Biochemical Pharmacology, Vol. 41, No. 8, pp. 1209–1215, 1991.
Printed in Great Britain.

0006-2952/91 $3.00 + 0.00
© 1991. Pergamon Press plc

DIAMINE UPTAKE BY RAT LUNG TYPE II CELLS *IN VITRO*

VICTOR I. C. OREFFO,* ROBERT A. JOHN and ROY J. RICHARDS
Department of Biochemistry, University of Wales, College of Cardiff, Cardiff CF1 1ST, U.K.

(*Received* 16 *April* 1990; *accepted* 22 *October* 1990)

Abstract—Lung epithelial type II cells are responsible for synthesising and secreting pulmonary surfactant which reduces surface tension and prevents lung collapse. Type II cells replace type I cells and can proliferate in response to alveolar injury. An important aspect of this proliferation may be the ability of type II cells to accumulate amines actively, particularly the endogenous diamine putrescine. Putrescine is accumulated into isolated alveolar type II cells by an energy-dependent process. The uptake obeys saturation kinetics for which an apparent K_m of 14.7 μM and V_{max} of 130 pmol/μg DNA/hr was derived. The inhibitory effects of structurally similar amines on putrescine accumulation are described. As the herbicide paraquat has been suggested to share the same uptake system as putrescine from lung slice studies, this phenomenon was investigated in type II cell cultures. The results demonstrated that paraquat is a partially competitive inhibitor of putrescine accumulation in the cells. The K_i for the inhibition of putrescine uptake by paraquat in type II cells was calculated to be 69 μM, a value which closely matches the K_m for paraquat (70 μM) predicted from lung slice studies. In molecular terms, the partial nature of the competition indicates that paraquat and putrescine do not occupy identical sites. Saturation of its site by paraquat reduced the affinity of putrescine 3.6-fold, but did not abolish it.

A number of endogenous amines and amino drugs are known to be accumulated by the lung. Putrescine, spermidine and spermine are found in all animal cells where they exist as organic cations and interact with proteins, phospholipids and nucleic acids. They have been implicated in a number of cellular processes including stabilisation and alteration of membranes, RNA replication, protein synthesis, DNA synthesis and cell division [1–3]. However, the role of the polyamine transport system in cellular function is not yet understood. Putrescine has been shown to be accumulated by lung slices *in vitro* [4], and it is thought that this uptake may be related to the control of cell proliferation [5–7]. The studies with lung slices show that a number of diamines and polyamines are accumulated by a process that obeys saturation kinetics. The amine uptake is reduced by the presence of metabolic inhibitors, incubation of the slices under nitrogen, or by low temperatures, suggesting that an active transport system is involved [6, 7].

The uptake of the herbicide paraquat (1,1-dimethyl-4,4-bipyridylium; methyl viologen) by the lung is considered to be mediated by the same uptake system as that used for putrescine [4, 5, 8]. The ability of the lung to accumulate paraquat [9] and retain the herbicide [10] coupled with the ability of the compound to deplete NADPH, redox cycle and generate reactive oxygen species [11] could well explain the mechanism of cellular injury. Alveolar type I and type II cells and the bronchiolar Clara cells are thought to play a major role in the accumulation of endogenous amines [4, 12]. Certainly accumulation of both putrescine and paraquat is decreased markedly in lung slices derived from rats in which type I and II cells have been selectively damaged with the herbicide [6, 13]. Using autoradiographic techniques, workers have reported that in mice injected intravenously with radiolabelled paraquat, the radioactivity is confined almost entirely to cells having the distribution of type II cells [14]. Similar, more recent studies on the rat [12] have also implicated the type II cell as a major site for the accumulation of paraquat, and other workers [15] using rat lung slices have suggested that putrescine and paraquat are accumulated by the same cell type.

Type II cells synthesise and secrete pulmonary surfactant which prevents lung collapse, and the cells also have a proliferative role at times of alveolar injury. Type II cell isolates have been shown to accumulate putrescine [16, 17] and thus represent a simple system for monitoring lung amine accumulation. The first objective of the present study was to determine if the type II cells provide a valid experimental model for assessing amine uptake. Thus, experiments were carried out to establish if putrescine was actively accumulated by type II cells and if its uptake could be inhibited by structurally related amines as had been demonstrated with lung slices. Second, the lung slice work had suggested that paraquat acts as a competitive inhibitor of putrescine accumulation, and therefore attention was focused on this system in type II cell cultures.

MATERIALS AND METHODS

Materials. Radiolabelled [^{14}C]putrescine (109 mCi/mmol) and internal standard [^{14}C]*n*-hexadecane were purchased from Amersham International, Amersham, Bucks, U.K. All other reagents used were of the highest available grade and were

* Address correspondence to: Dr. V. I. C. Oreffo, University of California, L. E. H. R. Facility, Davis, CA 95616, U.S.A.

purchased from the Sigma Chemical Co. or BDH, Poole, Dorset, U.K. Culture medium was obtained from Gibco Europe Ltd. and 24-well multiwell plates were from Becton Dickinson UK Ltd., Oxford, U.K.

Isolation and culture of type II cells. Type II cells were isolated and characterised from male, specific pathogen-free rats (CD strain) of 150–200 g body weight (Charles River, U.K. Ltd., Maidstone, Kent) in a manner identical to that described previously [17]. Freshly isolated cells were plated in a 24-well plate (1×10^6 cells/well) in 10% foetal bovine serum and Waymouth's medium containing gentamycin (50 μg/mL), penicillin (60 μg/mL) and ascorbic acid (50 μg/mL final concentration). The plates were then incubated for 48 hr in a gas phase of 95% air plus 5% CO_2 whereupon 40–60% of the cells attached and spread on the plastic substratum.

Uptake studies with type II cell cultures. Forty-eight-hour cultures of type II cells were used in all of the following studies as preliminary experiments established that this was an optimal time for amine accumulation [18]. The culture medium containing unattached cells was removed and discarded. The cell sheet was washed well with a balanced salt solution (133 mM NaCl, 5.2 mM KCl, 2.59 mM phosphate buffer, pH 7.4, 10 mM Hepes buffer, pH 7.4, and glucose 1 mg/mL) to remove any loosely attached or damaged cells. Radiolabelled putrescine (1 μM) plus carrier (non-radiolabelled) putrescine of required concentration was then added to the cells in fresh culture medium. In some experiments, metabolic inhibitors (potassium cyanide plus iodoacetate, oubain) or other amines (L-lysine-HCl, L-ornithine-HCl, spermine-4HCl, spermidine-3HCl, cadaverine-2HCl (1,5-diaminopentane), serotonin (5-hydroxytryptamine), parquat-2HCl or diquat-2Br of required concentration were added at this time and the final reaction mixture was of 1 mL volume. Incubations were then carried out at 37°, unless stated otherwise, for specified time intervals usually ranging between 5 and 45 min. At the appropriate time the culture medium was removed and the cell sheet washed with 1 mL of balanced salt solution to remove any associated free radiolabel. Attached cells were then removed from the plastic surface by means of a rubber policeman into a total volume of 1 mL of fresh balanced salt solution. The cell suspension was sonicated over ice (5×3 sec pulses in Branson B-30 sonifier on setting 6; Branson Power Co.). Duplicate 100-μL samples of the sonicate were counted using Phase Combining System (PCS) scintillant mixture and an Intertechnique SL 4000 Scintillation Counter. Quench correction was obtained by addition of the internal standard, and cell-associated counts were expressed as disintegrations per minute (dpm). When cell-associated radiolabel was measured at a single time point, the assays were performed on three separate cultures. In a number of experiments, duplicate 250-μL samples were also removed to determine cellular DNA [19].

Analysis of kinetic data. From the cell-associated radioactivity (dpm) the number of picomoles of putrescine incorporated at any external concentration of the amine was calculated. The rate of uptake (v) was expressed as picomoles of putrescine per culture or microgram DNA per given time. Applying Michaelis–Menten principles, a plot of v versus [S] (the external concentration of putrescine) permits the calculation of an apparent K_m (affinity constant for putrescine) and V_{max} (maximum velocity of uptake of the amine).

The Michaelis–Menten curve was fitted with unweighted analysis using a computational procedure developed by Wilkinson [20], and the Lineweaver–Burk [21] transformation of these data was used when relevant. The dissociation constant (K_i) for paraquat on putrescine uptake was also calculated and, applying principles well established in the field of enzyme kinetics [21], an interpretation was made of the competitive relationship between putrescine and paraquat for a type II cell receptor.

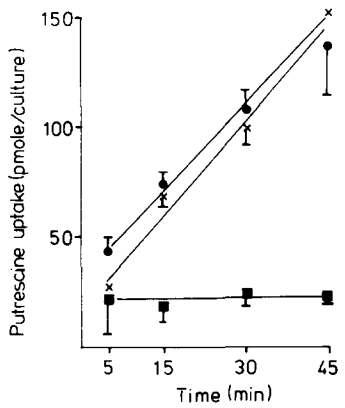

Fig. 1. Effect of metabolic inhibitors [oubain, 100 μM (×—×); potassium cyanide/iodoacetate, 1 mM (■—■)] on putrescine uptake by rat epithelial type II cells. Control cultures (●—●) were treated with putrescine (10 μM) alone. Results are means ± SD with three determinations for each time point.

RESULTS

Effects of metabolic inhibitors and low temperature on putrescine accumulation by type II cells. Oubain (100 μM), a potent inhibitor of the sodium/potassium ATPase pump, had little if any effect on putrescine (10 μM) uptake by type II cells (Fig. 1). In contrast, a combination of potassium cyanide (1 mM) and iodoacetate (1 mM) completely inhibited putrescine accumulation. Another metabolic inhibitor, rotenone, was ineffective unless the type II cells were preincubated in rotenone (plus DMF) for a 3-hr period prior to monitoring putrescine uptake. Under these conditions, the accumulation of putrescine over a subsequent period of 30 min was inhibited significantly (46%) (Table 1). Preincubation of the type II cells with the solubilising agent (DMF) alone produced a small (19%), but statistically insignificant inhibition of putrescine uptake. Putrescine accumulation with time was inhibited completely when the cultures were maintained at 4° irrespective of the exogenous concentration of the amine (Fig. 2). The passive diffusion of the putrescine

Table 1. Putrescine uptake by rat type II cells following preincubation of the cells in DMF alone or with rotenone plus DMF

Treatment	Putrescine uptake (pmol/culture/30 min)	% Inhibition
Control (untreated)	88.9 ± 12.7	0
DMF	71.8 ± 6.9	19 ± 8
Rotenone + DMF	48.1 ± 1.9	46 ± 2*

Values are means ± SD from three determinations.
* Significantly different from control ($P < 0.05$).

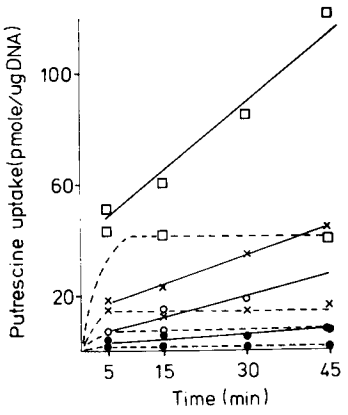

Fig. 2. Effects of temperature [4° (----); 37° (——)] on putrescine uptake by type II cells. Putrescine concentrations were 40 μM (□), 10 μM (×), 5 μM (○), and 1 μM (●). The results are the means of three determinations.

was a temperature-independent process occurring in less than 5 min and determined solely by the exogenous concentration of the putrescine. In contrast, active accumulation of putrescine was demonstrated at 37° (Fig. 2), and the calculated K_m value for putrescine was 14.7 μM and the V_{max} 130 pmol/μg DNA/hr.

Effects of structurally related amines on putrescine accumulation by type II cells. Lysine and serotonin did not inhibit significantly putrescine uptake by type II cells (Table 2). Ornithine, the precursor of putrescine in mammalian biosynthesis, did appear to inhibit putrescine uptake (by 34%), but this result was not statistically significant. Spermine, an endogenous amine, and cadaverine, a non-physiological amine, both proved to be effective inhibitors. Less potent, but significant inhibition of putrescine accumulation was found with paraquat and diquat (Table 2). In a similar experiment (data not shown), the endogenous amine, spermidine, inhibited putrescine (10 μM) uptake by 78%, and thus the effects of this compound were investigated further. Data from Lineweaver–Burk plots indicated that the K_m and V_{max} values for putrescine accumulation alone were 22 μM and 200 pmol/μg DNA/30 min, respectively, but that in the presence of 50 μM spermidine the K_m value for putrescine was increased to 100 μM (Fig. 3a). This 4.6-fold increase in K_m indicates a decreased affinity of putrescine for its receptor and with no change in V_{max} indicates that spermidine is a competitive inhibitor of putrescine uptake by the type II cells. An identical experiment carried out with 20 μM spermidine and putrescine produced a 3.8-fold change in the K_m value compared with that observed with putrescine alone (Fig. 3b).

Effect of paraquat on the accumulation of putrescine by type II cells. The effect of paraquat on the rate of uptake of putrescine was studied at a series of putrescine concentrations. Analysis of the results by the classical double-reciprocal method of Lineweaver and Burk [21] are shown in Fig. 4. The results show that the inhibition of the rate of putrescine uptake by paraquat was competitive. At the single concentration of paraquat (1 mM) used in this experiment the apparent K_m for putrescine was increased approximately 3-fold from 10 μM to 33 μM. If this is a case of simple competitive inhibition, then the dissociation constant for paraquat is calculated to be 460 μM. This calculation is based on the relationship of K_m apparent = K_m $(1 + I/K_i)$. Thus these observations, made using cells in culture, are qualitatively and quantitatively in near perfect agreement with results from similar experiments conducted with lung slices in other laboratories [15].

Classical competitive inhibition may also be analysed by methods based on varying the concentration of the putative competitive inhibitor at a single concentration of substrate [21]. Such a method yields a linear plot of $1/v$ against [I]. When this experiment was carried out with 10 μM putrescine at a range of paraquat concentrations, the results show that the $1/v$ versus [I] did not yield a linear relationship (Fig. 5a). In this case, the inhibition was more complex than that described by the simple competitive model. The effect of the inhibitor is perhaps more clearly illustrated when the rate of uptake of 10 μM putrescine is plotted directly against the varied paraquat concentration (Fig. 5b). This plot shows that the rate of putrescine uptake declined not to zero, but to a limiting value close to half of that observed in the absence of paraquat. The data are consistent with a system in which the effect of the inhibitor is not to increase K_i to infinity, as in fully competitive inhibition, but to a finite value as in partially competitive inhibition.

Full analysis of partially competitive inhibition requires determination of rates at a series of concentrations of both substrate and inhibitor. Such an analysis for the effects of paraquat on the uptake

Table 2. Inhibition of putrescine accumulation in type II cell cultures by structurally related amines

Added amine	Putrescine uptake (pmol/culture/30 min)	% Inhibition
Putrescine alone (control)	61.1 ± 5.1	
L-Lysine	51.2 ± 3.1	16
L-Ornithine	40.6 ± 16.9	34
Spermine	23.8 ± 2.9*	61
Cadaverine	28.4 ± 4.6*	54
Serotonin	54.8 ± 6.8	10
Paraquat	43.8 ± 0.9*	28
Diquat	42.3 ± 6.9*	31

Exogenous putrescine concentration was 10 μM and potential inhibitor concentration was 50 μM; putrescine uptake is expressed as means ± SD for triplicate assays. Inhibition was calculated for each amine relative to the control (putrescine alone = 100%).

* Significantly different from control ($P < 0.05$).

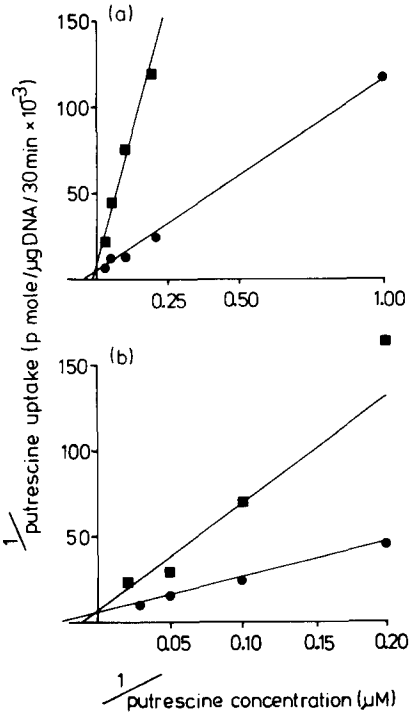

Fig. 3. Lineweaver–Burk plots to show the effect of spermidine [(a) 50 μM and (b) 20 μM] on putrescine (1–40 μM) uptake by type II cells. Key: putrescine alone (●—●); and putrescine and spermidine (■—■).

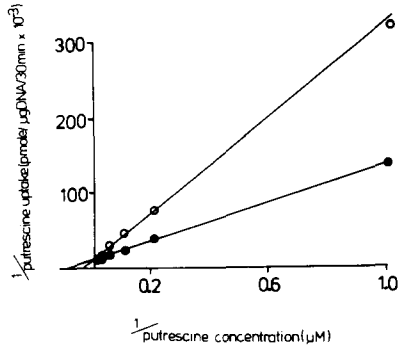

Fig. 4. Lineweaver–Burk plots to show the effect of paraquat (1 mM) on putrescine (1–60 μM) accumulation by type II cells. Key: putrescine alone (●—●); and putrescine and paraquat (○—○).

of putrescine by type II cells is shown in Fig. 6a. When the data at each inhibitor concentration were analysed by the Wilkinson method, V_{max} was found to be constant but the variation of apparent K_m (Fig. 6b) could not be reconciled with the linear relationship required by simple competitive inhibition. The observed hyperbolic dependence of apparent K_m on inhibitor concentration is that expected for partially competitive inhibition and the data have been analysed according to this model. The continuous line through the points in Fig. 6b is that expected for a system in which binding of inhibitor does not prevent uptake of substrate completely but simply reduces the affinity of the initial binding step. This model requires two inhibitor dissociation constants, one of which, K_i, characterises the binding of inhibitor to free receptor and the other K_i', characterises binding of inhibitor to the receptor saturated with putrescine. The calculated values for K_i and K_i' were 69 and 246 μM, respectively, showing that paraquat reduced the affinity for putrescine by 3.6-fold. Interpretation of these data in this way also resolves a paradox implicit in the results of earlier workers. The values of K_m reported for paraquat uptake by lung slices are 70 μM [9] or 119 μM [15] and yet the values of K_i reported for paraquat from competition experiments were 460 μM in the present study and 458 μM when calculated from other data [15]. The simple competitive model advanced to explain paraquat inhibition cannot accommodate this discrepancy. However, the relationships required by the proposed

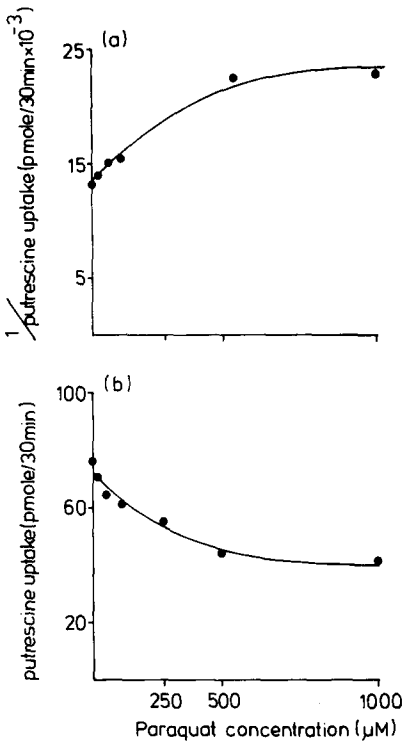

Fig. 5. (a) Dixon plot of the reciprocal of the rate of putrescine (10 μM) accumulation versus inhibitor concentrations of paraquat (0–1 mM). (b) The same data expressed as rate of putrescine uptake versus inhibitor concentration.

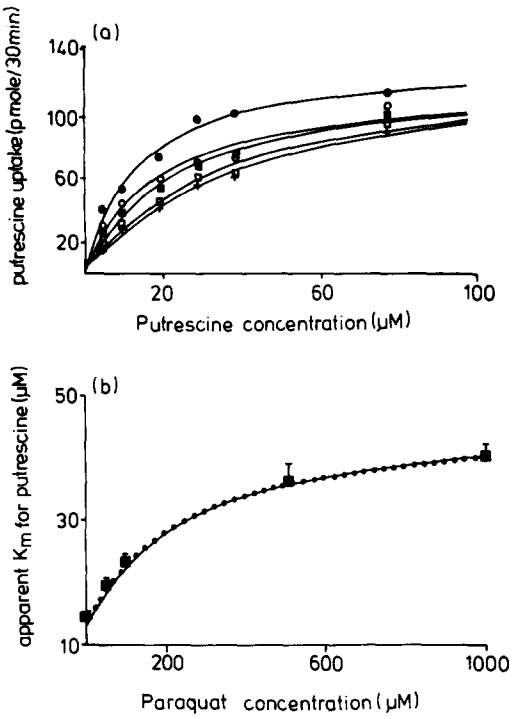

Fig. 6. (a) Michaelis–Menten plots of the velocity of putrescine accumulation versus exogenous putrescine concentration (5–80 μM) for type II cells in the presence of various concentrations of paraquat (50 μM to 1 mM). Paraquat concentrations were: 1000 μM (+), 500 μM (□), 100 μM (■), 50 μM (○), and 0 (●). (b) Plot of the apparent K_m value for putrescine versus inhibitor concentration. Values for K_m app were determined from Wilkinson analysis of data in Fig. 6a. The solid line connecting the small points is that predicted by the equation:

$$K_m \text{ app} = K_m (1 + I/K_i)/(1 + I/K_i')$$

where $K_m = 13\,\mu M$, $K_i = 69\,\mu M$, and $K_i' = 246\,\mu M$.

partially competitive inhibitor model allows a calculation of 69 μM for K_i in the absence of putrescine. This value is almost identical to the previously reported K_m value (70 μM [9]) for paraquat in the absence of competing putrescine.

DISCUSSION

The rate of uptake of the diamine putrescine by lung slices, previously shown to exhibit saturation kinetics [4], has now been demonstrated with isolated type II cells. Many similarities were found between the two systems in that putrescine accumulation was an active process which was temperature dependent and depressed by metabolic inhibitors of the electron transport chain. Oubain, an inhibitor of sodium/potassium ATPase, had no effect on putrescine uptake by type II cells or lung slices.

Specificity in the amine uptake system by type II cells was indicated by the inhibitor studies with serotonin. This monoamine is actively accumulated by lung slices with a K_m value determined at 4.5 μM [22] and the capillary endothelial cell is thought to be the uptake site [23]. Type II cell cultures did not accumulate serotonin (0.5 to 20 μM concentrations; [18]) and in the present study 50 μM concentrations of the monoamine had no significant inhibitory action on putrescine accumulation, indicating that they did not share a common uptake system. The high specificity of the uptake system in type II cells was further demonstrated by the inhibitor studies using structurally related amines. Cadaverine and ornithine have similar structures although the former was a much more potent inhibitor of putrescine uptake in type II cells. Other workers, using lung slices [5], have suggested that the carboxyl group on ornithine, and its absence on cadaverine, could explain the difference in inhibitory potencies between the two amines. The fact that any potential amine inhibitor may not necessarily be transported into the type II cells was indicated by the fact that diquat (5–1000 μM) was not accumulated [18] and yet acted as a weak inhibitor of putrescine uptake. Putrescine accumulation by type II cells was inhibited more strongly (78%) by 50 μM spermidine than accumulation of the same compound by lung slices (48%; [18]), although in both experimental systems the mode of inhibition was established as being competitive.

Previous experiments using lung slices have demonstrated that paraquat is a competitive inhibitor of the uptake of putrescine [4, 15], and the present

study has confirmed this observation using type II cells. However, our observations have been extended to indicate that paraquat does not behave as a simple competitive inhibitor. The results from experiments where both the putrescine and paraquat concentrations have been systematically varied demonstrate that the inhibition is of the type known in classical enzyme kinetics as partially competitive [21]. In fully competitive inhibition, which is the simplest type, extrapolation of the data obtained at a single putrescine concentration to infinite inhibitor (paraquat) concentration would show the rate of uptake falling to zero. However, extrapolation of these results indicates that at "infinite" (1 mM) paraquat concentration, putrescine was still being taken up at approximately half the velocity observed in the absence of paraquat.

In attempting to explain the molecular mechanism underlying such a process, the distinction between fully and partially competitive inhibition is important and can only be made by varying inhibitor (paraquat) concentrations as well as that of "substrate" (putrescine). The simplest mechanism to explain fully competitive inhibition is that both inhibitor and "substrate" occupy the same site on the receptor and therefore cannot be present on it at the same time. When, as in the present study, the inhibition is seen to be partially competitive, both inhibitor and "substrate" bind to the receptor simultaneously and therefore cannot both occupy the same site. While it is possible to explain this phenomenon with a model in which two sites overlap sterically so that binding interactions are distorted, a simpler and more plausible explanation is that the receptor has two separate sites and that the effects are mediated by conformational change.

There are alternative interpretations of the data much of which centralises on the mode or lack of accumulation of paraquat by the type II cell. Although paraquat accumulation has been demonstrated in lung slices [4, 8] and by rather a slow process *in vivo* [12] it may well be that cell types other than the type II have a prominent role. Indeed, autoradiographic studies do provide indirect evidence that type I and Clara cells, in addition to type II cells, represent the sites of polyamine uptake. In addition, paraquat accumulation has been reported in rat type II cells in primary culture [16]. However, both accumulation and autoradiographic studies are difficult to perform with paraquat as the herbicide does not bind to cellular constituents and is removed easily by washing [14]. In our own laboratory we have not been able to ascribe kinetic constants for the accumulation of paraquat by type II cells because of the variable response at different concentrations of the herbicide. Interestingly, however, the maximum linear rate of herbicide uptake by 48-hr cultures of type II cells was achieved at an 80 μM concentration which equates well with the K_m value of 70 μM reported for lung slices [9]. We cannot exclude the possibilities that (i) the type II cell has two separate uptake systems, one involving competitive inhibition of the diamine by the polyamine and the other solely for polyamine accumulation, (ii) the type II cell is selectively damaged during the isolation process and does not recover over 48 hr *in vitro* such that putrescine accumulation proceeds at maximal rates but paraquat does not, and (iii) that paraquat slowly diffuses into the cell, rapidly redox cycles, and depletes cellular constituents, such as reduced glutathione, and that such events affect putrescine uptake. Despite all these alternatives it is proposed that the receptor mediating putrescine uptake by type II cells has a separate site and that when this site is occupied by paraquat the affinity for putrescine is reduced approximately 3.6-fold.

Acknowledgements—V.I.C.O. would like to thank the Medical Research Council for financial support. We would also like to thank Lewis Smith and Ian Wyatt, ICI plc, Alderley Park, Macclesfield, U.K., for their constructive advice.

REFERENCES

1. Williams-Ashman HG and Canallakis ZW, Polyamines in mammalian biology and medicine. *Perspect Biol Med* **22**: 421–453, 1979.
2. Janne J, Poso H and Raina A, Polyamines in rapid growth and cancer. *Biochim Biophys Acta* **473**: 241–293, 1978.
3. Tabor CW and Tabor H, 1,4-Diaminobutane (putrescine), spermidine and spermine. *Annu Rev Biochem* **45**: 285–306, 1976.
4. Smith LL and Wyatt I, The accumulation of putrescine into slices of rat lung and brain and its relationship to the accumulation of paraquat. *Biochem Pharmacol* **30**: 1053–1058, 1981.
5. Gordonsmith RH, Brook-Taylor S, Smith LL and Cohen GM, Structural requirements of compounds to inhibit pulmonary diamine accumulation. *Biochem Pharmacol* **32**: 3701–3709, 1983.
6. Smith LL, The identification of an accumulation system for diamines and polyamines into the lung and its relevance to paraquat toxicity. *Arch Toxicol [Suppl]* **5**: 1–14, 1982.
7. Smith LL, Wyatt I and Cohen GM, The accumulation of diamines and polyamines into rat lung slices. *Biochem Pharmacol* **31**: 3029–3033, 1982.
8. Smith LL, The identification and characterization of a polyamine accumulation system in the lung. *Biochem Soc Trans* **13**: 332–334, 1985.
9. Rose MS, Lock EA, Smith LL and Wyatt I, Paraquat accumulation: Tissue and species specificity. *Biochem Pharmacol* **25**: 419–423, 1976.
10. Sharp CW, Ottolenghi A and Posner HS, Correlation of paraquat toxicity with tissue concentrations and weight loss of the rat. *Toxicol Appl Pharmacol* **22**: 241–251, 1972.
11. Bus JS, Cagen SZ, Olgaard M and Gibson JE, A mechanism of paraquat toxicity in mice and rats. *Toxicol Appl Pharmacol* **35**: 501–513, 1976.
12. Wyatt I, Soames AR, Clay MF and Smith LL, The accumulation and localisation of putrescine, spermidine, and paraquat in the rat lung: *In vitro* and *in vivo* studies. *Biochem Pharmacol* **37**: 1909–1918, 1988.
13. Lock EA, Smith LL and Rose MS, Inhibition of paraquat accumulation in rat lung slices by a component of rat plasma and a variety of drugs and endogenous amines. *Biochem Pharmacol* **25**: 1769–1772, 1976.
14. Wadell WJ and Marlowe C, Tissue and cellular disposition of paraquat in mice. *Toxicol Appl Pharmacol* **56**: 127–140, 1980.
15. Karl PI and Friedman PA, Competition between paraquat and putrescine for accumulation by rat lung slices. *Toxicology* **26**: 317–323, 1983.

16. Forman JH, Aldrich TK, Posner MA and Fisher AB, Differential paraquat uptake and redox kinetics of rat granular pneumocytes and alveolar macrophages. *J Pharmacol Exp Ther* **221**: 428–433, 1982.
17. Richards RJ, Davies N, Atkins J and Oreffo VIC, Isolation, biochemical characterization and culture of lung type II cells of rat. *Lung* **165**: 143–158, 1987.
18. Oreffo VIC, The metabolism of the pulmonary epithelial type II cell. Ph.D. Thesis. University of Wales, Cardiff, 1988.
19. Leyva A Jr and Kelly WN, Measurement of DNA in cultured human cells. *Anal Biochem* **62**: 173–179, 1974.
20. Wilkinson GN, Statistical estimations in enzyme kinetics. *Biochem J* **80**: 324–332, 1961.
21. Dixon M and Webb EC, *Enzymes*, 3rd Edn. Academic Press, London, 1979.
22. Aldridge VN and Nemry B, Toxicology of triakylphorothioates with particular reference to lung toxicity. *Fundam Appl Toxicol* **4**: S215–S223, 1984.
23. Strum J and Junod AF, Radiographic demonstration of 5-hydroxytryptamine-^{3}H uptake by pulmonary endothelial cells. *J Cell Biol* **54**: 456–467, 1972.