and C-13 protons of berberine hydrochloride appearing at δ 10.35 and δ 9.4 respectively. Similar reduction of berberine hydrochloride with tritium labeled sodium boro[^{3}H]hydride in methanol provided after column chromatography the desired tritium labeled [^{3}H]tetrahydroberberine ([^{3}H]**2**).

Our attempts for direct iodination of berberine using conventional procedures were unsuccessful. Consequently, we chose to attach ^{125}I to a functional group attached to berberine. The approach which appeared to be relatively simple for preliminary evaluation studies was the attachment of ^{125}I in the form of *p*-[^{125}I]iodobenzyl moiety to position 8 of berberine. Treatment of berberine hydrochloride with *p*-bromobenzylmagnesium bromide (**3**) gave 8-(*p*-bromobenzyl)dihydroberberine (**4**). Similar benzylation reactions of berberine have previously been reported from other laboratories [10,15]. Sodium borohydride reduction of **4** gave 8-(*p*-bromobenzyl)tetrahydroberberine (**5**). The radiolabeled compound, 8-(*p*-[^{125}I]iodobenzyl)tetrahydroberberine ([^{125}I]**6**) was prepared on a microscale (10 μCi) by iodine-125 exchange (~ 1%) of bromine in **5**. Our attempts to increase the yield (exchange) by changing the reaction conditions and/or by increasing the temperature were unsuccessful and resulted only in the decomposition of **5**. Compound [^{125}I]**6** showed a single radioactive spot which, as expected, had the same mobility as **5** when examined on tlc.

Biological Evaluation.

Tissue distribution studies in female Fischer 344 rats were performed as described previously [8]. The radioactive compounds, [^{3}H]**2** and [^{125}I]**6**, were formulated in normal saline solution containing tween 80 (1%) and ethanol (10%). Four animals per time period were used and the distribution of radioactivity in brain, blood, liver, lungs and kidneys was determined after intravenous administration of the radioactive compounds (Table 1). After 5 minutes the [^{3}H]tetrahydroberberine showed 2.2% brain uptake (brain:blood ratio 6:1) which declined to ~ 0.13% within 60 minutes apparently due to the washout of the activity from the brain. The loss of activity could be partly attributed to the anticipated loss of tritium label from [^{3}H]**2** due to the facile oxidation of [^{3}H]**2** to the quaternary compound **1** in the brain. The chemical form (e.g. as ^3H_2O, [^{3}H]**2** or as a tritium metabolite) of the tritium release was not determined in these preliminary studies. The iodobenzyl derivative [^{125}I]**6**, synthesized and evaluated to overcome such loss of radiolabel due to oxidation, showed relatively low brain uptake (Table 1) as compared to the parent unsubstituted compound [^{3}H]**2**. This observation, however, is similar to an earlier report [11] describing a lesser degree of potency as a central depressant for 8-benzyltetrahydroberberine as compared to tetrahydroberberine. The data indicate that the attachment of a radioiodide bearing benyl-type bulky groups in tetrahydroberberine results in diminished specificity for the brain and the effect of radioactive iodine or bromine attachment directly to the berberine skeleton on brain specificty should be evaluated.

EXPERIMENTAL

General.

All chemicals and solvents were analytical grade and were used without further purification. The iodine-125 and sodium boro[^{3}H]hydride were purchased from New England Nuclear, Inc., (North Billierica, MA). The melting points (mp) were determined in capillary tubes using a Buchi SP apparatus and are uncorrected. The low-resolution mass spectra (ms) were recorded at 70 eV using a Kratos MS 25 instrument. The thin-layer chromatographic analyses (tlc) were performed using 250 μm thick layers of silica gel G PF-254 coated on glass plates (Analtech, Inc.). The radiochemical purity of ^{125}I-labeled compounds was determined on tlc using a Technical Associate Radiochromatogram scanner. The proton nuclear magnetic resonance spectra (nmr) were obtained at 60 MHz with a Varian 360-L instrument and the resonances (ppm) are reported downfield (δ) from the internal tetramethylsilane standard. The elemental analyses were determined by Galbraith Laboratories, Knoxville, Tennessee.

Table 1

Distribution of Radioactivity in Tissues of Female Fischer 344 Rats Following Intravenous Administration of Radiolabeled Tetrahydroberberines [a]

Compound	Time after injection (Minutes)	Mean percent injected dose/gram Tissue				
		Brain	Blood	Liver	Lungs	Kidneys
[^{3}H]**2**	5	2.20	0.36	3.44	1.40	1.88
	30	0.27	0.16	1.74	0.81	0.75
	60	0.13	0.09	1.06	0.22	0.38
[^{125}I]**6**	5	0.42	1.81	4.06	1.81	1.58
	30	0.26	1.37	2.35	1.02	1.00

[a] In respective experiments each animal received 19.45 μCi of [^{3}H]**2** or 0.14 μCi of [^{125}I]**6** by tail vein injection.

Animal Tissue Distribution Studies.

The distribution of radioactivity was determined in tissues of 10-12 week old female Fischer 344 rats (170-200 g) after intravenous administration of the radiolabeled compounds. The animals were allowed food and water *ad libitum* prior to and during the course of the experiment. The radioiodinated compounds were formulated in a normal saline solution containing tween 80 (1%) and ethanol (10%). The solution was filtered through a 0.22 μm Millipore filter and injected *via* a lateral tail vein into the ether anesthetized animals. After the times indicated, the animals were killed by cervical fracture, and blood samples were obtained by cardiac puncture. The organs were then removed, rinsed with saline solution, and blotted dry to remove residual blood. For [^{125}I]**6** the organs were weighed and counted in a NaI autogamma counter (Packard Instruments). For the [^{3}H]**2** experiment, 100 mg samples of the tissues were digested in 1 ml of protosol solution (New England Nuclear, Inc.) by heating overnight at 55°. The samples were decolorized by adding benzoyl peroxide solution (20% in toluene, 0.25 ml) and heating at 55° for 1 minute. Aliquots (100 μl) were diluted in scintillation fluid and counted in a Tri-Carb liquid scintillation spectrometer. Samples of the injected radioactive solutions were also assayed as standards to calculate the percent injected dose per gram of tissue values.

Tetrahydroberberine (**2**).

Sodium borohydride (76 mg, 2 mmoles) was added to a stirred suspension of berberine hydrochloride hydrate (372 mg, 1 mmole) in methanol (5 ml) cooled in an ice bath. The solution was stirred for 15 minutes and then evaporated under vacuum. The residue was dissolved in chloroform and passed through a column of silica gel packed in chloroform. Elution with 2% methanol in chloroform gave 170 mg (50%) of **2** which was recrystallized with ethyl ether; mp 170-171° (Lit [13] 171-172°); ms: m/e 339.

Anal. Calcd. for $C_{20}H_{21}O_4N$: C, 70.78; H, 6.24; N, 4.13; Found: C, 70.76; H, 6.30; N, 4.13.

Dihydro-8-(*p*-bromobenzyl)berberine (**4**).

The Grignard reagent was prepared as follows. Magnesium turnings (0.58 g, 24 mmoles), were added to a solution of *p*-bromobenzylbromide (6.0 g, 24 mmoles) in absolute ethyl ether (35 ml) under argon atmosphere. After the vigorous reaction had ceased, the reaction mixture was refluxed for 2 hours. The mixture was then cooled with an ice-bath and berberine hydrochloride (0.89 g, 2.4 mmoles) was slowly added. The reaction mixture was allowed to warm to room temperature and stirred overnight. The reaction mixture was poured in ice, acidified with 6 *N* hydrochloric acid and extracted with ether. The aqueous portion was cooled, treated with concentrated ammonium hydroxide to *p*H 12-14 and extracted with chloroform (50 ml x 3). The combined chloroform layers were dried (sodium sulfate) and evaporated *in vacuo* affording a pale green oil, which on addition of ether afforded pale yellow crystals of **4**, yield 80% mp 123-124°. Recrystallization from methanol gave the analytical sample (mp 141-142°); ms: m/e 505.

Anal. Calcd. for $C_{27}H_{24}BrNO_4$: C, 64.04; H, 4.78, N, 2.77; Br, 15.78. Found: C, 64.21; H, 5.00; N, 2.58; Br, 15.98.

Tetrahydro-8-(*p*-bromobenzyl)berberine (**5**).

Sodium borohydride (60 mg, 1.6 mmoles) was slowly added to a solution of compound **4** (330 mg, 0.65 mmole) in anhydrous methanol (30 ml). The reaction mixture was stirred at room temperature for 2 hours and then poured into ice and acidified with 6*N* hydrochloric acid to dissolve the inorganic salts. The solution was adjusted to *p*H 8 by adding concentrated ammonium hydroxide at such a rate so that the temperature did not rise above 10°. The precipitated white crystals were collected by filtration (yield 92%). An analytical sample was obtained by recrystallization from methanol-water, mp 168-169°; ms: m/e 507.

Anal. Calcd. for $C_{27}H_{26}BrNO_4$: C, 63.78; H, 5.16; N, 2.76; Br, 15.72. Found: C, 64.10; H, 5.10; N, 2.63; Br, 15.61.

Synthesis of Radiolabeled Compounds.

Tetra[^{3}H]hydroberberine([^{3}H]**2**).

The [^{3}H]**2** was prepared from **1** (37.2 mg, 0.1 mole) following the procedure described for the unlabeled compound **2** except that sodium boro[^{3}H]hydride (25 mCi, 7.8 mg, specific activity (121 mCi/mmole) was used for reduction. The product [^{3}H]**2** was identified by comparing with the unlabeled standard on tlc.

8-(*p*-[^{125}I]Iodobenzyl)tetrahydroberberine ([^{125}I]**6**).

A solution of compound **5** (1.0 mg) and Na[^{125}I] (1 mCi) in 2-butanone was heated in a sealed tube for 16 hours at 90°. The mixture was applied on a 20 x 20 cm (250 μ thickness) preparative silica gel plate and developed in 25% acetone in hexane. The uv absorbing and radioactive band corresponding to **5** was scraped and eluted with ethyl ether to yield [^{125}I]**6** (10 μCi, 1% exhange) for tissue distribution studies. The radiochemical purity for [^{125}I]**6** was determined using a tlc scanner which showed a single peak for the radioactive compound having the same tlc mobility as **5**.

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