5-HT moved from an accessible to an inaccessible compartment. This may occur by translocation of bound [^{3}H]-5-HT across the plasma membrane into cell cytoplasm or storage vesicles. Furthermore, it appears as if the presence of imipramine or unlabeled 5-HT during incubation at 0° and in the initial stages of the washing procedure was sufficient to prevent this transition, allowing the surface-associated [^{3}H]-5-HT to be diluted out in the fresh medium.

It is of interest that when the platelet uptake of [^{3}H]-5-HT was blocked by imipramine, resuspension of the cells in fresh medium resulted in the resumption of uptake. Under these conditions any cell-associated imipramine not covalently bound or inside the cell membrane would be expected to equilibrate with the imipramine-free buffer and be greatly reduced in concentration. Thus, the inhibitory effect of imipramine appears to be secondary to a reversible association of the molecule with the cell surface. One would expect a similar dilutional loss of any surface-associated [^{3}H]-5-HT during the two washing steps described by Schick and McKean [1], and its absence is consistent with our suggestion that the bulk of the labeled material is not accessible to the external medium. It seems possible that measurement of the imipramine-sensitive and -resistant [^{3}H]-5-HT retained after washing may provide a more useful index of 5-HT uptake sites, and of cell function, than simple measurement of the total amount of wash-resistant [^{3}H]-5-HT.

In summary, [^{3}H]-5-HT remaining associated with human platelets following incubation at 0° and one wash could not be removed when imipramine or excess 5-HT was added to the washed cells. Thus, the cell-associated [^{3}H]-5-HT does not appear to represent material bound to the external membrane surface, but rather [^{3}H]-5-HT that has been transported into the platelet.

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Biochemical Pharmacology, Vol. 30, No. 18, pp. 2623–2625, 1981.
Printed in Great Britain.

0006-2952/81/182623-03 $02.00/0

Characterization of α-adrenergic receptors in rat lung membranes: presence of α_1 but not α_2 receptors

(*Received* 8 *December* 1980; *accepted* 5 *March* 1981)

In 1948, based on physiological studies, Ahlquist [1] suggested that adrenergic receptors be divided into α and β subtypes. About two decades later, β-adrenergic receptors were subdivided into β_1 and β_2, again on the basis of physiological experiments [2]. More recent experimental evidence has indicated the existence of subtypes of α-adrenergic receptors, i.e. α_1 and α_2 [3–5]. Using various ^{3}H-ligands, α-adrenergic receptor binding has been characterized in a variety of mammalian tissues, including brain [6, 7], kidney [6, 8], liver [9–11], platelet [12–14], uterus [15, 16], adipose tissue [17–19], heart [20] and salivary gland [21, 22]. The lung, however, has received little attention, particularly regarding the identification and characterization of its α-adrenergic receptor subtypes [23, 24].

In this communication we report the characterization of rat pulmonary α_1 adrenergic receptors. In addition, we present evidence indicating a lack of α_2-adrenergic receptors in this tissue.

Materials and methods

Randomly chosen Sprague–Dawley rats (200–300 g) were decapitated. Lungs were removed immediately, placed on ice, and then minced after discarding large bronchi. The tissue was homogenized with a Tekmar Tissumizer (model SDT) at maximum speed for two 20-sec periods (with a 10-sec interval) in 20 vol. of ice-cold 50 mM Tris–HCl (pH 8.0 at 25°). The homogenate was centrifuged at 50,000 *g* for 10 min, and the pellet was homogenized and centrifuged again. The pellet was then suspended in 30 ml of buffer and filtered through a 53-μm nylon mesh. The filtrate was centrifuged as before and the final pellet was resuspended in an appropriate volume of Tris–HCl buffer so that the final suspension contained approximately 0.2 mg protein/ml.

[^{3}H]WB4101 (24.7 Ci/mmole), [^{3}H]dihydroergocryptine (DHE) (30.4 Ci/mmole), [^{3}H]clonidine (22.2 Ci/mmole), and [^{3}H]yohimbine (81.6 Ci/mmole) were obtained from the New England Nuclear Corp. (Boston, MA). They were stored at −20° and appropriate dilutions were prepared daily just prior to use in the binding assay.

The following drugs were donated by the indicated company: (+)-norepinephrine, (+)-epinephrine, phenylephrine and (−)-isoproterenol (Sterling-Winthrop, Rensselaer, NY); phentolamine (Ciba–Geigy Corp., Summit, NJ); prazosin (Pfizer, Inc., New York, NY); clonidine (Boehringer Ingelheim, Elmsford, NY); and (−)-propranolol (Ayerst Laboratories, New York, NY). (−)-Epinephrine, (−)-norepinephrine, dopamine and yohimbine were

obtained from the Sigma Chemical Co. (St. Louis, MO).

α-Adrenergic receptors were assayed essentially as described previously [22, 25]. For saturation experiments, fresh rat lung homogenate was incubated in triplicate with increasing concentrations of a radioligand at 23°. The ^{3}H-ligand concentration ranges and incubation times were: 0.08 to 4.8 nM [^{3}H]WB4101 for 30 min; 0.1 to 3.8 nM [^{3}H]DHE for 45 min; 0.8 to 11.2 nM [^{3}H]clonidine for 30 min; and 0.5 to 5.8 nM [^{3}H]yohimbine for 30 min. Incubations were terminated by rapid filtration through Whatman GF/B glass fiber filters. The filters were washed with 12 ml of ice-cold buffer, except for [^{3}H]DHE experiments in which the wash buffer temperature was 23°. The radioactivity remaining on the filters was determined by scintillation spectroscopy at an efficiency of 37 per cent. Specific binding was defined as the difference between the binding in the presence and absence of 100 μM (−)-norepinephrine. Radioligands and unlabeled antagonists were diluted in 5 mN HCl, whereas unlabeled agonists were diluted in 5 mN HCl containing 1 mg/ml ascorbic acid.

Saturation data were calculated by Rosenthal analysis [26], using an unweighted linear regression of bound/free versus bound. For each drug, the IC_{50} value (the concentration of unlabeled drug that inhibits 50 per cent of the specific binding) was calculated from inhibition experiments by logit-log analysis using six to ten concentrations of the drug. Inhibitory constants or K_i values were calculated using the equation, $K_i = IC_{50}/(1 + L/K_D)$ where L and K_D represent the concentration of radioligand and its equilibrium dissociation constant, respectively. Protein concentration was determined by the method of Lowry *et al.* [27].

Results and discussion

Binding experiments with [^{3}H]WB4101 and [^{3}H]DHE showed that the α-adrenergic receptor binding sites of rat lung membranes were saturable and speicific. Saturation experiments with [^{3}H]WB4101 indicated a maximum number of binding sites (B_{max}) of 59 fmoles/mg protein and an equilibrium dissociation constant (K_D) of 0.27 nM (Fig. 1). Similarly we found a B_{max} of 57 fmoles/mg and a K_D of 1.58 nM for [^{3}H]DHE binding. [^{3}H]WB4101 has been shown to label α_1 adrenergic receptors in a variety of tissues [5, 6, 20], whereas [^{3}H]DHE appears to label both α_1 and α_2 adrenergic receptors [14, 16]. Linear Rosenthal plots and nearly identical B_{max} values for [^{3}H]WB4101 and [^{3}H]DHE binding indicated the presence of only α_1 adrenergic receptors in rat lung membranes (Fig. 1). However, since [^{3}H]WB4101 also appears to label α_2 receptors under certain conditions [15], we studied the binding of [^{3}H]clonidine, an α_2 agonist, and [^{3}H]yohimbine, an α_2 antagonist. Specific binding of these radioligands was not detectable in rat lung membranes (Fig. 1), supporting the existence of only α_1 receptors. Table 1 presents the results of three to five separate experiments for each ligand. The K_D values are comparable with those reported for α-adrenergic binding in other mammalian tissues [6, 7, 10, 20, 28]. The distribution of the α_1 receptors among the various tissues of the lung cannot be determined from these experiments. Presumably, most of these receptors are on bronchial smooth muscle, although some of them may be on vascular smooth muscle and possibly other cell types.

Inhibition of [^{3}H]WB4101 binding by various unlabeled agonists and antagonists was also consistent with the presence of only α_1 receptors in the rat lung (Table 2). Oxymetazoline was the most potent inhibitor of [^{3}H]WB4101 binding, followed by (−)-norepinephrine and (−)-epinephrine. Dopamine and (−)-isoproterenol, on the other hand, inhibited [^{3}H]WB4101 binding only at high concentrations. Prazosin, a very potent and selective α_1 antagonist, inhibited [^{3}H]WB4101 binding with a K_i value of 0.5 nM, whereas yohimbine, a selective α_2 antagonist, had a K_i of 740 nM. The observed K_i values are in good agreement with those obtained from [^{3}H]WB4101 binding to rat kidney [6] and heart membranes [20].

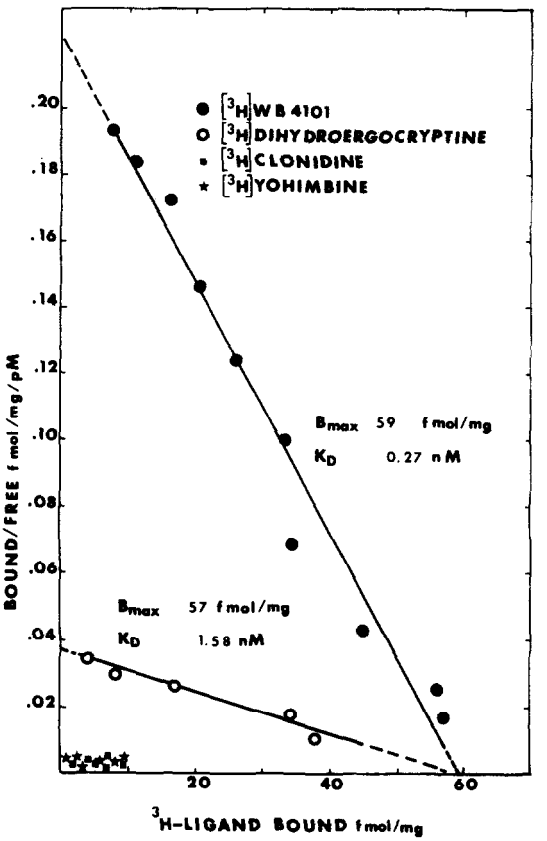

Fig. 1. Saturation experiments with various ^{3}H-ligands and rat lung membranes. Membrane particulate fractions of the lung were incubated with increasing concentrations of [^{3}H]WB4101 (0.08 to 4.8 nM), [^{3}H]DHE (0.1 to 3.8 nM), [^{3}H]clonidine (0.8 to 11.2 nM), or [^{3}H]yohimbine (0.5 to 5.8 nM) and then filtered, and the remaining radioactivity on the filters was determined. Data for saturation experiments were plotted by the method of Rosenthal using unweighted linear regression analysis. The B_{max} was determined from the intercept with the abscissa, and the K_D from the negative reciprocal of the slope of the line. Each point is a mean of triplicates from single representative experiments. The ratio of total binding (specific plus nonspecific binding) to free concentration of either [^{3}H]clonidine or [^{3}H]yohimbine never exceeded 0.006.

We have shown recently that rat submanidbular gland, like the lung, does not contain a detectable level of α_2 adrenergic receptors. Chronic administration of reserpine, however, results in the appearance of α_2 receptors in this salivary gland [22]. By contrast, α_2 receptor binding was not detected in the lung of reserpinized rats (J. Latifpour and D. B. Bylund, unpublished observation).

A malfunction of the pulmonary adrenergic regulatory system in some obstructive airway diseases was suggested a number of years ago [29]. In addition, recent evidence suggests that alterations in the characteristics of adrenergic receptors may occur in these disorders [24, 30, 31]. Thus, an understanding of the relative densities of pulmonary α-adrenergic receptor subtypes may have therapeutic implications. In preliminary experiments, we found the composition of adrenergic receptors in human lung to be similar to that of rat. If this absence of α_2 receptors in human lung is confirmed, the use of a selective α_1 adrenergic antagonist in certain pulmonary diseases, such as bronchial asthma, should be considered.

Table 1. Binding characteristics of various adrenergic radioligands to rat lung membranes

^{3}H-Ligand	N	B_{max} (fmoles/mg protein)	K_D (nM)
[^{3}H]WB4101	3	57 ± 2.5	0.33 ± 0.03
[^{3}H]Dihydroergocryptine	4	60 ± 7	1.7 ± 0.29
[^{3}H]Clonidine	5	ND*	
[^{3}H]Yohimbine	3	ND	

* Not determined. The amount of specific binding was too low to be reliably quantitated, as can be seen in Fig. 1.

Table 2. Inhibition of [^{3}H]WB4101 binding by various adrenergic agonists and antagonists*

Agent	N	K_i (nM)
Agonist		
(−)-Norepinephrine	6	110 ± 20
(−)-Epinephrine	4	200 ± 50
(+)-Norepinephrine	3	8,500 ± 2,300
(+)-Epinephrine	3	6,200 ± 1,000
(−)-Phenylephrine	4	1,800 ± 400
Oxymetazoline	4	50 ± 18
Clonidine	3	480 ± 100
(±)-α-Methyl-(±)-norepinephrine	3	7,500 ± 2,500
Dopamine	3	20,700 ± 4,700
(−)-Isoproterenol	3	22,000 ± 5,000
Antagonist		
Phentolamine	5	1.6 ± 0.2
Prazosin	4	0.5 ± 0.2
Yohimbine	3	740 ± 200
(−)-Propranolol	3	600 ± 150

* K_i values (mean ± S.E.) were calculated from the equation $K_i = IC_{50}/(1 + L/K_D)$ with $K_D = 0.33$ nM and L equal to the concentrations of [^{3}H]WB4101 in each experiment (0.6 to 1.0 nM).

In summary, the similarity in the B_{max} values for [^{3}H]WB4101 and [^{3}H]DHE indicates that rat lung membranes contain only α_1 adrenergic receptors. Furthermore, we were unable to detect any specific binding of either [^{3}H]clonidine, an α_2 agonist, or [^{3}H]yohimbine, an α_2 antagonist. In addition, the K_i values for adrenergic agonists and antagonists in inhibiting [^{3}H]WB4101 binding to rat lung membranes were consistent with the presence of only α_1 adrenergic receptors.

Acknowledgements—This investigation was supported, in part, by NSF Grant BNS 7824715.

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