RADIOBROMINE LABELLING OF 3,4-DIHYDROXY-PHENYL-L-ALANINE AND DISTRIBUTION STUDY IN MOUSE BRAIN

Hu Fang (胡 芳) and Li Yongjian (李永键)

(Shanghai Institute of Nuclear Research, Academia Sinica, Shanghai 201800, China)

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ABSTRACT

The synthesis of 3, 4-dihydroxy-6-bromo-phenyl-L-alanine (6-Br-L-dopa), preparation of 3,4-dihydroxy-6-[^{77}Br] bromo-phenyl-L-alanine(6-^{77}Br-bromo-L-dopa) and its distribution study in mouse brain are described. The radiochemical yield and radiochemical purity of 6-^{77}Br-bromo-L-dopa were 8.5 % and 93 %, respectively. Its distribution in mouse brain after one hour injection indicates that it does pass blood brain barrier. The ratio of uptake in striatum to that in cerebellum was 2.45±0.12. The results appeared that 6-^{77}Br-bromo-L-dopa could be used as brain tracer for visualizing dopamine-containing brain structures by using single photon tomography.

Keywords: 6-bromo-L-dopa 6-^{77}Br-bromo-L-dopa SPECT-radio-pharmaceutical Dopamine-brain-structure

1 INTRODUCTION

Several brain disorders, such as Parkinson's, Huntington's diseases and Schizophrenia are believed to involve change in dopaminergic system[1]. The turnover rate of dopamine is a useful measure of neuronal activity. This kind of measurement not only can be of aid in basic studies of neuronal function but also provide a potential diagnostic tool for clinical use.

Dopamine itself does not penetrate the blood-brain-barrier, but its metabolic precursor L-dopa labelled with radioisotopes (such as ^{75}Br, ^{77}Br and ^{18}F) can pass blood-brain-barrier and allow to be decaboxylated in vivo[2], therefore, radiolabelled analogues of L-dopa is significant for studying the turnover of L-dopa in brain.

Firnau *et al* had developed a radiolabelled analogue of L-dopa, 6-^{18}F-fluoro-L-dopa for imaging DA-containing structures in the human brain by positron emission tomography (PET). Because of lack of radiospecificity of direct fluorinated procedure, the yields of 6-^{18}F-fluoro-L-dopa were low (1—4 %)[3-5]. The bromination of L-dopa, on the other hand, leads exclusively to produce

6-bromo-L-dopa[6]. Thus it appears that a radiobrominated analogue of L-dopa might be served as a more convenient tracer for visualizing DA-containing brain structures.

In this report, 6-bromo-L-dopa was synthesized, L-dopa was labelled with ^{77}Br and the distribution of 6-^{77}Br-bromo-L-dopa in mouse was studied. Bromination of L-dopa did give the sole product of 6-bromo-L-dopa. The distribution study in mouse brain confirmed that 6-^{77}Br-bromo-L-dopa can pass blood brain barrier and enter DA-containing brain structures. These results indicated that 6-^{77}Br-bromo-L-dopa would be of useful for DA-containing structures imaging by SPECT.

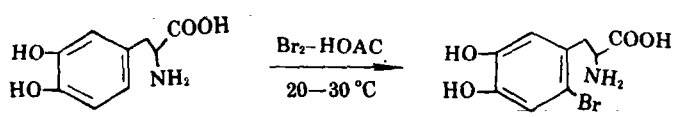
2 MATERIALS AND METHODS

2.1 Material

3,4-dihydroxy-phenyl-L-alanine (L-dopa) and all other chemicals are A.R grade. Each mouse was weighed around 20 g. Hitachi 638-50 model high performance liquid chromatography (HPLC) was used to monitor brominating reaction and to analyse product. The system consisted of Dupont ODS. 4.6 mm × 150 mm column, uv detector (280 nm) (the mobile phase used was MeOH in 0.1 % H_3PO_4(50:50)), FJ-2109 model γ-ray scanning equipment, 4096 channel analyser and well type γ-ray counting equipment.

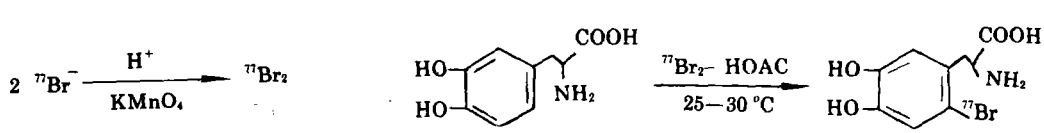
2.2 Procedures

2.2.1 Preparation of 6-Br-L-dopa Its synthesized reaction is as follows:



L-dopa of 0.59 g was dissolved in 30 ml glacial acetic acid with several drops of HCl added to complete the solution. Three ml of 1 mol/l Br_2 in HOAC was added in drop-wise at 20-30 °C. One μl sample each of the reaction mixture was analysed by HPLC at different time intervals during the reaction. When the reaction was completed, the solvent was evaporated almost to dryness and distilled water and sodium bicarbonate added to make pH 5.0. The bromo-dopa product was crystallized at 4 °C, vacuum filtered, washed with distilled water, and finally dried. The purity of the product was determined by HPLC, NMR and mass spectrometry.

2.2.2 ^{77}Br labelling of L-dopa Its labelling reaction process is as follows.



The diagram of reaction equipment is showed in Fig.1. At first, flask B was colded

with liquid nitrogen. In flask C, 1 g $Na_2S_2O_3$ in 10 ml water was added. 92.5 MBq $Na^{77}Br$/200 μl H_2O, 10 drops of 0.143 mol/l $KMnO_4$, 10 drops of H_2SO_4(98 %) and 5 mg NaCl/50 μl H_2O were added to flask A. Passing N_2 for 5 min to carry ^{77}Br to flask B, then stopping N_2 gas, 200 μl glacial HOAC was added to flask B. When it was molten, 10 mg dopa in 500 μl HOAC were added to flask B.

2.2.3 Purification The reaction solution in flask B was purified by silica-gel loaded paper chromatography (SLPC). The eluants were n-butanol:glacial acetic acid:water = 3:1:1(V/V). In comparison to standard sample developed with 0.2 % ninhydrin methanol solution, solica-gel loaded paper containing radiolabelled product was cut down and extracted. The radiochemical purity was determined by SLPC.

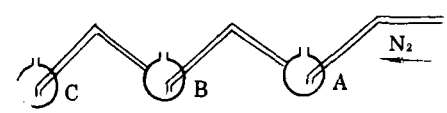


Fig.1 The diagram of reaction equipment

2.3 Mice distribution study of the radiopharmaceutical

The eluate was filtered and the solvent was partly evaporated. Sodium bicarbonate was added to adjust pH 5.0.

The mice were injected with 6-^{77}Br-bromo-L-dopa solution through the tail vein. Each mouse was injected 0.5 ml containing 340000 cpm of 6-^{77}Br-bromo-L-dopa. Mice were killed at 15 min, 60 min and 4 h after injection, and the tissues were sampled, weighed and counted.

3 RESULTS AND DISCUSSION

3.1 The identification of brominated L—dopa

The NMR of the direct brominated L-dopa showed aromatic proton peak at 7.09 ppm and 7.14 ppm, with para coupling. The mass spectrometry showed characteristic m/e peaks of 3,4-dihydroxy-6-Br-phenyl-$^-$CH and 3,4-dihydroxy-6-Br-phenyl-$^+$ CH_2 with equal area, m/e values of 201,203 and 202,204 is in consistent with bromine isotopic ratio. These analyses indicated the existence of a mono-brominated product, 6-bromo-L-dopa.

Using the same procedure for ^{77}Br labelled L-dopa, the brominated L-dopa synthesized through gas-liquid reaction possessing NMR spectrum in agreement with 6-bromo-L-dopa. R_f value of ^{77}Br labelled L-dopa was the same as 6-bromo-L-dopa in HPLC and SLPC.

3.2 Effect of reaction parameters on direct bromination

3.2.1 Effect of reaction time on yield (Fig.2) Using HPLC analysis, the yield of the direct bromination at 20 °C after 5 min was 65 %, and no more increased after 47 min.

3.2.2 Effect of reaction temperature on yield (Fig.3) At reaction time of one hour,

the yield was increased with temperature, reached maximum value of 100 % above 25 °C.

3.2.3 Effect of reaction temperature on equilibrium time Fig.4 shows that the higher the reaction temperature, the less the equilibrium time from 20 °C to 25 °C. At 25 °C, equilibrium time approaches to a constant value.

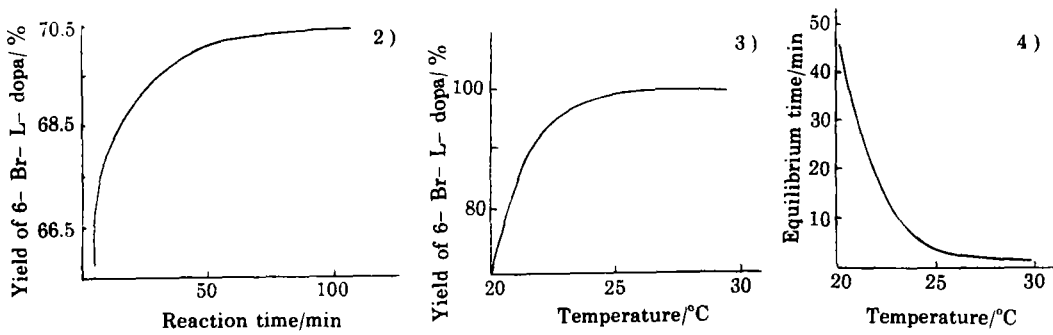


Fig.2—4 Effect of reaction time on yield (2) and of reaction temperature on yield (3) as well as on equilibrium time (4)

3.3 Distribution of 6—^{77}Br—bromo—L—dopa in mice

The uptakes in tissues of mice one hour after injection is showed in Table 1. The

Table 1

The uptake in tissues of mice one hour after injections

Mouse No.	Striatum	Cerebellum	Blood	Liver	Heart	Lung	Spleen	Kidney
1	2.28	0.93	3.29	1.54	1.07	2.92	1.46	5.97
2	1.04	0.44	3.30	1.58	0.89	2.71	1.49	3.10
3	1.05	0.51	2.56	1.33	1.24	1.97	1.60	5.67
$x \pm S$	1.46±0.71	0.63±0.27	3.05±0.42	1.48±0.13	1.07±0.18	2.33±0.37	1.50±0.09	4.88±1.54

Table 2

The ratio of uptake in striatum to that in cerebellum of mouse at 15, 60, 240 min after injection

Time/min	15			60			240		
Mouse No.	A_1	A_2	A_3	B_1	B_2	B_3	C_1	C_2	C_3
Ratio	0.78	0.77	0.74	2.49	2.68	2.45	0.25	0.53	1.43
$x \pm S$	0.76±0.02			2.54±0.12			0.74±0.62		

uptakes of kidney and blood are the highest, next is lung, striatum is at the third place on the same level of uptake as liver and spleen, but much higher than

cerebellum. Table 2 shows the ratio of uptake in striatum to that in cerebellum of mice at 15, 60 and 240 min after injection.

Fig.5 shows that the ratio of uptake in striatum to that in cerebellum has the highest value of 2.45±0.12 at one hour after injection. This result indicats that 6-^{77}Br-bromo-L-dopa entered striatum of DA-containing structure and concentrated in striatum at one hour post i.v. Before 15 min post injection 6-^{77}Br-bromo-L-dopa couldn't be detected in striatum and after 4 h, it was almost vanished.

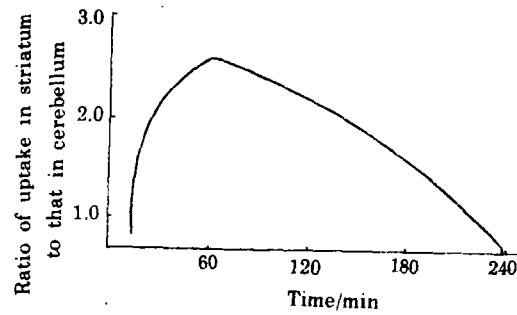


Fig.5 The ratio of uptake in striatum to that in cerebellum vs time

4 CONCLUSION

In this study, through direct gas-liquid bromination, the only product of 6-bromo-L-dopa was obtained, the oxidant and precursor were kept in separate solution, thus the possibility of precursor oxidation could be prevented and radiochemical yield of 8.5 % and radiochemical purity of 95 % were obtained. The distribution of 6-^{77}Br-bromo-L-dopa in mice indicated that it did pass blood-brain barrier and enter striatum of brain DA-containing structure. The ratio of uptake in the striatum to that in the cerebellum one hour after injection was 2.45± 0.12. Our methods are different from Ref.[2], in which the oxidant and precursor are in the same solution, no radiochemical purity is given and the uptake ratio of the target to the nontarget tissue is lower (1.25± 0.4). In conclusion, 6-^{77}Br-bromo-L-dopa is easily prepared through gas-liquid bromination reaction, it would be a potential brain tracer for visualizing DA-containing brain structure.

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