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INHIBITION OF PARAQUAT ACCUMULATION IN RAT LUNG SLICES BY A COMPONENT OF RAT PLASMA AND A VARIETY OF DRUGS AND ENDOGENOUS AMINES

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Abstract—The accumulation of paraquat by slices of rat lung has been shown to be inhibited *in vitro* by the addition of rat plasma. For a given concentration of plasma, inhibition was constant with time and the amount of inhibition increased with increasing concentration of plasma. This suggests that there are components of rat plasma which inhibit paraquat accumulation by rat lung slices in a concentration-dependent manner. An ultrafiltrate of plasma also inhibited paraquat uptake, indicating that the inhibitor is a small molecular weight compound. A number of endogenous amines including noradrenaline, 5-hydroxytryptamine and histamine have been shown to reduce the concentration of paraquat accumulated into lung slices, as have several other drugs including imipramine, propranalol, burimamide and betazole. The relevance of these findings to the prevention of paraquat accumulation by lung is discussed.

The herbicide paraquat (1,1-dimethyl 4,4′-bipyridilium) can produce widespread oedema and fibrosis in the human lung after accidental ingestion [1–3]. Paraquat has been shown to have a similar effect in experimental animals, the lungs being the primary target organ [4–6]. The discovery of an energy-dependent accumulation of paraquat by slices of rat lung *in vitro* [7] and the ability of rat lung to accumulate paraquat from a low plasma concentration *in vivo* [8, 9] suggests a possible reason for the selectivity of paraquat for the lung. Tissue slices from other organs have little capacity to accumulate paraquat [9] whereas lung slices from other species including man, do possess this capacity to accumulate paraquat [8, 9]. The rate of accumulation of paraquat by rat lung from plasma *in vivo*, following oral dosing of paraquat was about one seventh of that predicted by *in vitro* studies [9]. This suggested that inhibitors of paraquat accumulation might be present in the circulation. In this study the effect of rat plasma, several endogenous amines, amino acids and drugs on paraquat accumulation by rat lung slices have been examined.

MATERIALS AND METHODS

Animals. Male, Alderley Park (Wistar derived) specific pathogen-free rats were used throughout. Animals with body wt in the range 170–200 g were used for preparing lung slices and larger animals (300–400 g body wt) were used for obtaining plasma.

Special chemicals. [Methyl-^{14}C] paraquat of specific radioactivity 30 mCi/m-mole was purchased from the Radiochemical Centre, Amersham, Bucks, U.K. It was diluted with water to give a stock solution of 5 μCi/ml. The following compounds were used: adrenaline (Sigma); betazole hydrochloride (Eli Lilly); burimamide* (Smith, Kline & French); chlorophenirамine maleate (May & Baker); histamine hydrochloride (Sigma); L-histidine (Koch–Light); 5-hydroxytryptamine oxalate and creatinine sulphate complex (Sigma); imipramine hydrochloride (Geigy Pharm. Ltd.); L-lysine hydrochloride (Sigma); metiamide* (Smith, Kline & French); L-noradrenaline (Sigma); promethazine hydrochloride (May & Baker); D-propranolol (ICI Pharmaceuticals); mepyramine maleate (May & Baker); spermine tetrahydrochloride (Sigma); tryptamine hydrochloride (Sigma); tyramine (Sigma); L-tryptophan (Sigma); L-tyrosine hydrochloride (Sigma). Crystalline paraquat dichloride and diquat dichloride (1,1′-ethylene 2,2′-bipyridilium) were obtained from Plant Protection Division, Jealott's Hill Research Station, Berks.

Methods. Rats were killed with halothane, the lungs were removed and slices prepared (20–60 mg wet wt) by free-hand slicing or using a Stadie–Riggs tissue slicer. Only slices with two cut surfaces were used. For studies of the effect of plasma, slices were weighed and incubated in a modified Krebs–Ringer bicarbonate medium containing NaCl (121 mM); KCl (4.9 mM); $CaCl_2$ (1.7 mM); $MgSO_4$ (1.21 mM); KH_2PO_4 (1.21 mM); $NaHCO_3$ (25 mM) and glucose (11 mM) which had been gassed with 5% CO_2 in oxygen for 10 min prior to use. In addition the incubation medium contained rat plasma (0.25–1 ml), [^{14}C]paraquat (0.1 μCi) together with the required concentration of unlabelled paraquat, the final volume being 3 ml. Incubation was carried out under 5% CO_2, 95% oxygen to prevent pH changes [10], with shaking at 37°. For studies where plasma was not used, slices were incubated in a modified Krebs–Ringer phosphate medium [9]. The incubation medium also contained [^{14}C]paraquat (0.1 μCi) unlabelled paraquat (10 μM) and various concentrations

* The asterisks indicate the drugs were donated.

of test compounds. Incubation was carried out under air, with shaking at 37°. In all experiments one lung slice was incubated in 3 ml medium.

Paraquat measurement in slices. Slices were removed from the incubation medium and washed by transferring them to fresh medium which was free of paraquat. They were then carefully blotted, dissolved in 1 ml soluene (Packard Instrument Co Ltd) and radioactivity measured after addition of 10 ml Dimilume (Packard Instrument Co. Ltd.) using a liquid scintillation spectrometer. Samples of media (0.1 ml) were diluted to 1 ml with water and radioactivity measured after addition of 10 ml Instagel Scintillator (Packard Instrument Co. Ltd.). Counting efficiency was determined by the addition of internal standards and all counts were converted to disintegrations per minute.

Preparation of ultrafiltrate of plasma. Freshly obtained rat plasma was placed in an ultrafiltration cell (Model 52, Amicon, Lexington, Mass. USA) containing a Diaflo ultrafiltration membrane, type UM05 (which retains substances with a molecular weight in excess of 500 [11]). A pressure of 1.75 kg/cm^2 of N_2 was applied to the cell (by means of a pressurized cylinder), and the colourless ultrafiltrate which passed through the membrane collected at room temperature. The protein content of plasma and plasma ultrafiltrate was measured by the biuret method [12].

RESULTS AND DISCUSSION

Rat lung slices have been shown to accumulate paraquat *in vitro* by an energy-dependent process [7] to concentrations many times that present in the incubation medium. From this data the rate of accumulation of paraquat by lung slices is 30–40 nmoles of paraquat/g wet wt/hr from a concentration of 10 μM in the incubation medium. Following oral dosing of 680 μmoles of paraquat/kg body wt to rats a paraquat concentration of approx 10 μM was maintained in the plasma for several hours yet the rate of accumulation into the lung was 4–6 nmoles of paraquat/g wet wt/hr [8, 9]. This difference in rate of uptake *in vivo* compared with that *in vitro* could be due to several causes:

(1) to a difference in the rate of efflux of paraquat from lung tissue *in vivo* compared with that *in vitro* resulting in an apparent difference in the rate of accumulation.

(2) to differences in the behaviour of lung *in vivo* compared with lung tissue *in vitro* because of the non physiological nature of lung slices.

(3) to binding of paraquat by components of plasma thus reducing the concentration of free bipyridyl available for accumulation.

(4) to the presence of endogenous substances in plasma which inhibit the uptake of paraquat *in vivo*.

We have shown that rat plasma reduces the accumulation of paraquat by rat lung slices in a concentration dependent way (Fig. 1), 1 ml of plasma causing approximately 40 per cent inhibition, and that the uptake of paraquat into lung slices in the presence of a given concentration of rat plasma is linear with time (Fig. 2). When paraquat was added to a mixture of rat plasma (1 ml) and Krebs–Ringer bicarbonate under conditions identical with those used for the study of accumulation, all the paraquat was found to be freely dialysable, demonstrating that there was

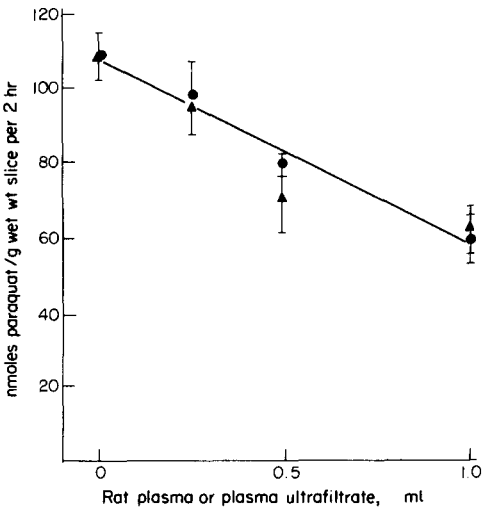

Fig. 1. The effect of rat plasma and rat plasma ultrafiltrate on paraquat accumulation by rat lung slices. Slices of rat lung were incubated in Krebs–Ringer bicarbonate–glucose medium as described in the Methods section, with 10 μM paraquat and either rat plasma (●) or rat plasma ultrafiltrate (▲) in the amounts indicated. The points represent the mean ± S.E.M. with four slices per point.

no binding to plasma macromolecules. An ultrafiltrate of rat plasma with a protein concentration of 0.5 mg/ml (compared with 74.1 mg/ml for plasma) also reduced the accumulation of paraquat by lung slices in a concentration-dependent manner (Fig. 1) and this inhibition was constant for a given concentration of ultrafiltrate (Fig. 2). Hence, the difference observed between the rates of paraquat accumulation *in vitro* and *in vivo* may at least partly be explained by the presence of endogenous small molecular weight compounds circulating in the plasma which inhibit the accumulation of paraquat into the lung.

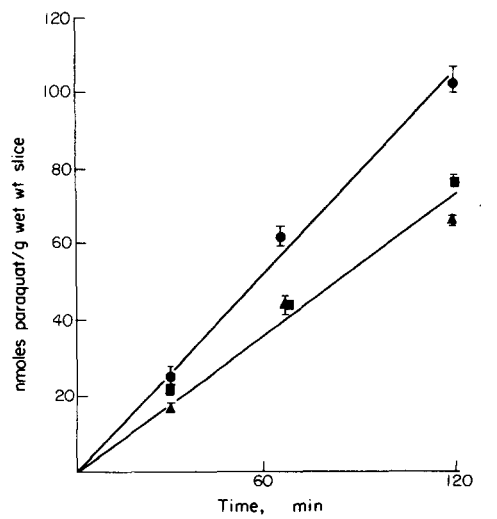

Fig. 2. The effect of rat plasma and rat plasma ultrafiltrate on paraquat accumulation by rat lung slices. Slices of rat lung were incubated in Krebs–Ringer bicarbonate–glucose medium as described in the Methods section, with 10 μM paraquat (●) or with paraquat and rat plasma (1 ml) (▲) or with paraquat and rat plasma ultrafiltrate (1 ml) (■). The points represent the mean ± S.E.M. with four slices per point.

Table 1. Inhibition of paraquat accumulation in rat lung slices by endogenous amines and amino acids

Compound	Concentration (mM)	Inhibition (% of control)
D-Adrenaline	1	44 (34–66)
Noradrenaline	0.01	31 (25–37)
	0.1	63 (58–69)
	1	87 (83–90)
5-Hydroxytryptamine	0.01	0–10
	0.1	32 (24–46)
	1	80 (76–82)
Tryptamine	0.01	14 (0–30)
	0.1	51 (27–64)
	1	92 (90–94)
Tryptophan	1	0–10
Tyramine	0.01	26 (17–30)
	0.1	81 (80–82)
	1	93 (92–94)
Tyrosine	1	0–10
Histamine	0.01	0–10
	0.1	51 (37–68)
	1	86 (84–89)
Histidine	1	0–10
Lysine	0.1	35 (33–41)
	1	72 (68–77)
Spermine	0.01	46 (40–51)
	0.1	88 (87–89)
	1	94 (93–95)

Slices of rat lung were incubated in Krebs–Ringer phosphate glucose medium, with 10 μM paraquat and the concentration of compounds indicated. Compounds were added at zero time and incubation carried out for 2 hr at 37°. The results are expressed as mean with the range of inhibition in brackets. At least three slices were used per concentration.

The lung plays an important role in the regulation of blood concentrations of several vasoactive substances [13, 14]. Studies using isolated perfused lungs have indicated that the endogenous amines 5-hydroxytryptamine [15, 16] and noradrenaline [17] are accumulated by the lung. Paraquat uptake into lung slices was inhibited by several endogenous amines including noradrenaline, 5-hydroxytryptamine, histamine and was also inhibited by the amino acid lysine (Table 1). Lysine is present in the plasma of Alderley Park rats at a free concentration of approximately 100 μM (M. Earlam, unpublished observation) which is sufficiently high to inhibit paraquat accumulation and may, therefore, contribute to the observed difference between *in vitro* and *in vivo* accumulation of paraquat. The amino acids tryptophan, tyrosine and histidine at 1 mM did not reduce paraquat accumulation significantly (Table 1).

Several exogenous compounds including D-propranolol, imipramine, diquat and betazole also inhibited the accumulation of paraquat *in vitro* (Table 2). The H_2 antagonist burimamide and the H_1 antagonist chloropheniramine reduced paraquat accumulation whereas the H_2 antagonist metiamide and the H_1 antagonist mepyramine did not (Table 2), indicating that the pharmacological activity of these compounds is not related to their inhibitory activity.

From this study it appears that a number of amines have the capacity to inhibit the accumulation of paraquat into rat lung. No precise structural requirement has emerged for compounds which inhibit the accumulation of paraquat, although the substitution of a carboxyl group on the α carbon carrying the amino group to given an amino acid (eg tyramine to tyrosine, histamine to histidine) abolishes the ability of the amine to inhibit paraquat. However, the amino acid lysine is effective in reducing paraquat accumulation, possibly due to the ϵ amino group.

It must be emphasised that although paraquat accumulation can be inhibited by various compounds, it does not follow that compounds which inhibit are themselves accumulated, or that they necessarily interact directly with the lung at the site of the paraquat accumulation process. For example, diquat is not accumulated by lung slices *in vitro* [7, 9] but reduces paraquat accumulation (Table 2). The structural similarity between paraquat and diquat would, therefore, suggest that this inhibition results from competition between the compounds for a recognition site on the cell membrane. We have also demonstrated two types of inhibition; the inhibition produced by noradrenaline (Fig. 3) imipramine, diquat and lysine which is linear with time and may be explained by simple competition between paraquat and inhibitor at the uptake site, and a non-linear inhibition produced by betazole (Fig. 3) and histamine. The explanation of this latter type of inhibition may involve the interaction of the inhibitor with the accumulation site or non-specific

Table 2. Inhibition of paraquat accumulation in rat lung slices by various drugs

Compound	Concentration (mM)	Inhibition (% of control)
D-Propranolol	0.01	0–10
	0.1	39 (38–41)
	1	89 (87–90)
Imipramine	0.1	58 (45–76)
Betazole	0.01	22 (0–40)
	0.1	50 (47–53)
	1	90 (88–92)
Burimamide	0.01	0–10
	0.1	39 (24–56)
	1	76 (73–83)
Metiamide	1	0–10
Chloropheniramine	1	46 (41–50)
Mepyramine	1	0–10
Promethazine	0.1	58 (57–60)
Diquat	0.01	0–10
	0.1	34 (9–55)
	1	52 (44–63)

Slices of rat lung were incubated in Krebs–Ringer phosphate glucose medium, with 10 μM paraquat and the concentration of compounds indicated. Compounds were added at zero time and incubations carried out for 2 hr at 37°. The results are expressed as mean with the range of inhibition in brackets. At least three slices were used per concentration.

damage to lung cells which results in a reduction of paraquat uptake, increase in efflux or a combination of both.

An understanding of the mechanism of inhibition of paraquat uptake brought about by different compounds is of paramount importance in the search for agents that reduce the uptake of paraquat into human lung. Compounds which will be of therapeutic value will be those which can be given in quantities sufficient to inhibit paraquat accumulation into the lung, until such time as the paraquat is excreted from the body or removed by other therapeutic measures.

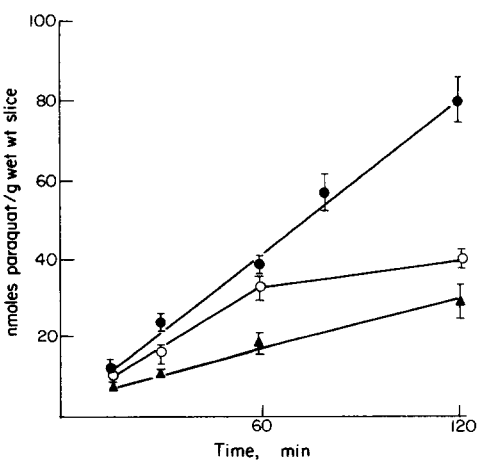

Fig. 3. Inhibition of paraquat accumulation into rat lung slices by noradrenaline and betazole. Slices of rat lung were incubated in Krebs–Ringer phosphate glucose medium, with paraquat 10 μM (●) or with paraquat and betazole 100 μM (○) or with paraquat and noradrenaline (100 μM) (▲). The points represent the mean ± S.E.M. with at least four slices per point.

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