Note

Preparation of [^3H]6-Nitroquipazine, a Potent and Selective 5-Hydroxytryptamine Uptake Inhibitor[†]

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The synthesis of 6-nitroquipazine, a very potent and selective 5-hydroxytryptamine (5-HT; serotonin) uptake inhibitor, labeled with tritium is described. High specific activity [^3H]6-nitroquipazine could be prepared by the nitration of [^3H]quipazine using a mixture of equal volumes of sulfuric acid and nitric acid. The radiochemical yield was approximately 50% based on [^3H]quipazine. The radiochemical purity was more than 95% from high performance liquid chromatography (HPLC) and thin layer chromatography (TLC) determinations. [^3H]6-Nitroquipazine would be a new suitable radioligand for studying 5-HT transporter complex in brain and platelets.

Key Words: tritium labeled 6-nitroquipazine, 5-hydroxytryptamine uptake inhibitor, nitration

1. Introduction

6-Nitroquipazine (DU 24565; 6-nitro-2-piperazinylquinoline) is a very potent and selective 5-hydroxytryptamine (5-HT; serotonin) uptake inhibitor^{1),2)}. Furthermore, 6-nitroquipazine inhibited very potently *in vivo* [^3H]paroxetine binding to 5-HT uptake sites in the mouse brain³⁾.

[^3H]Imipramine and [^3H]paroxetine have been widely used as radioligands for studying 5-HT transport system. It was reported that 6-nitroquipazine was a more potent 5-HT uptake inhibitor than imipramine or paroxetine^{1),2)}. To our knowledge, of all 5-HT uptake inhibitors available today, 6-nitroquipazine has the highest affinity toward 5-HT uptake sites. [^3H]6-Nitroquipazine might be a new suitable radioligand for studying 5-HT

transport system. The preparation of [^3H]6-nitroquipazine with high specific activity is, therefore, necessary for the binding assay studies of 5-HT transport system. In the present study, we report the preparation of high specific activity [^3H]6-nitroquipazine by the nitration of [^3H]quipazine.

2. Materials and Methods

2.1 Materials

[^3H]Quipazine (2264.4 and 2519.7 GBq/mmol) was purchased from New England Nuclear (Boston, MA, U.S.A.). 6-Nitroquipazine maleate was synthesized by condensation of 2-chloro-6-nitroquinoline with piperazine in refluxing xylene as described previously⁴⁾. Other chemicals were purchased commercially.

2.2 Preparation of [^3H]6-nitroquipazine

[^3H]Quipazine was dissolved in a mixture of sulfuric acid (0.1 ml) and nitric acid (0.1 ml) at 4°C and the reaction mixture was allowed to stand at room temperature for 5 min.

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^3H -Quipazine ^3H -6-Nitroquipazine

* : Position of tritium

To the reaction mixture was carefully added cold water (0.2 ml), following by 50% sodium hydroxide (0.4 ml). [^3H]6-Nitroquipazine was separated by high performance liquid chromatography (HPLC) (column; Shim-pack CLC-ODS (5 μm , 6.0 mm I.D. $\times$ 15 cm), mobile phase; $\text{H}_2\text{O} : \text{CH}_3\text{CN} : \text{Et}_3\text{NH} = 60 : 40 : 0.2$, flow rate; 1.5 ml/min, detector: UV (254 nm)). [^3H]6-Nitroquipazine was further purified by HPLC (column; TSKgel ODS-80 Tm (5 μm , 4.6 mm I.D. $\times$ 15 cm), mobile phase; $\text{H}_2\text{O} : \text{CH}_3\text{CN} : \text{Et}_3\text{NH} = 60 : 40 : 0.2$, flow rate; 1.0 ml/min, detector; UV (254 nm)). The specific activity of [^3H]6-nitroquipazine was determined by HPLC and liquid scintillation counter (Aloka, LSC 1000). The radiochemical purity was determined by HPLC (column; TSKgel ODS-80 Tm (5 μm , 4.6 mm I.D. $\times$ 15 cm), mobile phase; $\text{CH}_3\text{CN} : 1\%$ aqueous triethylamine acetate (pH 4.0) = 4 : 6, flow rate; 1.0 ml/min, detector; UV (254 nm)) and thin layer chromatography (TLC) (silicagel; $\text{CHCl}_3 : \text{MeOH} : \text{NH}_4\text{OH} = 9 : 1 : 0.1$, R_f value = 0.72).

3. Results and Discussion

The direct nitration of [^3H]quipazine was carried out using a mixture of nitric acid and sulfuric acid ("mixed acid"), as shown in Fig. 1. The reaction was completed after 5 min at room temperature. The reaction mixture was separated by HPLC, and [^3H]6-nitroquipazine was further purified by HPLC. The radiochemical yield was approximately 50% based on [^3H]quipazine. The specific activity of [^3H]6-nitroquipazine was determined by HPLC and liquid scintillation counting. The specific activity of [^3H]6-nitro-

quipazine was almost the same as that of the starting [^3H]quipazine. The radiochemical purity of [^3H]6-nitroquipazine was more than 95% from HPLC and TLC determinations.

Moreover, it has been demonstrated that the high affinity [^3H]6-nitroquipazine binding sites in the rat brain⁵⁾ and human platelets exist from the binding assay *in vitro* (submitted).

In conclusion, high specific activity [^3H]6-nitroquipazine could be prepared by the direct nitration of [^3H]quipazine using the "mixed acid". [^3H]6-Nitroquipazine would be a new excellent radioligand for studying 5-HT transport system in brain and platelets.

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