Journal of Pharmaceutical & Biomedical Analysis
Vol. 7, No. 12, pp. 1467–1472, 1989
Printed in Great Britain

0731–7085/89 $3.00 + 0.00

Lung tissue binding of iodobenzyl-propanediamine: involvement of beta-adrenergic receptors*

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Abstract: The basic compound *N-N-N'*-trimethyl-*N'*-(2-hydroxy-3-methyl-5-iodobenzyl)-1,3-propanediamine (HIPDM) accumulates in human and rabbit lungs, where it forms a slowly effluxable pool. In isolated perfused rat lung, HIPDM is taken up by a saturable, energy-independent mechanism, which is competitively inhibited by imipramine, chlorpromazine and propranolol. To ascertain whether beta-adrenergic receptors are involved in the binding process of HIPDM to lung tissue, the ability of unlabelled HIPDM to displace the beta-adrenergic receptor ligand [^{125}I]iodocyanopindolol (ICYP) from rabbit lung beta-receptors was examined. Lung microsomal membrane fractions (75 $\mu g\ ml^{-1}$) were incubated at 37°C for 3 h with 68 pM ICYP (with or without 1 μM of (±)-propranolol) in the presence of HIPDM (10^{-10}–10^{-3} M). Bound and free radioactivity were separated through glass-fibre filters and the retained radioactivity was counted in a gamma-spectrometer. HIPDM competed with ICYP for beta-adrenoceptors (13% displacement at 10^{-5} M, 50% at 5×10^{-5} M, and 90% at 2×10^{-4} M). The inhibition curve of ICYP binding by HIPDM was similar to that observed for (−)-noradrenaline. Although the results of the *in vitro* studies cannot be extrapolated to *in vivo* conditions, they suggest that beta-adrenergic receptors may be involved in the observed lung uptake of the basic amine HIPDM.

Keywords: *Iodobenzyl-propanediamine; rabbit lung; beta-adrenergic receptors; ICYP.*

Introduction

The lung is able to remove from the circulation a variety of endogenous and exogenous amines [1]. Carrier-mediated, sodium-dependent transport systems have been described for the lung uptake of the biogenic amines serotonin and noradrenaline, which are inactivated by the lung microvascular endothelial cells [1]. In contrast, most exogenous basic amines are taken up by the lung with saturable, sodium- and energy-independent mechanisms and do not appear to be appreciably metabolized [1]. Basic amines with amphiphilic character, such as chlorcyclizine, methadone, imipramine and propranolol, are stored by the lung in a slowly effluxable pool [2–4]. The persistence of these compounds in the lung has been attributed to the presence of specific binding sites whose nature and location have not been precisely identified.

*Presented at the "Third International Symposium on Drug Analysis", May 1989, Antwerp, Belgium.
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Recently, the present authors evaluated the lung kinetics of *N-N-N'*-trimethyl-*N'*-(2-hydroxy-3-methyl-5-iodobenzyl)-1,3-propanediamine (HIPDM), a basic amphiphilic amine which can be easily labelled with radio-iodine by an isotope-exchange reaction [5]. Although HIPDM was originally synthetized for brain perfusion imaging [5], biodistribution studies in various animal species indicated that the lung is the primary site of HIPDM accumulation [5, 6]. Experimental data obtained in humans have shown that intravenously injected HIPDM was distributed in the lung according to the regional blood flow [7]. Moreover, ^{123}I-HIPDM lung clearance, as assessed by external detection, was characterized by a slow decline of radioactivity (mean time about 7 h) that was compatible with a relatively stable cellular binding [7]. A similar rate of HIPDM lung clearance was observed in rabbits [7]. In these animals, subcellular fractionation of lung homogenates showed that HIPDM was almost entirely associated with particulate fractions [8]. In isolated, perfused rat lung, Slosman *et al.* [9] reported that the pulmonary uptake of HIPDM is saturable, sodium- and energy-independent and competitively inhibited by other basic amines such as imipramine, chlorpromazine and propranolol.

In vitro receptor binding studies have shown that the lung membranes from several animal species have the highest density of beta-adrenergic receptors of any other tissue, whereas the density of alpha-adrenoceptors and cholinergic-receptors is low [10]. Autoradiographic studies revealed that beta-adrenoceptors are predominantly distributed in the peripheral lung, over 90% being located in the alveolar walls, whereas alpha-adrenoceptors are equally distributed between tracheal smooth muscle and peripheral lung [10]. Conversely, muscarinic receptors are essentially found in the tracheal smooth muscle [10].

The aim of the present study was to discover whether beta-adrenergic receptors are involved in the process of HIPDM binding to lung tissue. Since labelled HIPDM of high specific activity was not available, the ability of unlabelled HIPDM to displace the beta-adrenergic receptor ligand [^{125}I]iodocyanopindolol (ICYP) from its receptors on rabbit lung crude microsomal membrane fractions was examined. Particulate membrane fractions were used to study the interaction of HIPDM with beta-adrenoceptors because it has been demonstrated that the evaluation of specific binding to beta-adrenergic receptors on intact cells is compromised by substantial receptor-independent, non-stereospecific sequestration of radioligands [11, 12].

Experimental

Chemicals

(−)[^{125}I]iodocyanopindolol (sp. act. 2200 Ci mmol^{-1}) was purchased from New England Nuclear Corp. (−)-Isoprenaline bitartrate, (+)-isoprenaline bitartrate, (−)-propranolol, (+)-propranolol and (±)-propranolol hydrochloride, (−)-adrenaline acid tartrate and (−)-noradrenaline acid tartrate were from Sigma Chemical Company (St. Louis, MO, USA). Phentolamine was kindly provided by Ciba-Geigy Corp. (Italy). All other chemicals were of reagent grade and were purchased from Merck.

Preparation of receptor tissue

Lung membranes from New Zealand albino rabbits were prepared as described by Brodde *et al.* [13], with some modification. Animals were killed by air embolization and their lungs were removed, dissected free of major airways, minced and homogenized in

10 vol of ice cold homogenization buffer (250 mM sucrose and 1 mM $MgCl_2$, 5 mM Tris–HCl, pH 7.4 at 4°C) with a Polytron PT 10-35 homogenizer (2 × 10 s bursts at maximum speed). The homogenate was filtered through two layers of cheesecloth and undisrupted cells and nuclei were removed by centrifugation at 2000*g* for 10 min at 4°C. The supernatant was centrifuged at 30,000*g* for 15 min, and the resultant pellets were washed 3 times in incubation buffer (10 mM $MgCl_2$ and 50 mM Tris–HCl, pH 7.5 at 37°C) by recentrifugation and resuspension. The final pellets were resuspended in the same buffer at a protein concentration of 6–8 mg ml^{-1}, as assessed by the Pierce BCA Protein Assay Reagent (Pierce Chemical Company, IL, USA), and were kept frozen at −70°C until used.

Binding assay

The binding assays were carried out in duplicate in polypropylene tubes. Aliquots of the lung membrane suspension (200 μl) were incubated at 37°C with 50 μl of ICYP with or without 50 μl of competing ligands in a final volume of 500 μl of incubation buffer. Bound and free ICYP were separated by addition of 10 ml of incubation buffer at 37°C followed by rapid filtration through Whatman GF/C glass fibre filters. Each filter was rapidly washed with additional 3 × 5-ml portions of the same buffer and the retained radioactivity was measured in a gamma-scintillation spectrometer at about 75% counting efficiency. Non-specific binding was assessed in the presence of 1 μM (±)-propranolol and was subtracted from the total binding to calculate the specific binding.

In order to determine the potency of HIPDM in displacing ICYP from beta-adrenergic receptors, lung membranes were incubated with ICYP (60–80 pM) in the presence of unlabelled HIPDM in the concentration range from 10^{-10} to 10^{-3} M; specific binding was determined as previously described.

Unless stated otherwise, the experimental data reported in the text and figures represent values of the mean ±SEM (standard error of the mean).

Results and Discussion

In preliminary experiments, previous observations were confirmed that the high-affinity beta-adrenergic ligand ICYP is bound to membrane fractions from rabbit lungs by interactions consistent with binding to the beta-adrenergic receptor [13].

ICYP binding was time-, ligand- and protein-concentration dependent, saturable, of high affinity and stereoselective. Specific ICYP binding increased linearly with increasing membrane protein concentration up to 80 μg ml^{-1}. With an ICYP concentration of 50 pM and a protein concentration of about 30 μg ml^{-1}, specific binding attained a plateau in about 3 h, remaining stable thereafter (data not shown). An incubation time of 180 min and a protein concentration of 35 μg ml^{-1} were therefore routinely used.

Scatchard analysis [14] of ICYP specific binding data (Fig. 1) resulted in linear plots which suggest one class of binding sites. The affinity of ICYP (K_D 54.6 ± 3.1 pM) was very similar to that determined by Brodde *et al.* [13] (K_D 69.6 ± 8.5 pM) and the number of binding sites (B_{max} 942 ± 18 fmol mg^{-1} of protein), although greater, was of a similar order of magnitude as that reported by Brodde *et al.* [13] (B_{max} 399.6 ± 44.8 fmol mg^{-1} of protein).

Inhibition of ICYP binding (Fig. 2) by agonists showed a rank order of potency characteristic of the beta-adrenergic receptors: (−)-isoprenaline ≫ (−)-adrenaline ≈ (−)-noradrenaline. The alpha-antagonist phentolamine caused inhibition of binding only

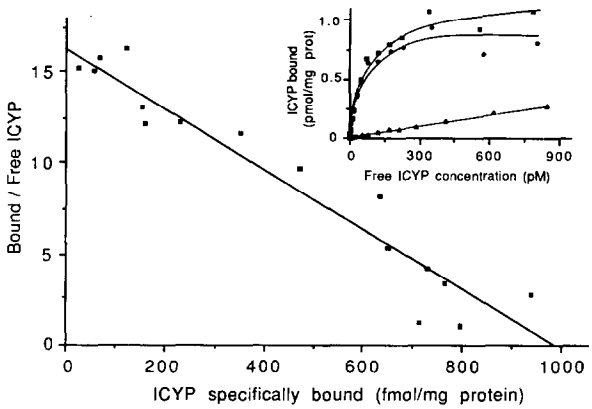

Figure 1
Scatchard analysis of specific ICYP binding to rabbit lung membranes. Bound and free ICYP are expressed as fmol mg^{-1} of protein and pM, respectively. $R = 0.96$. Inset: Total (■), specific (●), and non-specific (▲) binding of ICYP to rabbit lung membranes as function of increasing ICYP concentrations. Each value is the mean of three experiments with a SEM <6%.

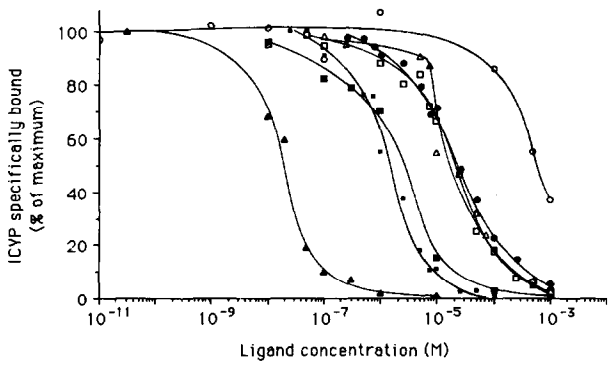

Figure 2
Inhibition of specific ICYP binding to rabbit lung membranes by unlabelled ligands: (−)-propranolol (▲), (−)-isoprenaline (□), (+)-propranolol (■), (−)-adrenaline (□), (−)-noradrenaline (●), (+)-isoprenaline (△), phentolamine (○). Each value is the mean of at least two experiments.

at concentrations 400 times higher than its dissociation constant for the alpha-adrenergic receptors. (+)-Isoprenaline and (+)-propranolol were less potent than their respective (−)-isomers, confirming the stereospecificity of binding (Fig. 2).

HIPDM was found to inhibit specific ICYP binding to rabbit lung membranes in a dose-dependent manner in the concentration range 10^{-6}–10^{-3} M. Fifty percent inhibition was observed at a HIPDM concentration of about 3×10^{-5} M (Fig. 3).

Ouabain, in a concentration of 10^{-3} M, had no effect on the inhibition of ICYP binding by HIPDM (data not shown).

The concentration range (10^{-6}–10^{-3} M) over which HIPDM shows inhibition of specific ICYP binding is similar to that reported by Slosman *et al.* [9] for the dose-dependent extraction of ^{125}I-HIPDM by isolated perfused rat lungs.

Transformation of competition curves into Hofstee plots [15], i.e. by plotting % inhibition versus % inhibition divided by the concentration of the competing ligand,

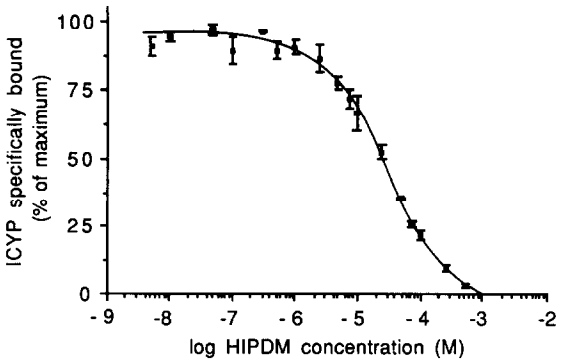

Figure 3
Inhibition of specific ICYP binding to rabbit lung membranes by HIPDM (mean ± SEM of four experiments).

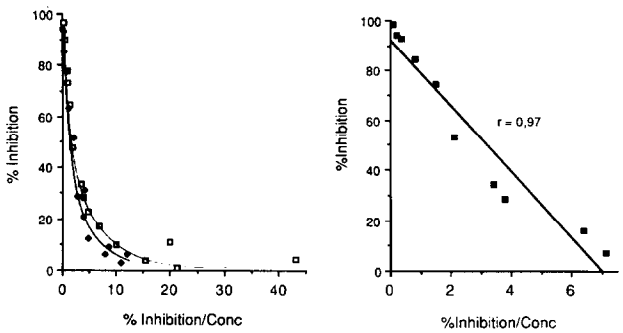

Figure 4
Hofstee plots for inhibition of specific ICYP binding by HIPDM (□), (−)-noradrenaline (●) and (−)-adrenaline (■).

showed that the inhibition of the specific ICYP binding to rabbit lung membranes by HIPDM was similar to that of the biogenic amine (−)-noradrenaline (Fig. 4). As previously reported [13], Hofstee plot for (−)-adrenaline was linear (Fig. 4), with an inhibition constant of specific ICYP binding of 5.19 ± 0.48 μM. In contrast, (−)-noradrenaline produced curvilinear plots, consistent with the coexistence of both $beta_1$- and $beta_2$-adrenoceptors in the rabbit lung [13] and with the different affinity of (−)-noradrenaline for the two receptor subtypes [16].

Thus, HIPDM seems to interact with beta-adrenergic receptors in the rabbit lung in a way similar to that observed for (−)-noradrenaline.

The *in vitro* studies do not imply that the *in vivo* uptake of HIPDM follows the mechanism described for (−)-noradrenaline (sodium-dependent, carrier-mediated transport) [1]. In fact, according to Slosman *et al.* [9], the mechanism of uptake of HIPDM in the perfused rat lung is similar to that of imipramine [17] and propranolol [18], i.e. it is little affected by low temperature and low sodium concentration in the perfusion medium. Likewise, from the observed interaction between HIPDM and beta-adrenergic receptors, it cannot be inferred that HIPDM behaves as a beta-adrenergic agonist or antagonist. In this respect, it should be considered that the possible pharmacological properties of HIPDM have not as yet been investigated.

The nature of the binding process of exogenous amines to lung tissue is still largely undefined. Some investigators [19, 20] proposed that this binding process could result from an interaction of basic amines with tissue phospholipids, possibly related to the amphiphilic character of these moieties. Others [12, 21], studying the interaction of beta-adrenergic ligands with intact cells, advanced the hypothesis of a possible sequestration of the ligands within intracellular acidic compartments.

Although the results of the present *in vitro* studies cannot be extrapolated to *in vivo* conditions, they suggest that beta-adrenergic receptors may be involved in the observed lung uptake of the basic amine HIPDM.

The possibility of labelling HIPDM at a high specific activity should provide a useful approach to elucidate the rôle of receptors and/or other binding sites in the lung uptake of exogenous basic amines.

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[*Received for review 16 May 1989*]