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HIGH-AFFINITY [^{3}H]6-NITROQUIPAZINE BINDING TO THE 5-HYDROXYTRYPTAMINE TRANSPORT SYSTEM IN RAT LUNG

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Abstract—[^{3}H]6-Nitroquipazine bound to rat lung membranes at 37° with a dissociation constant (K_d) of 0.310 ± 0.13 nM and a maximal number of binding sites (B_{max}) of 1752 ± 334 fmol/mg protein (mean ± SD, N = 4). The binding was saturable, of high affinity and sodium dependent. Drug inhibition studies indicated that [^{3}H]6-nitroquipazine binding in the lung is similar to that already reported in the rat brain and human platelets. Scatchard analysis indicated that 5-hydroxytryptamine (5-HT) inhibited [^{3}H]6-nitroquipazine binding to rat lung membranes in a competitive manner. The present results suggest that [^{3}H]6-nitroquipazine binding sites in the rat lung are associated with the uptake system of 5-HT.

The 5-hydroxytryptamine (5-HT†; serotonin) transport system in the mammalian brain and human platelets has been studied extensively using radioligands such as [^{3}H]imipramine and [^{3}H]paroxetine. Also, the presence of high-affinity binding sites for [^{3}H]imipramine or [^{3}H]paroxetine has been reported in the rat lung [1], mouse lung [2] and human lung [3].

A major pulmonary function is to regulate the systemic concentration of certain vasoactive amines (e.g. 5-HT) by selective removal or metabolism [4–6]. It has been reported that the uptake of 5-HT by the pulmonary endothelium is saturable and sodium dependent, and monoamine oxidases (MAO) mediate the deactivation [7]. Also, the lung does not store 5-HT which, once taken up, is metabolized rapidly by MAO [7]. Thus, the uptake of 5-HT in the lung is an important inactivating mechanism for 5-HT in circulating blood. The binding sites labeled by [^{3}H]imipramine and [^{3}H]paroxetine may play an important physiological role in the control of 5-HT levels by regulating the 5-HT uptake into the lung [1].

Recently, we reported that [^{3}H]6-nitroquipazine is a suitable radioligand for studying the 5-HT transporter in rat brain [8, 9] and human platelets [10], and that 6-nitroquipazine is more potent than imipramine and paroxetine for inhibiting [^{3}H]6-nitroquipazine binding [8, 10]. We felt that it would also be of interest to study the 5-HT transporter in the lung using [^{3}H]6-nitroquipazine. In the present study, we examined [^{3}H]6-nitroquipazine binding to rat lung membranes at a physiological temperature of 37°.

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† Abbreviations: 5-HT, 5-hydroxytryptamine; MAO, monoamine oxidases; and NE, norepinephrine.

MATERIALS AND METHODS

Materials. [Piperazinyl-^{3}H]6-nitroquipazine (2.51 TBq/mmol, 67.8 Ci/mmol) and 6-nitroquipazine maleate were synthesized as described previously [11]. The drugs were obtained from the following sources: paroxetine hydrochloride (Beecham Pharmaceutical Ltd., Surrey, U.K.), citalopram hydrochloride (Lundbeck A/S, Copenhagen, Denmark), fluoxetine (Eli Lilly, Indianapolis, IN, U.S.A.), *Z*-zimelidine dihydrochloride and *E*-zimelidine oxalate (Astra Alab AB, Södertälje, Sweden), maprotiline hydrochloride (Nippon Ciba-Geigy Ltd., Takarazuka, Japan), desipramine hydrochloride, and 5-HT creatinine sulfate (Sigma Chemical Co., St. Louis, MO, U.S.A.), and imipramine hydrochloride and norepinephrine (NE) hydrochloride (Wako Pure Chemical Industries Ltd., Tokyo, Japan). Other chemicals were purchased commercially.

Membrane preparation. Male Wistar rats (200–250 g) were killed by decapitation and the lungs were removed rapidly. The lungs were homogenized in 50 vol. of ice-cold buffer (50 mM Tris–HCl, 120 mM NaCl, 5 mM KCl, pH 7.4) with a Kinematica Polytron homogenizer (Lüzern, Switzerland) at setting 5 for 30 sec. The homogenates were centrifuged at 30,000 *g* for 10 min. The resulting pellet was suspended in the same buffer and centrifuged. The washing procedure was repeated twice. The final pellet was resuspended in the same buffer to a protein concentration of approximately 1 mg/mL. Protein concentrations were measured by the method of Lowry *et al.* [12] using bovine serum albumin as standard.

[^{3}H]6-*Nitroquipazine binding.* [^{3}H]6-Nitroquipazine binding was determined by incubating aliquots of the lung homogenate with [^{3}H]6-nitroquipazine at 37° in a final volume of 1.5 mL for 1 hr. After the addition of 4 mL of ice-cold buffer, the homogenates were filtered rapidly through Whatman GF/C filters pretreated with 0.05% polyethyleneimine using a 24-channel cell harvester (Brandell, Gaithersburg, MD, U.S.A.). The filters were washed with three 5-mL

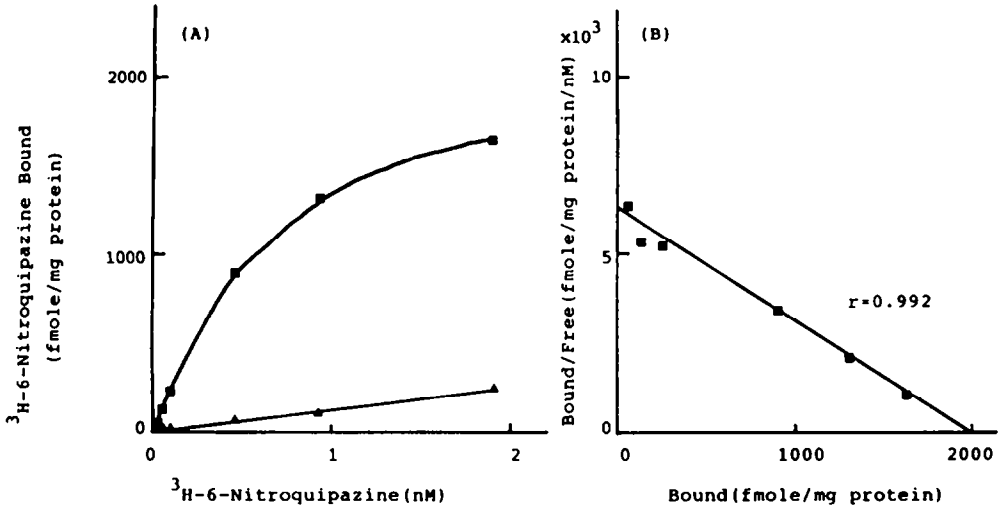

Fig. 1. Binding of [^{3}H]6-nitroquipazine to rat lung membranes. Membranes were incubated with various concentrations of [^{3}H]6-nitroquipazine (20–1900 pM) for 1 hr at 37°. Non-specific binding was estimated in the presence of 1 μM paroxetine. The results are from a typical experiment, and the values are the averages of duplicate determinations. (A) Saturation binding isotherm showing specific binding (■) and non-specific binding (▲). (B) Scatchard plot analysis of [^{3}H]6-nitroquipazine binding in this typical experiment gave a K_d of 0.324 nM and a B_{max} of 1977 fmol/mg protein.

rinses of ice-cold buffer. The radioactivity trapped by the filters was determined by a liquid scintillation counter (Aloka, LSC-1000). Non-specific binding was estimated in the presence of 1 μM paroxetine.

Data analysis and statistics. Values for the maximum number of binding sites (B_{max}) and the apparent equilibrium dissociation constant (K_d) were determined by Scatchard analysis where the best-fitting lines to the data were calculated by the least-squares linear regression method.

The inhibition experiments were analyzed by conventional graphical techniques by means of the iterative non-linear least squares methods, and drug IC_{50} values were defined as the concentration that resulted in a 50% inhibition of specific binding. The K_i values for drugs were calculated subsequently according to Cheng and Prusoff [13].

The statistical significance of results was evaluated by Student's *t*-test.

RESULTS

[^{3}H]6-Nitroquipazine binding to rat lung membranes at 37° was saturable (Fig. 1A). Scatchard analysis of the equilibrium saturation binding data gave a straight line, indicating a single population of binding sites with an apparent equilibrium dissociation constant (K_d) of 0.310 ± 0.13 nM and a maximal number of binding sites (B_{max}) of 1752 ± 334 fmol/mg protein (mean ± SD, N = 4) (Fig. 1B).

Inhibition of the specific [^{3}H]6-nitroquipazine binding to rat lung membranes was studied at 37° with 0.2 nM [^{3}H]6-nitroquipazine. The K_i values for the nontricyclic 5-HT uptake inhibitors (6-nitroquipazine, paroxetine, citalopram and fluoxetine) were in the low nanomolar range for inhibition of [^{3}H]6-nitroquipazine binding to rat lung membranes (Table 1). The tricyclic antidepressants imipramine and desipramine gave K_i values of 82 and 550 nM, respectively (Table 1). The inhibition of [^{3}H]6-nitroquipazine binding to rat lung membranes was stereoselective. The *Z*-form of zimelidine, which is the isomer active *in vitro* on 5-HT uptake, was more potent than the *E*-form for inhibiting [^{3}H]6-nitroquipazine binding (Table 1). The K_i value of maprotiline, a very weak 5-HT uptake inhibitor, was

Table 1. Drug inhibition of [^{3}H]6-nitroquipazine binding to rat lung membranes

Drugs	K_i (nM)	Hill coefficient
6-Nitroquipazine	0.397	1.02
Paroxetine	0.547	1.06
Citalopram	5.44	0.82
Fluoxetine	21.9	1.05
Imipramine	82.2	0.87
Z-Zimelidine	203	0.87
Desipramine	550	0.98
E-Zimelidine	1,782	1.11
Maprotiline	24,773	1.01
5-HT	1,506	1.00

The inhibition of [^{3}H]6-nitroquipazine binding by various drugs was determined with 0.2 nM [^{3}H]6-nitroquipazine. Ten concentrations of the drugs were used for each determination. K_i values for the various drugs were determined as described in Materials and Methods. The values shown in the table represent the means of three determinations done in duplicate.

24,773 nM. Furthermore, the inhibition of [^{3}H]6-nitroquipazine binding by the tricyclic and non-tricyclic 5-HT uptake inhibitors was monophasic with a pseudo-Hill coefficient close to unity. The K_i value of 5-HT was 1506 nM (Table 1). The inhibition of [^{3}H]6-nitroquipazine binding by 5-HT appeared to be monophasic and gave a pseudo-Hill coefficient close to unity. NE was inactive at displacing the [^{3}H]6-nitroquipazine binding.

The [^{3}H]6-nitroquipazine binding to rat lung membranes was sensitive to sodium ions. The K_d in the absence of added sodium was significantly greater than that in the presence of 120 mM sodium chloride [with 120 mM NaCl, $K_d = 0.450 \pm 0.15$ nM (mean ± SD, N = 3), while with zero Na^+, $K_d = 7.50 \pm 2.5$ nM (mean ± SD, N = 3), $P < 0.01$]. In the absence of sodium, the B_{max} of [^{3}H]6-nitroquipazine binding on the rat lung was not significantly different.

Scatchard analysis indicated that 5-HT inhibited [^{3}H]6-nitroquipazine binding to rat lung membranes in a competitive manner. The K_d value of [^{3}H]6-nitroquipazine binding was increased significantly by the addition of 5 μM 5-HT [with 5-HT (5 μM), $K_d = 5.350 \pm 1.55$ nM (mean ± SD, N = 4); without 5-HT, $K_d = 0.350 \pm 0.25$ nM (mean ± SD, N = 3), $P < 0.01$]. The B_{max} value of [^{3}H]6-nitroquipazine binding in the rat lung was not changed significantly by the addition of 5 μM 5-HT [with 5 μM 5-HT, $B_{max} = 1900 \pm 250$ fmol/mg protein (mean ± SD, N = 3); without 5-HT, $B_{max} = 2100 \pm 230$ fmol/mg protein (mean ± SD, N = 3)].

DISCUSSION

Our results indicate the presence of high-affinity binding sites for [^{3}H]6-nitroquipazine in rat lung membranes at 37°. The data suggest that the [^{3}H]6-nitroquipazine binding sites on rat lung membranes are associated with the 5-HT transport complex as follows. The [^{3}H]6-nitroquipazine binding to rat lung membranes was inhibited selectively by both the tricyclic and the nontricyclic 5-HT uptake inhibitors. The inhibition of [^{3}H]6-nitroquipazine binding in rat lung was stereoselective. Furthermore, the good correlation between the potency of various drugs to inhibit [^{3}H]6-nitroquipazine binding to rat lung membranes and to inhibit [^{3}H]6-nitroquipazine binding to rat brain membranes [8] gave a correlation coefficient of 0.998 ($P < 0.001$) with a slope of 1.00. The absence of sodium in lung membrane preparations and in the incubation medium increased the K_d value of [^{3}H]6-nitroquipazine binding to rat lung membranes, indicating that the [^{3}H]6-nitroquipazine binding is dependent on sodium ions. Scatchard analysis in the absence or presence of 5-HT (5 μM) indicated that 5-HT inhibits [^{3}H]6-nitroquipazine binding to rat lung membranes in a competitive manner.

It has been reported that [^{3}H]imipramine binding to rat lung membranes is inhibited competitively by tricyclic antidepressants but in a complex manner by 5-HT and non-tricyclic 5-HT uptake inhibitors [1]. However, the present results suggest that all drugs used in the current study inhibited [^{3}H]6-nitroquipazine binding to rat lung membranes in a competitive manner.

It has been recognized that lungs are a site for the uptake, accumulation and/or metabolism of numerous endogenous compounds [4–6]. Furthermore, it was reported that in addition to the lung being the major site for its inactivation, the presence of several specific 5-HT receptors may be related to some of the known actions of 5-HT in the lung [14]. To regulate the systemic concentration of biogenic amines by selective removal or metabolism is one of the important functions of the lung [4–6]. Pulmonary endothelial cells regulate the uptake, accumulation and metabolism of biologically active endogenous compounds, and the MAO system, which is concentrated in the mitochondria of endothelial cells, plays an important role in the metabolism of 5-HT [4–6]. Thus, 5-HT metabolism by lung may be under physiological regulation. Furthermore, the recognition sites labeled with [^{3}H]6-nitroquipazine may play an important physiological role in the control of 5-HT levels [1]. Since the K_d value for [^{3}H]6-nitroquipazine binding to rat lung membranes at 37° is very low, [^{3}H]6-nitroquipazine may be a suitable radioligand for the *in vivo* study of the 5-HT transport system in the lung. *In vivo* studies of the 5-HT transport system in mouse lung with [^{3}H]6-nitroquipazine are now in progress.

In conclusion, [^{3}H]6-nitroquipazine would be a suitable radioligand for studying the 5-HT transport system in the lung at physiological temperature.

REFERENCES

1. Raisman R and Langer SZ, Specific high affinity [^{3}H]imipramine binding sites in rat lung are associated with a non-neuronal uptake site for serotonin. *Eur J Pharmacol* **94**: 345–348, 1983.
2. Hashimoto K and Goromaru T, Evaluation of [^{3}H]paroxetine as a radiopharmaceutical for lung function. *Jpn J Nucl Med* **26**: 879–885, 1989.
3. Morin D, Zini R, Lange J and Tillement JP, Evidence for high affinity [^{3}H]imipramine binding sites in human lung. *Biochem Biophys Res Commun* **120**: 926–932, 1984.
4. Bend JR, Serabjit-Singh CJ and Philpot RM, The pulmonary uptake, accumulation, and metabolism of xenobiotics. *Annu Rev Pharmacol Toxicol* **25**: 97–125, 1985.
5. Gillis CN and Pitt BR, The fate of circulating amines within the pulmonary circulation. *Annu Rev Physiol* **44**: 269–281, 1982.
6. Iwasawa Y and Gillis CN, Pharmacological analysis of norepinephrine and 5-hydroxytryptamine removal from the pulmonary circulation: Differentiation of uptake sites for each amine. *J Pharmacol Exp Ther* **188**: 386–393, 1974.
7. Junod AF, Uptake, metabolism and efflux of ^{14}C-5-hydroxytryptamine in isolated perfused rat lungs. *J Pharmacol Exp Ther* **183**: 341–355, 1972.
8. Hashimoto K and Goromaru T, High-affinity [^{3}H]6-nitroquipazine binding sites in rat brain. *Eur J Pharmacol* **180**: 273–281, 1990.
9. Hashimoto K and Goromaru T, High affinity [^{3}H]6-nitroquipazine binding to rat cortical membranes: Inhibition by 5-hydroxytryptamine and 5-hydroxytryptamine uptake inhibitors. *Neuropharmacology*, **30**: 113–117, 1991.
10. Hashimoto K and Goromaru T, High affinity binding of [^{3}H]6-nitroquipazine to 5-hydroxytryptamine transporter in human platelets. *Eur J Pharmacol* **187**: 295–302, 1990.

11. Hashimoto K and Goromaru T, Preparation of [^{3}H]6-nitroquipazine, a potent and selective 5-hydroxytryptamine uptake inhibitor. *Radioisotopes* **39**: 168–169, 1990.
12. Lowry OH, Rosebrough NJ, Farr AL and Randall RJ, Protein measurement with the Folin phenol reagent. *J Biol Chem* **193**: 265–275, 1951.
13. Cheng Y-c and Prusoff WH, Relationship between the inhibition constant (K_i) and concentration of inhibitor which causes 50 per cent inhibition (IC_{50}) of an enzymatic reaction. *Biochem Pharamacol* **22**: 3099–3108, 1973.
14. Das DK and Steinberg H, Serotonin receptors in rat lung. *Respiration* **49**: 27–36, 1986.