Biochemical Pharmacology, Vol. 43, No. 3, pp. 431–437, 1992.
Printed in Great Britain.

0006-2952/92 $5.00 + 0.00

THE ACCUMULATION OF PENTAMIDINE INTO RAT LUNG SLICES AND ITS INTERACTION WITH PUTRESCINE

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(*Received* 15 *April* 1991; *accepted* 22 *October* 1991)

Abstract—The aromatic diamidine, pentamidine, accumulated into rat lung slices by an uptake system that obeyed saturation kinetics, with an average K_m value of 554 μM and a V_{max} value of 4077 nmol/g lung wet wt/30 min, respectively. This system was not inhibited by metabolic inhibitors but was greatly diminished by lowering the temperature from 37° to 4°. Both compounds, pentamidine and putrescine, inhibited the uptake of the other and the inhibition of pentamidine accumulation by putrescine was demonstrated to be non-competitive. Uptake of putrescine was inhibited by increasing concentrations of pentamidine. As putrescine accumulates in epithelial type 1 and type 2 cells and in Clara cells, it is likely that pentamidine is also accumulated in these cell types but does not utilize the pulmonary uptake system for polyamine transport. Within the time period studied, toxic effects of the drug were not observed.

Since the 1940s, pentamidine isethionate (4:4′-diamidinodiphenoxypentane di-(β-hydroxyethane sulphonate) has been widely used for the treatment of human tropical diseases such as Leishmaniasis and Trypanosomiasis [1, 2]. It is also one of the standard therapies for *Pneumocystis carinii* pneumonia (PCP‖), an opportunistic infection seen in debilitated infants and immunosuppressed patients including those suffering from acquired immunodeficiency syndrome (AIDS) [2]. Since 1981, the use of pentamidine in the treatment of AIDS-associated PCP has increased dramatically [3]. As parenteral administration of the drug results in severe adverse effects, particularly in causing renal damage, pentamidine has recently been given by inhalation. This inhalation route is claimed to produce much higher concentrations of the drug on the bronchoalveolar surface with little associated toxicity, thus enhancing efficacy [4].

Whilst toxicological considerations of pentamidine have led to extensive mechanistic studies of accumulation in kidney and liver cells [5], its cellular site of uptake within the lung, particularly with regard to inhaled administration, has not been established, even though this is likely to influence the efficacy of the drug following pulmonary administration. It has been shown that lung tissue possesses a saturable energy-dependent uptake system for the accumulation of the polyamines spermine and spermidine and their diamine precursor putrescine [6–8]. It has also been concluded that the accumulation of putrescine, spermine and spermidine occurs in the alveolar epithelial type 1 and type 2 cells and Clara cells [9]. As pentamidine has the structural potential expected of a compound which could inhibit pulmonary diamine accumulation [10], it is possible that the drug is accumulated in the lung via the polyamine uptake system. The aims of this study were therefore, to (1) establish whether pentamidine is accumulated into lung slices in a saturable, energy-dependent manner and (2) determine the target cells for the drug and investigate the mechanism of pentamidine transport using putrescine in inhibitory studies.

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‖ Abbreviations: PCP, *Pneumocystis carinii* pneumonia; KRP, Krebs–Ringer–phosphate.

MATERIALS AND METHODS

Materials

[ring-^{3}H]Pentamidine isethionate (8.81 GBq/mmol) and [1,4-^{14}C]putrescine dihydrochloride (4.37 GBq/mmol) were purchased from Amersham International plc (Amersham, U.K.). Pentamidine isethionate and sodium pentobarbitone "Sagatal" were supplied by May and Baker (Rhône-Poulenc Group, Dagenham, U.K.). Putrescine dihydrochloride was purchased from the Sigma Chemical Co. (Poole, U.K.), sodium cyanide from B.D.H. (Poole, U.K.), iodoacetate from the Aldrich Chemical Co. (Poole, U.K.) and Halothane B.P. from the Pharmaceuticals Division, Imperial Chemical Industries plc (Macclesfield, U.K.). The tissue solubilizer, Soluene-100 and the scintillation cocktails, Monophase-S and Emulsifier-Safe were obtained from Packard Instruments Ltd (Amersham, U.K.).

Animals

Male Wistar derived pathogen free rats (body weight 200–280 g) were used throughout and purchased from CDE (Porton Down, U.K.).

Methods

Preparation of lung slices. Rats were immobilized

with Halothane and killed with an i.p. injection of Sagatal. The lungs were immediately removed intact as detailed previously [11]. Lung slices, 0.5 mm thick were prepared using a McIlwain tissue chopper. Only slices with two cut surfaces were used.

Uptake of the diamines into lung slices. Modified KRP solution was prepared as described previously [6]. The uptake of pentamidine or putrescine was investigated by adding 3.7 kBq of either [^{3}H]-pentamidine or [^{14}C]putrescine to the appropriate media. Fresh lung slices, weighed after blotting (20–40 mg wet wt) were incubated in 1.0 mL KRP in a 37° oven unless stated otherwise.

Several preliminary experiments were performed before wider studies were undertaken.

(a) The accumulation of pentamidine was determined over a range of concentrations (10–50 μM) and over a time course (5–60 min). In each experiment, three rats were used with the lung slices from each rat being used to investigate all the concentrations and time points. Incubations were performed in multiwell plates at 37°. In addition, the effect of (a) the metabolic inhibitors sodium cyanide (1 mM) and iodoacetate (1 mM) and (b) temperature on the uptake of 25 μM [^{3}H]pentamidine was measured with time (5–30 min).

(b) Pentamidine uptake was also investigated at high concentrations (0.1–1 mM) over a time course (5–30 min) at 37°. The effect of the metabolic inhibitors NaCN (1 mM) and iodoacetate (1 mM) on each of these high drug concentrations was determined using a 30 min incubation period.

(c) The accumulation of pentamidine was also investigated in Erlenmeyer conical flasks (25 mL), an established protocol devised by Smith and Wyatt [6], in order to confirm that the data generated in multiwell plates were in accord with the data generated with this previously established method. The uptake of pentamidine was determined in an identical manner to that reported for multiwell plates above. Slices were incubated in 3 mL KRP at 37° in a shaking water bath at 70 c/min. Accumulation of pentamidine was determined over a range of concentrations (10–300 μM) and over a time course (5–120 min).

Analysis of the results from the above consecutive experiments conducted early in this programme, demonstrated that the measurement of the rate of uptake above 50 μM concentration of pentamidine was subject to large variation. For this reason, the number of experiments and data points were substantially increased.

The accumulation of pentamidine was determined over a range of concentrations (7–200 μM) at 37° using a 30 min incubation period. In all experiments, each concentration was investigated in triplicate with several experiments being performed. The effect of 20 μM putrescine on the uptake of pentamidine (7–200 μM) was also studied. Using [^{14}C]putrescine accumulation as a marker of epithelial cell integrity in the slices, possible toxic effects of pentamidine were studied. The slices were preincubated in various concentrations of pentamidine (25–500 μM) at 37° for 1 hr. After a thorough wash in KRP to remove any traces of pentamidine, they were incubated in 20 μM [^{14}C]putrescine at 37° over a time course (5–30 min).

Measurement of diamine concentration in the lung slices and incubation media. Following removal from the incubation medium, the lung slices were immersed in KRP, washed well and carefully blotted dry. They were solubilized in 1 mL Soluene-100 and either 15 mL of Monophase-S was added for ^{3}H-radiolabel or Emulsifier-Safe for ^{14}C-radiolabel. The appropriate scintillant was also added to a sample of medium (0.1 mL) in order to determine the specific activity of the medium. The radioactivity was determined by liquid scintillation counting using a LKB Rackbeta scintillation counter. The radioactivity was expressed as disintegrations per minute (dpm) by the use of ^{3}H and ^{14}C external standards.

Analysis and expression of results. At each concentration of the diamine, the number of nanomoles of either pentamidine or putrescine that had been incorporated into the rat lung slice were calculated from the radioactivity (dpm) that was associated with the slice using the specific activity of the corresponding incubation medium. The rate of accumulation was expressed as nanomoles of compound per gram wet lung weight per given time. The apparent kinetic constants, K_m (concentration at $\frac{1}{2} V_{max}$) and V_{max} (maximum velocity of pentamidine or putrescine uptake) were determined using the Hane plot method [12] where S (exogenous diamine concentration) is plotted against S/V (V = the rate of diamine accumulation) and were also calculated and best-fit to the relevant theoretical equations using the curve-fit analysis and presentation programme available in the software of Sigmaplot 4 (Jandel Corporation, Corte Madera, CA).

Validation of the use of radioactivity to measure diamine concentration. Experiments were carried out to determine if pentamidine was metabolized by lung slices. The slices were incubated in 25 μM (7.4 kBq) [^{3}H]pentamidine for periods up to 1 hr after which they were washed (KRP), blotted dry and homogenized in 1.5 mL 0.1 M HCl for 5 min. The homogenate was centrifuged for 10 min at 1500 g and the supernatant retained for investigation. A sample of the incubation medium was also acidified and processed for analysis. Samples of the supernatant, medium and [^{3}H]pentamidine standard (0.17 kBq, 4 mg/mL cold pentamidine) were applied to a cellulose-coated, plastic TLC plate (0.1 mm thick, Merck, Darmstadt, Germany). The solvent system consisted of ethanol:ammonia:water (80:4:16). After separation, the standard was localized using ninhydrin (0.5% w/v) in water-saturated n-butanol and the corresponding R_f areas were removed for each sample. Monophase-S (10 mL) was added and the radioactivity was determined as described previously.

Using this technique, approximately 70% of the sample from the lung slice homogenate and the incubation medium co-chromatographed with the [^{3}H]pentamidine standard. The remaining radioactivity was found to be evenly distributed between the origin and the area immediately preceding that which co-chromatographed with the standard. No other major spots were detected. It was therefore

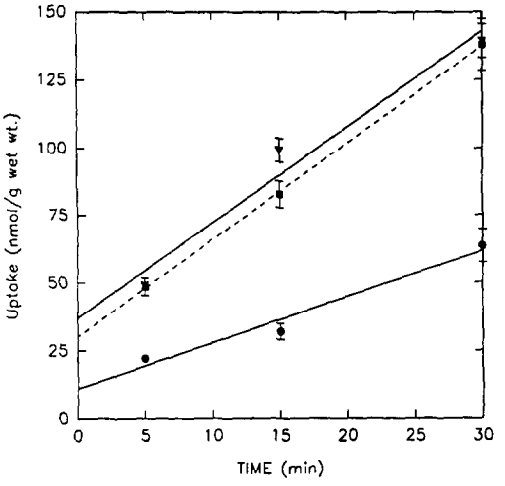

Fig. 1. The accumulation of pentamidine by rat lung slices. The lung slices were incubated in KRP containing either 25 μM [^{3}H]pentamidine (-■- and - - -), 25 μM [^{3}H]-pentamidine plus sodium cyanide (1 mM) and iodoacetate (1 mM) (-▼-) (both at 37°) or 25 μM [^{3}H]pentamidine at 4° (-●-). Incubations were performed over a time course (5–30 min) in a shaking water bath. The accumulation of pentamidine was derived from tissue radioactivity. The results are expressed as the mean values ± SEM of three observations per time point.

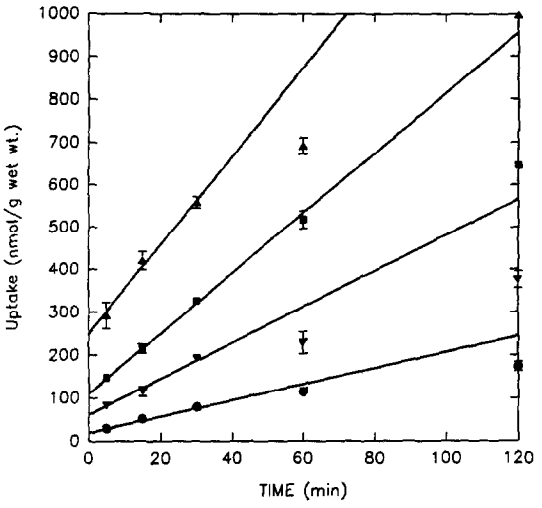

Fig. 2. The uptake of pentamidine with time. The lung slices were incubated in 10 μM pentamidine (-●-), 25 μM pentamidine (-▼-), 50 μM pentamidine (-■-) and 100 μM pentamidine (-▲-) over a time course (5–120 min) in Erlenmeyer conical flasks (25 mL) at 37° in a shaking water bath. The results are presented as mean values ± SEM of three determinations per time point.

concluded that pentamidine was not metabolized in the lung slices during the time period studied. This observation is in agreement with those of numerous workers who have also concluded, using a wide range of techniques, that pentamidine is inert and not metabolized in any of the major body organs including lung and in several species including rats [13–16]. It has recently been shown, however, that pentamidine is metabolized by rat liver fractions resulting in the formation of seven metabolites [17]. Should this metabolism occur in lung slices, it is possible that the TLC separation employed would not be able to differentiate these species from the parent molecule. It is significant to note, therefore, that if the major metabolites are similar to those in the liver, then in our present study, quantification of the radiolabel could only be associated with pentamidine and/or its metabolites.

The second radiolabel used, namely [^{14}C]-putrescine is not metabolized in 1 hr incubations of lung slice preparations [6].

RESULTS

Accumulation of pentamidine by rat lung slices

The preliminary experiments demonstrated that when rat lung slices were incubated in 25 μM [^{3}H]-pentamidine at 37° and in a shaking water bath, it was observed that pentamidine accumulated in a linear, time-dependent manner over the period from 5 to 30 min, with a small amount of additional uptake also occurring during the initial few minutes (Fig. 1). Treatment with the metabolic inhibitors sodium cyanide (1 mM) and iodoacetate (1 mM) had no effect on the accumulation of pentamidine but when the slices were incubated at 4°, a reduction in pentamidine uptake was noted (Fig. 1). It was observed that using conical flasks containing rat lung slices, the accumulation of pentamidine was linear with time for 30 min for concentrations of 10–300 μM, but levelled off beyond this incubation period (Fig. 2). As similar observations were noted the multiwell plate protocol, future studies were not performed beyond the 30 min time period and the kinetic parameters thus presented were, therefore, determined using points up to and including 30 min. With flask incubations, the apparent K_m and V_{max} values for the accumulation of pentamidine in rat lung slices were determined as 93 μM and 610 nmol/g wet lung wt/30 min respectively (Table 1, Fig. 3). Almost identical results for K_m and V_{max} (100 μM and 625 nmol/g wet lung wt/30 min, respectively) were calculated from multiwell incubations (Table 1). The more convenient multiwell plate method was therefore chosen for further studies.

Kinetics of pentamidine in rat lung slices at high concentrations

Data from the Hane plot used for the derivation of kinetic constants for pentamidine uptake revealed that at concentrations of the drug above 100 μM, a considerable deviation from the straight line occurred (Fig. 3). The possibility that pentamidine was transported differently above 100 μM was investigated by incubating lung slices in high concentrations of the drug (0.1–1 mM) and the results are shown in Fig. 4. Pentamidine was accumulated in a linear time-dependent manner over 30 min and saturation kinetics applied to the rates of uptake thus determined revealed an apparent K_m and V_{max} value

Table 1. Kinetic constants for the accumulation of pentamidine into rat lung slices

Pentamidine concentration in medium (μM)	Rate of accumulation (nmol/g wet wt lung/30min)	Apparent K_m (μM)	V_{max} (nmol/g wet wt lung/30min)
10	59 ± 7.6 (3)		
25	128 ± 10.1 (3)		
50	213 ± 11.9 (3)	93	610
100	323 ± 21.9 (3)		
300	975 ± 66.5 (3)		
10*	58 ± 1.5 (3)		
25*	125 ± 16.7 (3)	100	625
50*	208 ± 14.7 (3)		

The lung slices were incubated in the above concentrations of [^{3}H]pentamidine over a time course at 37° in either a shaking water bath or in multiwell plates (*). The concentration of pentamidine in the lung slice was determined as described in Materials and Methods. The rate of accumulation was determined using linear regression against time.

The results are expressed as mean values of three determinations made for each time point and concentration ± SEM with the number of observations in parentheses.

The apparent K_m and V_{max} values were determined using the Hane plot method, i.e. plotting S/V against S.

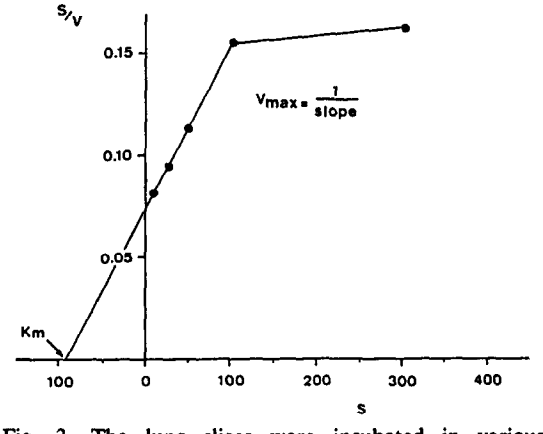

Fig. 3. The lung slices were incubated in various concentrations of [^{3}H]pentamidine (10–300 μM) over a time course at 37° in a shaking water bath. The rate of accumulation of pentamidine (V) was calculated as described in Materials and Methods. The kinetic constants were determined using the Hane plot where S (μM pentamidine) is plotted against S/V. The apparent K_m and V_{max} values were calculated as 93 μM and 610 nmol/g wet lung wt/30 min, respectively, using the first four points, i.e. S = 10, 25, 50 and 100 μM.

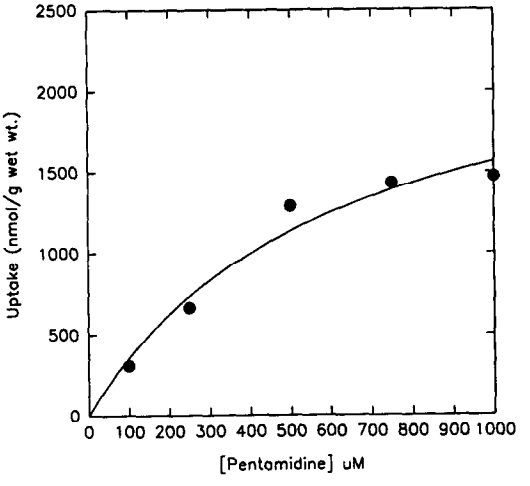

Fig. 4. The accumulation of high concentrations of pentamidine by rat lung slices. The lung slices were incubated in various concentrations of [^{3}H]pentamidine (0.1–1 mM) over a time course (5–30 min) at 37° in multiwell plates. The rate of accumulation was calculated using linear regression against time, each rate being derived from three determinations for each concentration and time point. The results have been analysed using Sigmaplot 4 and are expressed as a Michaelis–Menten curve. Apparent K_m and V_{max} values of 607 ± 121 μM and 2513 ± 282 nmol/g wet lung wt/30 min were calculated (mean ± SD).

of 607 ± 121 μM and 2513 ± 282 nmol/g wet lung wt/30 min, respectively, for pentamidine.

The metabolic inhibitors had no effect on the rate of pentamidine uptake at high concentrations (0.1–1 mM) over 30 min, as the values obtained for pentamidine accumulation plus inhibitors were virtually identical to those obtained with pentamidine alone (data not shown).

The kinetic results obtained from the experiments shown in Figs 3 and 4 were analysed and best-fit to theoretical equations using Sigmaplot 4. An attempt was made to fit the data from Fig. 3 to a double Michaelis–Menten equation:

$$v = V_{max}[S]/(K_m + S) + V'_{max}[S]/(K'_m + S)$$

i.e. combination of a (high affinity) active uptake system together with another (low affinity) process which obeyed saturation kinetics. It was found that the data presented in Fig. 3 (10–300 μM) did not fit

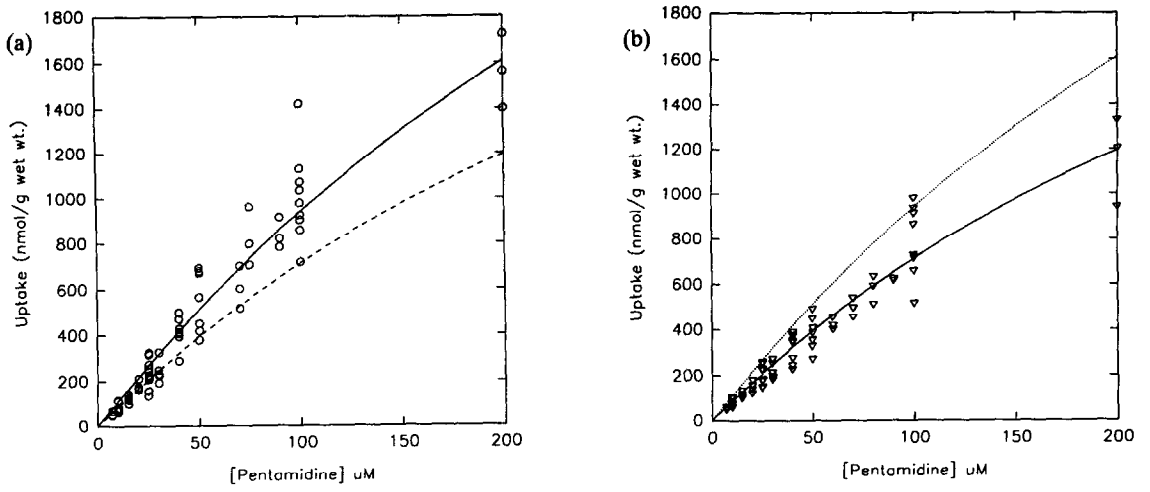

Fig. 5. The lung slices were incubated in various concentrations of pentamidine (7–200 μM) alone (-○- and · · · ·) or plus 20 μM putrescine (-▽- and – – –). Incubations were performed at 37° for 30 min in multiwell plates. The results represent a combination of data points from four separate experiments including the initial studies (N varies between 3 and 10 determinations). For pentamidine alone (a) the apparent K_m and V_{max} values of 500 ± 121 μM and 5640 ± 1131 nmol/g wet lung wt/30 min (mean ± SD), respectively, and the presentation of the data were determined using the Sigmaplot 4 analysis and best-fit routine for a single Michaelis–Menten equation. The apparent K_m and V_{max} values for pentamidine plus 20 μM putrescine (b) were calculated as 420 ± 88 μM and 3707 ± 635 nmol/g wet lung wt/30 min (mean ± SD) and also presented exactly as described above. Statistical differences between the K_m and V_{max} values calculated for pentamidine alone and pentamidine plus 20 μM putrescine above were determined using a Student's *t*-test. It was seen that no difference occurred between the K_m values, however, a significant decrease ($P > 0.05$) was determined in the V_{max} value for pentamidine plus 20 μM putrescine compared with that of the control value.

a double Michaelis–Menten equation. The uptake of pentamidine at the lower concentrations (10–100 μM) however, did fit a single Michaelis–Menten curve yielding K_m and V_{max} values of 95.0 ± 21.2 μM and 1242 ± 169 nmol/g wet lung wt/30 min, respectively (cf. Table 1). The data shown in Fig. 4 was also found to fit a single Michaelis–Menten curve and K_m and V_{max} values of 607 ± 121 μM and 2513 ± 282 nmol/g wet lung wt/30 min respectively, were obtained.

An attempt was made to curve-fit the data in Fig. 3 to a Michaelis–Menten plus the Hill equation for a sigmoid curve i.e.

$$v = V_{max}[S]/(K_m + S) + V'_{max}[S^n]/(K'_m + S^n).$$

Whilst a fit was obtained (not shown), it became apparent that further studies were required to derive meaningful kinetic parameters.

Uptake of pentamidine and the effect of putrescine on its kinetic constants in rat lung slices

The possible existence of two uptake systems for the drug was investigated by performing numerous experiments covering a wide range of pentamidine concentrations (7–200 μM) and the results from several separate experiments were combined (Fig. 5). First, it was observed that the scatter of data points from each experiment was interspersed with that from each of the others, i.e. no one experiment had all low values or all high values. Secondly, the variability from the mean value for the rate of accumulation between 7 and 40 μM concentrations was extremely small but that this variability increased considerably with increasing concentration (Fig. 5). Analysis and best curve-fit to a single Michaelis–Menten equation gave K_m and V_{max} values of 500 ± 121 μM and 5640 ± 1133 nmol/g wet lung wt/30 min.

The presence of 20 μM putrescine in the incubation medium resulted in a small but significant decrease in V_{max} but no change in the K_m values for pentamidine (Fig. 5), i.e. $K_m = 420 \pm 88$ μM, $V_{max} \pm 3707 \pm 635$ nmol/g wet lung wt/30 min. It was noted that, as with pentamidine alone, the variability from mean values increased with increasing concentration (Fig. 5). Again, the individual values for each concentration from each experiment were well interspersed between all the experiments.

The effect of pentamidine on putrescine uptake in rat lung slices

When pentamidine is present in the incubation medium, the accumulation of putrescine is inhibited (Table 2). When the concentration of pentamidine is increased, the percentage inhibition of putrescine is also increased (Table 2).

Putrescine accumulation in rat lung slices following their pre-incubation in pentamidine

It was observed that when lung slices were pre-incubated in various concentrations of pentamidine (25–500 μM) over a 60 min time period, no effect

Table 2. The effect of pentamidine on the accumulation of putrescine by rat lung slices

Pentamidine concentration (μM)	Putrescine concentration (μM)				
	2	5	10	20	50
25	20.7 ± 3.8	22.4 ± 1.3	5.4 ± 1.1	10.6 ± 6.1	13.1 ± 5.7
100	28.7 ± 5.0	25.1 ± 6.3	32.3 ± 3.9	19.2 ± 6.1	22.4 ± 2.9
250	50.7 ± 3.9	47.8 ± 4.6	55.0 ± 3.6	40.2 ± 5.3	43.9 ± 4.1

The lung slices were incubated in varying concentrations of putrescine (2–50 μM) containing 0–25 μM concentrations of pentamidine. Incubations were performed for 30 min at 37°.

The results are presented as the mean values ± SEM of three determinations and are expressed as the percentage inhibition of putrescine uptake when pentamidine is present in the medium.

The control values for 2, 5, 10, 20 and 50 μM putrescine uptake were 15.0 ± 0.9, 33.9 ± 2.3, 68.1 ± 1.3, 125.5 ± 11.8 and 318.1 ± 19.9, respectively.

Table 3. The effect of pentamidine pre-incubation on the accumulation of putrescine in rat lung slices

Pentamidine concentration (μM)	Rate of putrescine accumulation (nmol g wet lung wt/hr)
0	22.0 ± 1.0
25	19.0 ± 2.0
50	16.5 ± 0.9
100	17.0 ± 1.0
300	17.5 ± 1.5
500	19.5 ± 1.3

The effect of pre-incubation of pentamidine on the accumulation of putrescine. Rat lung slices were pre-incubated in various concentrations of pentamidine (25–500 μM) and the accumulation of 20 μM putrescine was then determined over a time course (5–60 min) at 37°. Three observations per time point were performed for each pre-incubation pentamidine concentration and the rate of accumulation was calculated by linear regression against time. Results are expressed as the mean values ± SEM. Statistical differences were tested using analysis of variance (one-way) ($P < 0.05$) and it was shown that no significant difference existed between the control values and those determined for any pre-incubation pentamidine concentration.

on the subsequent accumulation of 20 μM putrescine was noted as compared to the control values (Table 3).

DISCUSSION

The results show that pentamidine accumulated in lung slices in a manner which obeyed saturation kinetics. Variations of the original methodology [6], that is the replacement of flask incubations with the more convenient plastic multiwell plates, had little effect on the results obtained. Pentamidine was accumulated by rat lung slices in a linear, time dependent manner over 30 min. This uptake was unaffected by metabolic inhibitors but was considerably diminished at low temperature. This suggests that the process of pentamidine accumulation is not energy dependent but its route of transport may be influenced by some conformational change in the cell membrane. It is well established that at low temperatures, membrane fluidity decreases due to the transitional temperatures of the constitutional fatty acids [18]. A more rigid membrane may result in a lower rate of uptake of pentamidine thus reported in this study. It has been noted that pentamidine accumulation in *Trypanosoma* is both temperature and pH dependent, V_{max} values decreasing with decreasing temperature and pH [19].

The distinct deviation from linearity that occurred after 30 min incubations was thought to be the result of damage by pentamidine on the target cell membranes, as the drug is known to have a number toxic side effects [5]. This was investigated using putrescine uptake as a marker for epithelial cell membrane integrity. It was seen that pre-incubation with even extremely high concentrations of pentamidine had no effect on the accumulation of putrescine, leading to the conclusion that within the time period studied, pentamidine toxicity did not play an important part in the observed and, therefore, unexplained fall from linearity which occurred with time.

The preliminary experimental data suggested that two different uptake processes were operative in the accumulation of pentamidine. When pentamidine was present in excess of 100 μM, there was a distinct deviation from the straight line Hane plot which was used to calculate kinetic constants. This prompted the investigation of the accumulation of high concentrations of pentamidine and hence the calculation of two sets of kinetic parameters for the drug with K_m values of 100 and 607 μM and V_{max} values of 625 and 2513 nmol/g wet lung wt/30 min, respectively. The possibility that two transport systems operated for the uptake of pentamidine would not be unique, i.e. a high affinity system plus a low (undefined) process as it has been reported that a similar biphasic system exists for the accumulation of cystamine by the lung [20]. In attempting to analyse, and hence define, the effects noted in the Hane plot by the application of the relevant theoretical equations, it became apparent that more data points were required to derive meaningful kinetic parameters.

Further numerous and detailed experiments demonstrated that the variability of the data

regarding the rate of uptake of pentamidine increased considerably with increasing exogenous pentamidine concentration and all the points for each experiment were interspersed with those from the other experiments. It was noted that the apparent deviation in the Hane plot was now concealed within the variability of data points, when the initial and later data was combined. The best interpretation of this observation is that pentamidine does not accumulate via high and low affinity transport systems but simply by single Michaelis–Menten saturation kinetics with an average K_m value of 554 μM and V_{max} value of 4077 nmol/g wet lung wt/30 min.

Pentamidine is an aromatic diamidine containing five central methylene groups and is therefore structurally related to endogenous amines that use the polyamine transport system [10]. In inhibitory studies with putrescine, it was observed that the K_m for pentamidine remained the same compared to control values but a decrease in V_{max} occurred. As putrescine uptake was also inhibited by pentamidine, this suggests that pentamidine acts in a non-competitive manner with the endogenous amine putrescine in rat lung slices. Pentamidine therefore, does not utilize the pulmonary system for polyamine transport but is probably accumulated by the same target cells which contain this system.

Isolated alveolar type 2 epithelial cells have been shown to accumulate putrescine [21], which correlates with autoradiographical studies indicating that the cellular sites of uptake for putrescine were the alveolar type 1 and 2 epithelia and the bronchiolar Clara cell [9, 22]. Thus, it seems likely that pentamidine will also target the same cells. Isolates of these cell types are currently being examined for their ability to accumulate pentamidine and to establish at which levels the drug exerts any damaging effects.

Acknowledgements—The authors wish to thank the Medical Research Council, U.K. for funding this project.

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