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THE ACCUMULATION OF DIAMINES AND POLYAMINES INTO RAT LUNG SLICES

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Abstract—The diamine cadaverine, and the polyamines spermidine and spermine have been shown to accumulate into rat lung slices by an uptake process which obeyed saturation kinetics. The apparent K_m values for the accumulation process of cadaverine, spermidine and spermine were 19, 11 and 15 μM respectively with V_{max} values of 937, 768 and 617 nmoles/g wet weight/hr respectively. The accumulation was KCN sensitive, indicative of an energy dependent process, although spermine did show some non-specific binding to lung tissue. Cadaverine, spermidine and spermine were not accumulated by slices of liver, kidney, heart and spleen to concentrations much greater than that in the medium. They were accumulated, however, by a KCN sensitive process into brain slices although the accumulation was much less than that which occurred in lung slices. The diamine, putrescine, exhibited a concentration-dependent inhibition of the ability of lung slices to accumulate cadaverine and the polyamines. These data have led us to conclude that the transport process in the lung, which has recently been shown to accumulate the diamine putrescine, is also capable of accumulating cadaverine, spermidine and spermine. Thus, by analogy with putrescine, there exists in specific lung cells a membrane receptor(s) which is selective in its acceptance and transport of diamines and polyamines.

The herbicide paraquat has been shown to accumulate into rat lung both *in vivo* [1] and *in vitro* [2]. This accumulation process obeys saturation kinetics and is energy dependent [2]. Recently, the diamine putrescine, has been shown to accumulate into rat lung slices by an apparently identical process to that which accumulates paraquat [3]. The uptake of both putrescine and paraquat into tissue slices from various organs is known to be selective for the lung although brain slices can accumulate either compound to some extent [3–6]. The discovery that putrescine is accumulated into rat lung slices resulted in part from the observation that this diamine was a very effective inhibitor of the accumulation of paraquat [3]. Several diamines and polyamines including cadaverine, spermidine and spermine have been shown to reduce the uptake of paraquat into the lungs [7]. We have, therefore, undertaken this study to determine whether these compounds are themselves accumulated into rat lung by a similar saturable energy dependent process and if there is a similar organ selective uptake to that of putrescine.

MATERIALS AND METHODS

Materials

[1,4-^{14}C]Putrescine dihydrochloride (116 mCi/mmole), [^{14}C]spermidine trihydrochloride (120 mCi/mmole), and [^{14}C]spermine tetrahydrochloride (112 mCi/mmole) were purchased from Amersham International Ltd (Amersham, U.K.). [1,5-^{14}C]Cadaverine dihydrochloride (106.3 mCi/mmole) was purchased from New England Nuclear (Dreieich, F.R.G.). Putrescine dihydrochloride, cadaverine dihydrochloride, spermidine trihydrochloride and spermine tetrahydrochloride were purchased from Sigma Chemical Co. (Poole, U.K.). KCN was purchased from BDH Ltd (Poole, U.K.) and halothane was obtained from Pharmaceuticals Division, ICI Ltd (Macclesfield, U.K.). Soluene 350 (a tissue solubiliser), Dimilume and Instagel (Scintillation cocktails) were purchased from Packard Ltd. (Poole, U.K.).

Animals

Male Alderley Park Wistar derived specific pathogen free rats (body-weights approximately 200 g) were used throughout.

Methods

Preparation of lung slices. Rats were killed with halothane, the lungs were removed, and slices 0.5 mm thick were prepared using a McIlwain tissue chopper.

Uptake of the diamines and polyamines into lung slices. Freshly prepared lung slices (20–40 mg) were incubated in 3.0 ml of modified Krebs–Ringer phosphate (KRP) containing NaCl (130 mM), KCl (5.2 mM), $CaCl_2$ (1.9 mM), $MgSO_4$ (1.29 mM), Na_2HPO_4 (10 mM) and glucose (11 mM). The pH of the buffer was adjusted to 7.4. The uptake of putrescine, cadaverine, spermidine and spermine

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was determined by adding 0.1 μCi ^{14}C-label of each compound to the respective media and the accumulation was studied for the following concentrations of each compound (1, 3, 10, 30 and 100 μM). Incubations were carried out at 37° under air in a shaking water bath at 70 c/min. The accumulation of each concentration was studied over a time course of 5, 15 and 30 min for 1, 3 and 10 μM and 15, 30 and 60 min for 30 and 100 μM. The slices of lung from one rat were used to investigate all concentrations and time courses for one compound (i.e. 15 flasks) and 4 rats were studied per compound.

The ability of 1, 10 and 100 μM putrescine to reduce the uptake of 10 μM [^{14}C]spermine, spermidine or cadaverine was determined after 30 min of incubation, with the slices from one rat lung used for all concentrations of putrescine studied including a flask for the control uptake of each compound. When the effect of the metabolic inhibitor KCN (1 mM) was studied, the uptake of 10 μM [^{14}C]spermine, spermidine or cadaverine was measured at 15 and 30 min and slices of lung from one rat were used for each time point and compound.

Uptake of diamines and polyamines into slices of various rat tissues. The uptake of 1 μM [^{14}C]spermine, spermidine or cadaverine was measured at 30 and 60 min in the presence or absence of KCN (1 mM). Slices of lung, heart and spleen were prepared and handled in a similar manner to that mentioned for lung slices, whilst slices of liver and kidney cortex and brain cortex were prepared by hand [3].

Determination of diamine and polyamine concentrations in tissue slices and medium. Tissue slices were removed from the incubation medium and washed by brief immersion in KRP. They were carefully blotted, dissolved in 1 ml Soluene 350 and 10 ml Dimilume was added. The radioactivity was determined by liquid scintillation spectrometry. A sample of the medium (0.1 ml) was made up to 1 ml with water and 10 ml Instagel scintillator added. The radioactivity was measured as described above. The counting efficiency was determined by the addition of an internal ^{14}C standard and all counts were then expressed as disintegrations per minute. The slice to medium ratio was calculated as the ratio of ^{14}C present per unit weight of slice to the ^{14}C present in the equivalent volume of medium. From this the amount of putrescine, spermine, spermidine or cadaverine present in the slice was calculated.

The validity of using radioactivity as a measure of the diamines and polyamines. The radioactivity from [^{14}C]-labelled putrescine accumulated into lung slices has been shown to be non-covalently bound and 95% of the radioactivity eluted from the ion exchange resin in a similar manner to a standard solution of putrescine [3]. On this basis the amount of [^{14}C]-label in the lung was taken to be putrescine. For [^{14}C]spermine, spermidine and cadaverine (10 μM), a similar evaluation was carried out as described in [3]. Also no metabolism to $^{14}CO_2$ from any of the compounds was detected after one hour of incubation. The [^{14}C]-label profile for the ^{14}C extracted from the lung slice (method of Rosenblum and Russell [8]) was identical to that for the respective elution profile for the standard for that compound (Fig. 1) with 87% of the ^{14}C-label applied to the columns being recovered. Therefore, the presence of ^{14}C-label in the slices of lung has been used as a measure of spermine, spermidine and cadaverine.

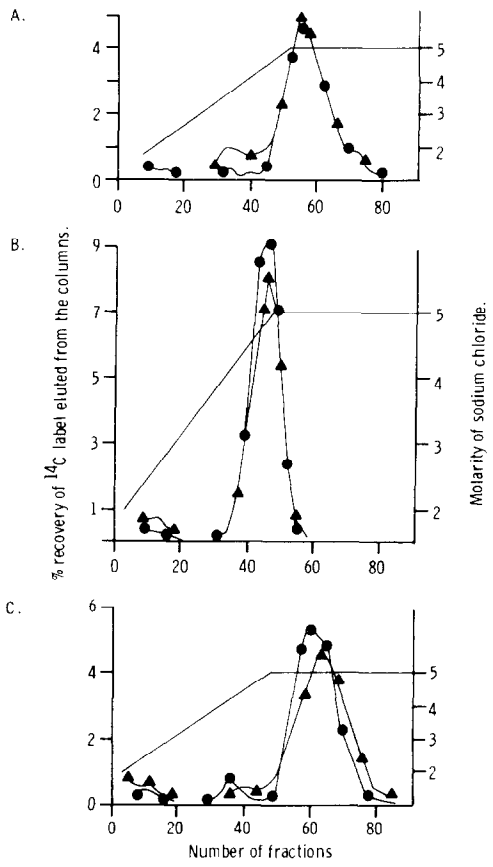

Fig. 1. Lung slices were incubated at 37° for 1 hr in KRP containing 10 μM [^{14}C]spermine, spermidine or cadaverine, and then the slices were prepared for the separation of the ^{14}C metabolites by ion exchange chromatography (see Materials and Methods). The columns were eluted initially with a 2–5 M linear NaCl gradient in 0.05 M sodium potassium phosphate buffer adjusted to pH 7.2 and finally with 5 M NaCl buffered as before. The elution profile for standard ^{14}C-labelled compounds is shown by (●) whilst the elution profile for the ^{14}C-label extracted from the lung slice is shown by (▲), and is an average of two experiments. Profile A is for spermine, B for spermidine and C for cadaverine.

RESULTS AND DISCUSSION

The ability of cadaverine, spermidine and spermine to reduce the accumulation of paraquat into the lung [7] suggested that these compounds were either able to prevent paraquat reaching the site of uptake or, as is the case with putrescine [3], are themselves accumulated. Using ^{14}C-labelled compounds we have shown that these compounds were accumulated into lung slices by a process which obeys saturation kinetics (Table 1). Both the apparent K_m and V_{max} values for the uptake process are similar for cadaverine, spermidine, spermine and putrescine (Table 1). It seems likely that the V_{max} for the uptake process

Table 1. The accumulation kinetics for the uptake of diamines and polyamines by slices of rat lung

Compound	Method of slice preparation	K_m (μM)	V_{max} (nmoles/g/hr)
Spermine	Tissue chopper	14.8	617 (463–923)
Spermidine	Tissue chopper	10.9	768 (726–814)
Cadaverine	Tissue chopper	19.0	937 (818–1096)
Putrescine	Tissue chopper	13.1	723 (598–914)
*Putrescine	Hand slice	7	330

Slices of rat lung were incubated at 37° in KRP glucose medium containing 1, 3, 10, 30 and 100 μM of either [^{14}C]spermine, spermidine, cadaverine or putrescine. Four observations were made at three time points for each concentration (see Materials and Methods), and the amount of compound in the slice was calculated from the slice to medium ratio. The rate of accumulation of each compound (nmoles/g/hr) for all concentrations was determined by using a weighted least squares regression on the four samples at each of the three time points. The fitting of a linear uptake model was significant $P < 0.05$ for 18 out of 20 equations, with the remaining two being $P < 0.10$. Using the nmole/g/hr uptake for each concentration, estimates of the Lineweaver–Burk relationship were obtained using weighted least square linear regression, and from the lines derived, the K_m and V_{max} were obtained for each compound. The figures in parentheses are 95% confidence limits.

* Data from L. L. Smith and I. Wyatt, *Biochem. Pharmac.* **30**, 1053 (1981).

can differ when lung slices are prepared by hand or tissue chopper, since the previously published V_{max} for putrescine (from our laboratory) differs from that in this study (Table 1). It is our experience that the use of a tissue chopper gives more consistent results compared with the free hand slicing. Marked differences in the uptake of spermine by slices of rat cerebral cortex have also been noted when slices of different thickness have been used [6].

The accumulation of cadaverine, spermidine and spermine can be inhibited by the addition of KCN to the incubation medium (Table 2). Thus as with the accumulation of putrescine [3] the uptake can be described as energy dependent.

Since the amines used in this study were selected, in part, because of their structural similarity to putrescine, it seemed probable that they would be accumulated into lung by the same process as that described for putrescine. In order to test this hypothesis, the accumulation of cadaverine, spermidine and spermine into the lung was determined in the presence of varying concentrations of putrescine in the incubation medium. Putrescine showed a concentration dependent inhibition of the accumulation of the other amines (Table 3). The degree to which putrescine was able to reduce the uptake of these three amino compounds was very similar (Table 3), as would be expected for compounds competing for the same uptake system and with similar K_m values (Table 1). Thus the ability of cadaverine, spermidine and spermine to accumulate in the lung by a saturable energy dependent process, together with the ability of putrescine to inhibit the accumulation of these compounds to a similar extent, is consistent with the conclusion that there exists in the lung a transport process which accumulates paraquat, putrescine, cadaverine, spermidine and spermine.

This transport process was revealed as a consequence of the search for endogenous compounds which are taken up into the lung by the process first described for the herbicide paraquat [2]. The selective toxicity which paraquat exhibits for the lung has in part been attributed to the accumulation of high concentrations of this herbicide in the lung [4]. It is

Table 2. The effect of KCN on the accumulation of spermine, spermidine and cadaverine by slices of rat lung

Compound (10 μM)	KCN present in medium (1 mM)	Accumulation of polyamines (nmoles/g wet wt) 15′	30′
Spermine	−	55.3 ± 0.3 (3)	124.0 ± 5.1 (4)
	+	24.0 ± 1.2 (4)	28.1 ± 1.9 (4)
Spermidine	−	62.9 ± 6.8 (4)	164.0 ± 10.8 (4)
	+	14.6 ± 1.3 (4)	17.3 ± 0.9 (4)
Cadaverine	−	58.8 ± 2.6 (4)	125.6 ± 18.1 (4)
	+	11.0 ± 0.74 (4)	13.2 ± 0.7 (4)

Lung slices were incubated at 37° in KRP glucose medium containing the above ^{14}C-labelled compounds and the amount of ^{14}C-label accumulated was measured at 15 and 30 min, using radiochemical techniques. The results are expressed as the mean ± S.E.M. with the number of observations in parentheses.

Table 3. The effect of putrescine on the accumulation of spermine, spermidine and cadaverine by slices of rat lung

Putrescine concentration	Accumulation of polyamines (% of control) Spermine	Spermidine	Cadaverine
100 μM	29.7 ± 1.6 (4)	26.0 ± 2.8 (4)	25.6 ± 0.8 (4)
10 μM	62.9 ± 0.6 (4)	66.4 ± 5.7 (4)	67.8 ± 1.5 (4)
1 μM	89.4 ± 3.4 (4)	94.4 ± 5.3 (4)	93.0 ± 5.4 (4)

Slices of rat lung were icubated at 37° for 30 min in KRP glucose medium in the presence of 10 μM [^{14}C]spermine, spermidine or cadaverine and the above concentrations of putrescine. The results are expressed as mean % of control accumulation for each compound ± S.E.M. with the number of observations in parentheses. The control levels of spermine, spermidine and cadaverine accumulated by lung slices over 30 min were 136, 158 and 130 nmoles/g wet wt respectively.

known that both paraquat [4] and putrescine [3] are not accumulated by tissue slices from other organs. An exception to this is brain cortex slices which accumulates both paraquat [4] and putrescine [3] although to a much lesser extent than lungs. In order to further examine the conclusion that cadaverine, spermidine and spermine are selectively accumulated by the system responsible for the uptake of putrescine, the uptake of these compounds into various tissue slices was studied (Table 4). Of the tissues examined only the brain cortex showed a marked ability to accumulate these amines and this was to a much lesser extent than lung (Table 4). The results, showing an uptake of cadaverine, spermine and spermidine into rat cerebral cortex slices, are in agreement with other studies [5, 6, 9]. In marked contrast to the brain and lung, the other tissues studied i.e., spleen, heart, kidney and liver showed only a slight time dependent increase in the amount of labelled amine present but this was also seen with slices incubated in the presence of KCN. Thus with cadaverine, spermidine and spermine there appeared to be a slight accumulation of label which was not energy dependent and hence differed from the transport process in the lung and brain.

The most probable reason for the pulmonary uptake of paraquat is its similarity to the diamines and polyamines with respect to the separation of the quaternary nitrogen atoms of paraquat and the amino groups of putrescine. Membrane transport systems are known to exhibit a high degree of structural specificity. However, exogenous compounds, which structurally closely resemble endogenous compounds may be transported by the carrier for the endogenous molecules. This has been well documented for several systems including the base transport system in the kidney [10] and the monoamine uptake system in the lung [11]. Furthermore, a num-

Table 4. The accumulation of spermine, spermidine and cadaverine into rat tissue slices and the effect of KCN

Tissue	KCN present in medium (1 mM)	Accumulation into tissue slices (nmoles/g) at times of incubation: Spermine 30′	Spermine 60′	Spermidine 30′	Spermidine 60′	Cadaverine 30′	Cadaverine 60′
Spleen	–	3.0 ± 0.4	5.3 ± 0.9	1.4 ± 0.2	2.9 ± 0.4	0.9 ± 0.8	1.1 ± 0.1
	+	2.3 ± 0.5	3.0 ± 0.3	1.0 ± 0.1	1.1 ± 0.1	0.7 ± 0.1	0.7 ± 0.1
Heart	–	2.9 ± 0.4	4.6 ± 0.4	1.5 ± 0.2	2.0 ± 0.2	1.3 ± 0.2	1.2 ± 0.1
	+	4.2 ± 0.4	5.6 ± 0.7	1.2 ± 0.1	2.0 ± 0.2	1.1 ± 0.2	1.1 ± 0.2
Kidney cortex	–	4.6 ± 0.4	7.5 ± 0.5	3.3 ± 0.6	4.5 ± 0.5	1.9 ± 0.1	2.5 ± 0.1
	+	3.7 ± 0.6	5.2 ± 0.3	1.4 ± 0.1	2.0 ± 0.2	1.2 ± 0.1	1.4 ± 0.1
Liver	–	2.6 ± 0.5	3.5 ± 0.3	1.3 ± 0.4	1.6 ± 0.1	1.0 ± 0.1	1.4 ± 0.2
	+	3.4 ± 0.3	4.2 ± 0.2	1.1 ± 0.1	1.3 ± 0.1	0.8 ± 0.1	0.8 ± 0.1
Brain cortex	–	5.2 ± 0.7 (4)	10.5 ± 1.8 (4)	4.0 ± 0.4 (7)	10.0 ± 2.2 (7)	2.8 ± 0.3 (6)	4.5 ± 0.2 (8)
	+	3.6 ± 0.2 (4)	4.0 ± 0.6 (4)	1.5 ± 0.1 (7)	2.3 ± 0.2 (7)	1.5 ± 0.0 (6)	1.8 ± 0.1 (8)
Lung	–	17.0 ± 2.8	45.9 ± 4.4	26.2 ± 4.3	72.2 ± 7.6	25.1 ± 3.5	43.7 ± 0.9
	+	4.1 ± 0.2	5.4 ± 0.8	3.2 ± 0.3	4.0 ± 0.6	2.2 ± 0.2	2.2 ± 0.2

Slices of the above tissue were incubated in KRP glucose medium, containing 1 μM of the above ^{14}C-labelled compounds, in the presence or absence of KCN (1 mM). The amount of ^{14}C-label accumulated was measured at 30 and 60 min using radiochemical techniques. The results are expressed as the mean ± S.E.M. with 4 animals for each observation except where the number of animals are in parentheses.

ber of monoamines inhibit the uptake of paraquat into the lung [12] and in turn paraquat can inhibit the uptake of bases into the kidney [13]. Imipramine can effectively block the monoamine uptake system in the lung [11] although it itself is not accumulated [14]. These observations suggest that the transport processes for monoamines, diamines/polyamines and bases in different tissues have receptors which recognise a range of structural conformations. Consequently several related compounds will have varying affinities for these receptors thereby blocking the accumulation of the preferred substrate to differing degrees. In addition, it is probable that some compounds will inhibit the accumulation of substrates as a consequence of their affinity for the transport receptor although they themselves are not accumulated. Alternatively, some compounds may both demonstrate an affinity for the receptor and be themselves accumulated into cells.

Our earlier work with both paraquat and putrescine have implicated the alveolar type I and type II cells as in part the sites of their accumulation [3, 15]. Recently, we have suggested that the type II cells may be of prime importance. The uptake system has also been differentiated from that of the monoamine 5-hydroxytryptamine [16] which is taken up by the pulmonary endothelial cells [11]. The present study infers that spermine, spermidine and cadaverine are also accumulated, at least in part, by the alveolar type I and type II cells. The reason(s) for this cellular specific pulmonary uptake of diamines and polyamines is unclear but the location of the site on the alveolar epithelial cells might suggest that its function is not the clearance of polyamines from the pulmonary circulation. The accumulation process may be related to the control of cell proliferation and differentiation in the lung as polyamines are known to be ultimately involved in these processes in other tissues [17, 18]. Why the alveolar epithelial cells in particular should have the ability to accumulate diamines and polyamines is unclear. Currently we are investigating the *in vivo* accumulation of diamines and polyamines into rodent lung and relating this with the ability of lung cells to synthesis the polyamines, spermidine and spermine.

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