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Mitochondria act as a reservoir for the basic amine HIPDM in the lung. M. Miniati, A. Paci, F. Cocci, G. Ciarimboli, S. Monti, M. Pistolesi. ©ERS Journals Ltd 1996.

ABSTRACT: The lungs are a site for the uptake, accumulation, and storage of exogenous basic amines. The compound N-N-N'-trimethyl-N'-(2-hydroxy-3-methyl-5-iodobenzyl)-1,3 propanediamine (HIPDM) is a basic amine that can be labelled with radioactive iodine and detected by external counting. Intravenously injected ^{123}I-HIPDM is extracted by the human lung, where it is retained in a slowly effluxable pool.

In the present study, we measured HIPDM lung kinetics and subcellular distribution in rabbits given *i.v.* ^{125}I-HIPDM. Rabbits were killed from 2 min to 5 h after injection, and the radioactivity retained in their lungs was measured. Subcellular lung fractions (nuclear, mitochondrial, lysosomal, microsomal, and postmicrosomal supernatant) were assayed for HIPDM radioactivity, protein content, and distribution of specific marker enzymes.

HIPDM lung clearance in rabbits was nearly identical to that of humans. Virtually all the HIPDM radioactivity in lungs (98±1%) was associated with subcellular membranous structures. The highest HIPDM specific radioactivity was found in the mitochondrial fraction, and the subcellular distribution profile closely resembled that of the mitochondrial marker enzyme succinate cytochrome c reductase. No redistribution of HIPDM among subcellular compartments was observed over a 5 h period after injection.

The data indicate that mitochondria act as reservoir for HIPDM in the lungs and contribute to the pulmonary persistence of this compound. HIPDM can be used to investigate the pulmonary uptake of basic amines in health and in lung disease.

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The lungs are a site for uptake, accumulation, and storage of a variety of exogenous chemicals, including drugs in clinical use. The available data on the pharmacokinetic function of the lung can be summarized as follows: 1) the compounds which concentrate most extensively in the lungs are amines with a pKa greater than 8, collectively grouped under the term of basic amines [1, 2]; 2) many of these molecules are amphiphilic in nature, inasmuch as they contain a large hydrophobic group and a side chain which is protonated at physiological pH [1, 2]; 3) in contrast to endogenous biogenic amines [3], a carrier-mediated, energy-dependent transport system is not involved in the pulmonary uptake of basic amines [4–6]; 4) no appreciable *in vivo* lung metabolism has been reported for most of the basic amines investigated so far [4, 6–12]; 5) basic amines with amphiphilic character, *e.g.* imipramine [8, 13], methadone [9, 13], chlorphentermine [13], propranolol [10], verapamil [11], and amiodarone [14], are retained in the lungs in a slowly effluxable pool. At present, there is no obvious explanation for the observed persistence of these drugs in the lungs.

The radioiodinated compound N-N-N'-trimethyl-N'-(2-hydroxy-3-methyl-5-iodobenzyl)-1,3-propanediamine (HIPDM) is an amphiphilic basic amine similar to pneumophilic drugs [15]. Although HIPDM was introduced as a radiopharmaceutical for brain perfusion imaging, preliminary biodistribution studies have indicated that the lungs, rather than the brain, are the main storage site for HIPDM [15]. As assessed in isolated perfused rat lungs, the pulmonary uptake of HIPDM is saturable, does not require an active transport system, and is competitively inhibited by other basic amines, such as imipramine, chlorpromazine and propranolol [16]. No significant *in vivo* metabolism of HIPDM has been detected in rat and rabbit lungs [17, 18].

Unlike other basic amines, the pulmonary uptake of HIPDM can be visualized by external detection [19, 20]. Intravenously injected ^{123}I-HIPDM is quickly extracted by the human lung, where it is distributed according to regional blood flow [20]. The resulting lung scan is indistinguishable from that obtained by conventional perfusion radiotracers. Furthermore, it has been observed that HIPDM is cleared from the human lung at a slow exponential rate [20]. The pulmonary persistence of HIPDM is increased in asymptomatic smokers and in patients with acute respiratory distress syndrome (ARDS) as compared to healthy nonsmokers [18, 20].

In the present study, HIPDM was used to investigate the mechanisms responsible for the pulmonary persistence of basic amines. The time course of HIPDM biodistribution was first evaluated in rabbits receiving *i.v.* bolus injection of labelled HIPDM. In additional experiments, the lung subcellular distribution profile of the compound was assessed by cell fractionation techniques.

Methods

Radiolabelling

The radiolabelling of HIPDM was carried out according to the method described by KUNG *et al.* [15]. Briefly, 2 mCi of carrier-free $Na^{125}I$ (Sorin Biomedica, Saluggia, Italy) were added to 0.25 mL of HIPDM solution (4 mg·mL^{-1} in 0.14 N HCl) in a sealed glass vial, and the reaction mixture was heated in a boiling water bath for 60 min. Radiochemical incorporation was evaluated by thin layer chromatography (Silica Gel 60-F; Merck, Darmstadt, Germany) using chloroform-ethanol-saturated ammonia (80:15:5 by volume) as solvent. The labelling yield was usually greater than 95% and the specific activity of the labelled compound was approximately 0.3 Ci·mmol^{-1}. Free iodine was removed by eluting the reaction mixture through anion exchange resin (Biorex AG 1-X8; Bio-Rad, Richmond, CA, USA).

Biodistribution studies

Forty six adult New Zealand albino rabbits (Stefano Morini Laboratories, Reggio Emilia, Italy) weighing 2–3 kg were used throughout the study. They were fed with a standard rabbit chow. The protocol was approved by the Institutional Animal Care Committee. The animals were injected, through the marginal ear vein, with a 0.5 mL bolus of saline containing an average of 37±9 µg (mean±SD) of labelled HIPDM. At preselected times, ranging from 2 min to 5 h after HIPDM injection, the animals were killed by an intravenous dose of sodium pentobarbital (50 mg·kg^{-1}) and their lungs, liver, kidneys, brain and spleen were quickly removed, blotted dry, and weighed. Samples of peripheral blood were also obtained. The excised organs were then minced and homogenized in saline using a Polytron PT10 homogenizer (Kinematica, Luzern, Switzerland). Weighed aliquots of the homogenates and of the blood, along with suitably diluted aliquots of the injected dose, were counted in a gamma scintillation spectrometer (Packard Instruments, Warrenville, IL, USA) at a counting efficiency of about 75%. Radioactivity per organ was expressed as percentage of the HIPDM injected dose. The fraction of the injected dose in the blood was estimated by using reference values of rabbit total circulating blood volume, as reported in the literature [21].

In three additional rabbits, HIPDM radioactivity was measured in bronchoalveolar lavage. Rabbits were given an *i.v.* bolus of ^{125}I-HIPDM, and were killed by sodium pentobarbital overdose at 2 min, 1 and 4 h after the administration of the labelled compound. The trachea was exposed and clamped shut. The lungs and the trachea were then removed intact and were dissected free of other tissues. A plastic tube was inserted into the trachea and the lungs were gently inflated with air until any sign of atelectasis had gone. Next, 60 mL of ice-cold phosphate-buffered saline were slowly instilled into the lungs *via* the endotracheal tube and were made to recirculate five more times [22]. The lungs were then turned upside down and the lavage liquid was allowed to drain into a tared tube. Recovery of the lavage liquid was 73±6% of the instillate. Cells present in the lavage effluent were sedimented by an integrated force of 5,000 g·min. The cellular sediment (>90% alveolar macrophages as determined by light microscopy examination) was resuspended in saline for *in vitro* counting. The supernatant of the retrieved lavage liquid was layered on a 0.75 M sucrose cushion and was centrifuged at 4,800,000×g·min [22]. The white band (alveolar surfactant) overlaying the sucrose cushion was collected and partitioned in 1 mL aliquots for *in vitro* counting. The lungs were then dissected free of the trachea and of major airways, minced, and homogenized in saline as described previously. The whole lung homogenate was assayed for HIPDM radioactivity. Counts in lung homogenate, lavage cells, and alveolar surfactant were expressed as percentage of the total HIPDM radioactivity recovered.

Lung cell fractionation studies

Twenty two adult New Zealand albino rabbits were used in these experiments. As for biodistribution studies, the rabbits received an *i.v.* bolus of labelled HIPDM and were killed with a lethal injection of sodium pentobarbital at various times (from 2 min to 5 h) after HIPDM administration. The lungs were excised, dissected free of major airways, weighed and minced. The lung mince was homogenized in 10 vol of ice-cold medium (0.25 M sucrose, 0.005 M Tris hydrochloride, 0.001 M $MgCl_2$, pH 7.4 at 4°C) using a Polytron PT 10 homogenizer for 2×10 s at maximum speed. The resulting slurry was strained through two layers of gauze and subjected to differential centrifugation by a modification of the method described by DE DUVE *et al.* [23]. Prior to centrifugation, weighed aliquots of the homogenate and of the filtrate were set aside for further determinations as described below. All the subsequent manipulations were conducted at 4°C in tared tubes. Undisrupted cells and nuclei, henceforth referred to as nuclear fraction, were sedimented by an integrated force of 20,000 g·min. The supernatant, which contained the cytoplasmic extract, was further fractionated and three particulate fractions, mitochondrial, lysosomal and microsomal, were successively sedimented by integrated forces of 88,000, 200,000 and 4,440,000 g·min, respectively. All four sediments were washed once in cold medium (0.05 M Tris hydrochloride, 0.01 M $MgCl_2$, 0.075 M NaCl, pH 7.4 at 4°C) by resuspension and recentrifugation. The washings were then discarded and the wet weight of each sediment was measured. Weighed samples of each sediment and of the final supernatant were taken for measuring the retained HIPDM radioactivity and the protein content. The latter was measured by the BCA Protein

Assay (Pierce Chemical Company, Rockford, IL, USA) using bovine serum albumin as a standard. The same determinations were carried out on the samples of the homogenate and of the filtrate laid aside previously.

Additional aliquots of the subcellular fractions were assayed for the following enzymatic activities: succinate cytochrome c reductase (mitochondrial marker enzyme); arylsulphatase B (lysosomal marker enzyme); and NADPH-cytochrome c reductase (microsomal marker enzyme). Succinate cytochrome c reductase was measured as described by SOTTOCASA *et al.* [24]. Arylsulphatase B was assayed by the spectrophotometric method of BAUM *et al.* [25], as modified by HALL *et al.* [26]. NADPH-cytochrome c reductase determination was carried out by a modification of the method of SILER MASTERS *et al.* [27].

Because the mitochondrial fraction of the mammalian lung is contaminated by lamellar bodies originating from alveolar type 2 pneumocytes [28], 10 additional lung cell fractionation experiments were carried out in which lamellar bodies were isolated from mitochondria in order to measure the partitioning of ^{125}I-HIPDM between these two subcellular components. In these experiments, rabbits were killed by sodium pentobarbital overdose at 5 min and at 3 h after HIPDM *i.v.* injection. For the isolation of lamellar bodies, the methods described by PAGE-ROBERTS [29] and by HASSETT *et al.* [30] were used, with some modification. Briefly, the crude mitochondrial sediment, obtained as described previously, was resuspended in 2 mL of 0.33 M sucrose in 0.01 M Tris hydrochloride, pH 7.4, at 4°C (0.33 M sucrose-Tris) and weighed. A weighed aliquot of the crude mitochondrial suspension was set aside for measuring HIPDM radioactivity. The residual suspension was layered on 5 mL of 0.80 M sucrose-Tris and centrifuged at 1,600,000×g·min. The procedure was conducted at 4°C in tared tubes. After centrifugation, there was a light amber-coloured mitochondrial sediment and a white band, containing lamellar bodies, at the interface between 0.33 and 0.80 M sucrose. The white band was gently removed, diluted with 0.22 M sucrose-Tris to a final volume of 8 mL, and centrifuged at 1,600,000 g·min to yield a tightly packed white pellet. Weighed aliquots of the purified mitochondrial and lamellar body sediments were assayed for HIPDM radioactivity.

In preliminary experiments, the two sediments were characterized biochemically by measuring the phospholipid:protein ratio. Lipids were extracted according to FOLCH *et al.* [31], using a chloroform-methanol mixture (2:1 by volume). Inorganic phosphorus in perchloric acid digests of the lipid extract was assayed according to SHIN [32]. The phospholipid content was then calculated by multiplying the lipid-phosphorus by 25 [33]. In the present experiments, the phospholipid:protein ratios of the lamellar body and of the purified mitochondrial sediment were 4.77±0.42 and 0.36±0.08, respectively. These values are in close agreement with those reported by GIL and REISS [28] for the lamellar body and mitochondrial fractions obtained from rat lung homogenate.

Results

Biodistribution studies

Biodistribution data of HIPDM in rabbits are presented in table 1. Two minutes after HIPDM *i.v.* administration, approximately 90% of the injected dose was recovered from the lungs. Radioactivity in the liver and in the total circulating blood volume accounted for 2 and 4% of the injected dose, respectively. Negligible amounts of radioactivity (<1% of the dose) were recovered from the other organs. As a function of time, the clearance of HIPDM from the lungs was biphasic, with an initial faster wash-out followed by a more gradual decline of radioactivity. In the liver and kidneys, the radioactivity increased progressively and, by 5 h after injection, averaged 12 and 5% of the injected dose, respectively. Throughout the study, the blood radioactivity declined to a constant value of about 1.5% of the injected dose; brain and spleen activity did not exceed 1% of the dose.

Figure 1 shows the percentage of HIPDM injected dose in the lungs, liver, brain, kidneys and spleen, normalized to the corresponding organ weight. It appears that, throughout the period of experimental observation, the concentration of HIPDM was highest in the

Table 1. – Biodistribution of HIPDM in rabbits

	HIPDM % injected dose					
Time min	Lungs	Liver	Kidneys	Brain	Spleen	Blood*
2	90±7	2.1±0.4	0.8±0.5	0.5±0.1	0	3.9±2.6
15	50±12	5.2±1.2	4.0±0.8	0.9±0.3	0.1±0.0	2.7±0.0
30	52±11	6.2±1.2	4.2±1.2	0.7±0.3	0.3±0.2	1.8±0.5
45	39±11	8.3±3.5	4.5±1.6	0.7±0.3	0.3±0.2	1.8±0.4
60	31±5	7.1±0.8	5.3±0.9	0.8±0.1	0.3±0.2	1.6±0.0
90	22±2	11±2	5.6±0.5	1.0±0.3	0.5±0.1	1.7±0.2
120	23±7	13±3	6.9±1.3	1.0±0.1	0.4±0.2	1.8±0.4
150	21±6	13±3	6.1±0.6	1.0±0.1	0.5±0.1	1.4±0.2
180	21±7	13±2	4.9±1.1	1.0±0.2	0.5±0.3	1.6±0.2
240	17±5	15±4	4.5±1.1	0.8±0.1	0.3±0.1	1.5±0.4
300	13±4	12±1	4.9±2.2	0.7±0.2	0.4±0.1	1.4±0.3

Values are presented as mean±SD of four different experiments. *: % of HIPDM injected dose in total circulating blood volume. HIPDM: N-N-N'-trimethyl-N'-(2-hydroxy-3-methyl-5-iodobenzyl)-1,3 propanediamine.

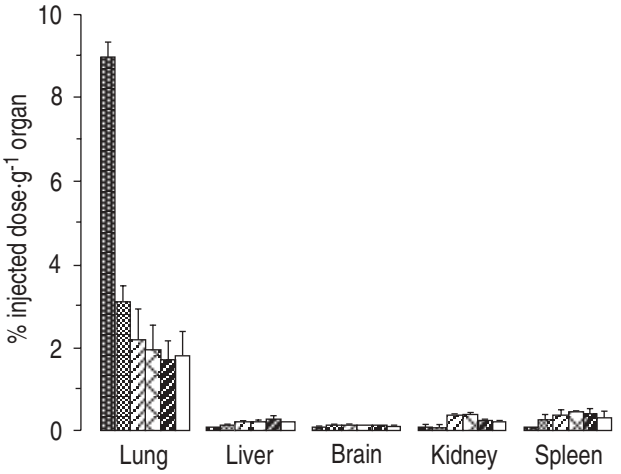

Fig. 1. – Biodistribution of HIPDM in rabbits. Bars representt the percentage of injected dose in the lung, liver, brain, kidney and spleen, normalized to the corresponding organ weight. Values are presented as mean±SD. For the sake of clarity, experimental data obtained at 15, 30, 45, 90 and 150 min are omitted. : 2 min; : 60 min; : 120 min; : 180 min; : 240 min; : 300 min. HIPDM: N-N-N'-trimethyl-N'-(2-hydroxy-3-methyl-5-iodobenzyl)-1,3 propanediamine.

lungs. Even at 5 h after injection, the radioactive concentration in the lungs was higher than that measured in the liver, brain, kidneys and spleen, by a factor of 10, 20, 8 and 6, respectively.

The time course of HIPDM clearance from the lungs was further characterized by applying nonlinear regression analysis to the experimental data. The efflux of HIPDM from the lungs was adequately described by a biexponential function (fig. 2). The half-times of the faster and of the slower exponential component were 18 min and 4.3 h, respectively (fig. 2). The mean times of

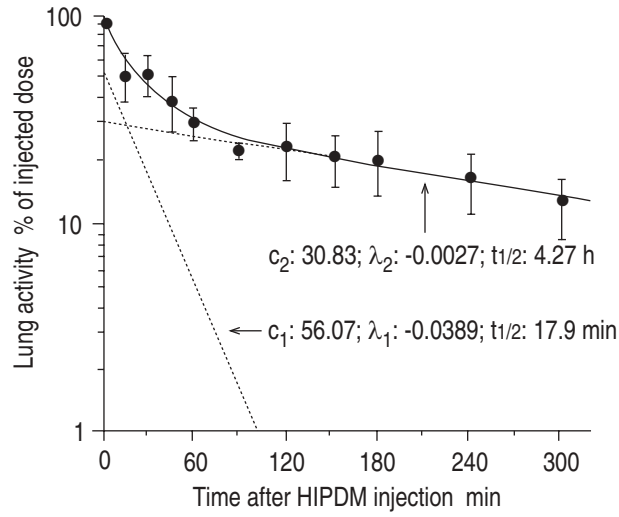

Fig. 2. – Efflux of HIPDM from the rabbit lung. The curve is obtained by *in vitro* counting of lung homogenates from rabbits killed at various times after ^{125}I-HIPDM *i.v.* injection. Data are reported on a semilog scale as percentage of the injected dose. Values at each time are the average±SD of four different experiments. The experimental curve has been resolved into two exponential components (dashed straight lines). λ_1 and λ_2 are the slopes (decay constants) of the two exponentials; C_1 and C_2 are the intercepts to the y-axis for the respective exponentials. The solid line represents biexponential fitting of experimental data. HIPDM efflux = $C_1e^{-\lambda_1 t} + C_2e^{-\lambda_2 t}$. For definitions see legend to figure 1.

the two exponential components, computed from the corresponding decay constants (λ), were 26 min and 6.2 h, respectively. The size of the slowly effluxable pool, estimated by backward extrapolation of the slower exponential component of HIPDM efflux, accounted for 30% of the injected dose.

In the three rabbits in which we did bronchoalveolar lavage (BAL), the radioactivity associated with lavage cells and alveolar surfactant did not exceed 3 and 0.2% of the total HIPDM activity recovered, respectively. Indeed, at any time after HIPDM *i.v.* injection, most of the radioactivity recovered (98±1%) was retained by the lung tissue after BAL.

Lung cell fractionation studies

The subcellular distributions of HIPDM and of protein, obtained by averaging the data of 22 consecutive experiments, are presented in table 2. HIPDM and protein in each subcellular fraction are expressed as a percentage of their respective cumulative amounts recovered from all the fractions. In these experiments, the cumulative radioactivity recovered from the fractions averaged 98±3% of the radioactivity measured in the filtrate prior to centrifugation, thus indicating no significant loss of tracer in the fractionation procedure.

Virtually all the radioactivity retained in the lungs at any time was associated with subcellular membranous structures, the nuclear and mitochondrial fractions containing the bulk of the compound. Less than 2% of the total radioactivity in the lungs was recovered from the final supernatant. As a function of the time elapsed from the administration of the compound, the percentage of total radioactivity in each sediment did not appreciably change, suggesting no redistribution of HIPDM in subcellular compartments.

The relative specific radioactivity of HIPDM in the subcellular fractions was calculated by dividing the percentage of total radioactivity in each fraction by the corresponding percentage of total protein [23]. The mean values of HIPDM relative specific radioactivity in the fractions are given in table 2. A more illustrative analysis of the results is obtained by plotting the mean relative specific radioactivity of the fractions against their

Table 2. – HIPDM and protein in subcellular fractions of rabbit lung

Fraction	HIPDM %	Protein %	HIPDM %/ protein %
Nuclear	55±4	37±7	1.6±0.3
Mitochondrial	31±2	5±1	6.5±1.2
Lysosomal	7.1±1.8	1.8±0.5	4.1±1.1
Microsomal	4.8±1.6	3.7±0.9	1.4±0.6
Supernatant	1.9±0.9	53±8	0.04±0.01

HIPDM and protein in each subcellular fraction are expressed as the percentage of their respective cumulative amounts recovered from all fractions. HIPDM %/protein %: HIPDM relative specific radioactivity. Data are presented as mean±SD of 22 determinations carried out at preselected times from 2 to 300 min after HIPDM *i.v.* injection. For individual time points and further definitions see legend to table 1.

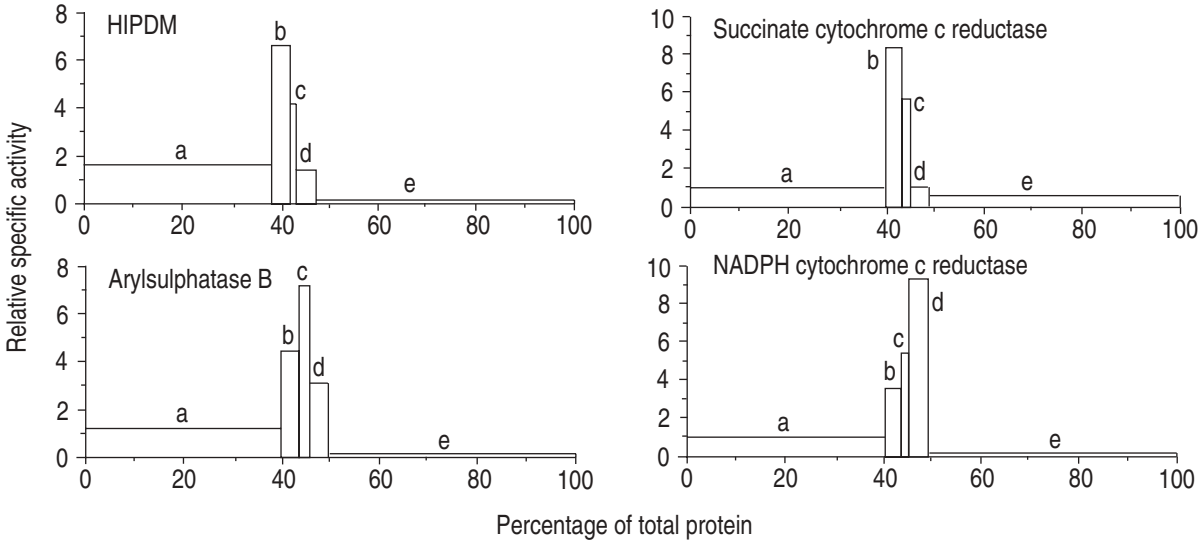

Fig. 3. – Lung subcellular distribution profiles of HIPDM and of marker enzymes for mitochondria (succinate cytochrome c reductase), lysosomes (arylsulphatase B), and microsomes (NADPH-cytochrome c reductase). Relative specific activity for HIPDM: % of total radioactivity/% of total protein in each fraction. Relative specific activity for marker enzymes: % of total enzymatic activity/% of total protein in each fraction. On the abscissa: fractions are represented by their relative protein content in the order in which they were isolated, *i.e.* from left to right, a) nuclear, b) mitochondrial, c) lysosomal, d) microsomal, and e) postmicrosomal supernatant. NADPH: nicotinamide adenine dinucleotide phosphate (reduced form). For further definitions see legend to figure 1.

relative protein content (fig. 3). The area of each block is, thus, proportional to the percentage of radioactivity recovered in the corresponding fraction, and its height to the relative HIPDM concentration. In figure 3, the subcellular distribution profiles of marker enzymes for mitochondria, lysosomes, and microsomes are reported for comparison, using the same graphic display. The highest relative specific radioactivity of HIPDM was found in the mitochondrial fraction. Indeed, the subcellular distribution profile of HIPDM closely resembled that of the mitochondrial marker enzyme, succinate cytochrome c reductase, thus suggesting a predominant localization of the compound in the mitochondria. Such preferential distribution remained unchanged as a function of time from the *i.v.* administration of the compound.

In 10 additional lung cells fractionation experiments, the crude mitochondrial sediment was purified from lamellar body contamination. The cumulative HIPDM radioactivity recovered from the purified mitochondrial and lamellar body fractions averaged 99±0.5% of the radioactivity measured in the crude mitochondrial sediment prior to density gradient centrifugation. After removing lamellar bodies, the purified mitochondrial sediment retained 82±1% of the HIPDM radioactivity measured in the crude mitochondrial sediment.

Discussion

The results of the present study indicate that the radioiodinated basic amine, HIPDM, is extracted by the rabbit lung from which it is cleared at a slow biexponential rate. As shown by previous experiments on the metabolism of HIPDM, more than 90% of the radioactivity recovered from the lungs of rats and rabbits is in the form of unmetabolized compound [17, 18]. By contrast, HIPDM is extensively metabolized in the liver [17, 18]. The above findings are consistent with the observation that rat and rabbit lungs are devoid of diamine oxidase activity [34]. Hence, the observed rate of clearance of radioactivity from the rabbit lung mostly reflects the behaviour of unmetabolized HIPDM. The pulmonary clearance of HIPDM in rabbits is similar to that observed in humans by external detection [20]. In fact, the mean time of the slower exponential component of HIPDM lung efflux in rabbits (6.2 h) approximates the value computed in normal nonsmokers (6.7±0.6 h) [20]. The amount of tracer cleared with the slower exponential component accounts for approximately 30% of the injected radioactivity. Thus, in the rabbit lung, a sizeable fraction of HIPDM is retained in a slowly effluxable pool. The formation of a slowly effluxable pool has been described, in isolated-perfused lungs, for a variety of basic amines [8–11, 14]. In these experiments, the size of the slowly effluxable pool was similar to that observed for HIPDM, ranging 30–55% of the drug accumulated by the lungs [8, 9, 11, 14]. For imipramine, a pulmonary persistent pool with a mean residence time in excess of 5 h has also been demonstrated *in vivo* in rabbits receiving *i.v.* bolus injection of the labelled compound [13].

Because only those basic amines with amphiphilic character are retained by the lungs in a slowly effluxable pool, it has been speculated that such a persistent pool could result from the interaction of these molecules with lung phospholipids, namely with the alveolar surfactant [1, 2, 8, 13]. The present data do not support this hypothesis, inasmuch as it was found that very little HIPDM radioactivity was associated with alveolar surfactant obtained by BAL. At any time after *i.v.* injection, most of the HIPDM radioactivity recovered from the lungs (98±1%) was associated with intracellular particulate fractions (table 2). The subcellular distribution profile of HIPDM, derived from lung cell fractionation studies, was consistent with a predominant localization of the compound in the mitochondria (fig. 3). After removing the lamellar body contamination from the crude mitochondrial fraction, most of the HIPDM

radioactivity was still retained in the purified mitochondrial sediment. Thus, it appears that the mitochondria are important sites for the accumulation of HIPDM in the lungs.

The findings of the present study are in agreement with those of YOSHIDA *et al.* [35], who evaluated the subcellular distribution of the basic amines imipramine, quinine, and metoclopramide in the rat lung. The accumulation of these drugs was highest in the crude mitochondrial fraction and was correlated to the degree of lipid solubility, imipramine (the most lipophilic compound) exhibiting the highest relative accumulation in mitochondria [35]. The subcellular distribution of basic amines, described by YOSHIDA *et al.* [35], was measured in isolated rat lungs after 60 min of perfusion. In the present experiments, the subcellular distribution of HIPDM was examined in rabbit lungs at various times after *in vivo i.v.* injection of the labelled compound. It was observed that the preferential distribution of HIPDM in the mitochondrial fraction was constant over a period of time ranging from 2 min to 5 h after the *i.v.* administration of the compound. Since, during this time HIPDM is slowly cleared from the lungs at a biexponential rate (fig. 2), the above finding suggests that the persistence of HIPDM in the lungs is accounted for by the interaction of the compound with lung mitochondria.

The preferential distribution of basic amines in the mitochondria may result from binding to specific phospholipids, such as cardiolipin and phosphatidylethanolamine, known to be rich in mitochondrial membranes [36]. However, the mitochondria have a distinctive property over any other intracellular organelle, *i.e.* they express a significant membrane potential with a negative charge inside [37]. Indeed, a proton gradient is established across the inner mitochondrial membrane as a result of active extrusion of protons driven by electron transport chains [37, 38]. This gradient has a chemical component (pH gradient) and an electric component (membrane potential). The mitochondria of mammalian cells express this electrochemical gradient mostly as membrane potential of about -180 mV and, to a lesser extent, as pH gradient of about 1 unit [37]. Lipophilic compounds with a delocalized positive charge (*e.g.* HIPDM) may, therefore, penetrate the hydrophobic core of the inner mitochondrial membrane and attain an equilibrium across the membrane as predicted by the Nernst equation [37].

The *in vivo* results of this study support the concept that the mitochondria act as reservoir for the basic amine HIPDM in the lungs and, as such, contribute to the persistence of this compound in the lung tissue. The observation of an increased persistence of HIPDM in the lungs of smokers [20] and of patients with acute respiratory distress syndrome [18] may reflect some biochemical alteration of mitochondrial membranes that warrants further investigation. In this connection, it is worth considering that an unusual retention of the mitochondria-specific, fluorescent probe rhodamine 123 has been described *in vitro* in a variety of carcinoma cells [39], which express a significantly higher mitochondrial membrane potential as compared to normal epithelial cells. HIPDM or related compounds can be used to further investigate the pulmonary uptake of basic amines in health and in lung disease.

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