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# PULMONARY ACCUMULATION OF METHYLGLYOXAL-BIS(GUANYLHYDRAZONE) BY THE OLIGOAMINE UPTAKE SYSTEM

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**Abstract**—The accumulation of methylglyoxal-bis(guanylhydrazone) (MGBG) into rat lung slices and its relationship to the accumulation of oligoamines has been investigated. MGBG was accumulated by rat lung slices by a process which obeyed saturation kinetics ($K_m$ 6.6 $\mu$M; $V_{max}$ 75.3 nmoles/g wet wt lung/hr). The uptake process appeared to be identical to those described for the accumulation of oligoamines and paraquat, being both KCN- (1 mM) and temperature-sensitive but insensitive to ouabain (100 $\mu$M). Pulmonary MGBG accumulation was found to be sodium-independent, either being enhanced or unaffected by sodium chloride-deficient media, so distinguishing the process from that described for the monoamine, 5-hydroxytryptamine. The ability and nature of various rat tissue slices to accumulate MGBG generally followed that of the oligoamines. Slices of lung, brain cortex and seminal vesicles accumulated MGBG by a KCN-sensitive and temperature-dependent process. These observations, together with the ability of MGBG to inhibit pulmonary oligoamine accumulation, indicate that it is the uptake system for the oligoamines which is mainly responsible for the *in vitro* accumulation of MGBG.

Due to its low therapeutic index, the cytotoxic agent, methylglyoxal-bis(guanylhydrazone) (MGBG), has limited therapeutic activity against acute myelogenous leukaemia [1–3], lymphomas [3] and oesophageal cancer [4]. Recently, however, there has been a renewed interest in MGBG as a potential anticancer agent stemming from a greater understanding of its pharmacodynamics. Using revised dosage regimens MGBG has been shown to be effective against various solid tumours accompanied with a lowered host toxicity [5].

Although the exact mechanism of the antiproliferative activity of MGBG is unknown, MGBG causes a depletion of cellular ATP pools [6–8], and extensive mitochondrial damage [7–10]. It also influences the levels of the endogenous oligoamines (putrescine, spermine and spermidine) [11–13] which in turn may affect a large number of cellular activities including mitochondrial function [14, 15] and macromolecular synthesis [16–18]. In addition, elevated oligoamine levels have been associated with actively proliferating cells [16] and with tumour growth [17]. Thus the effects of MGBG on the endogenous oligoamines, which may be due to inhibition of either putrescine activated *S*-adenosyl-L-methionine decarboxylase [12, 19, 20] or diamine oxidase [12, 21] may be of importance in its antiproliferative activity.

Depletion of endogenous oligoamines by either MGBG or difluoromethylornithine (DFMO), an irreversible inhibitor of ornithine decarboxylase, may result in the activation of homeostatic compensatory mechanisms. These mechanisms may include an increase in the biosynthesis [22], uptake [23] and a decrease in the excretion [24] of the oligoamines. Of particular interest is the inducible increase in oligoamine uptake as structurally related compounds, including MGBG, are known to utilize such systems for their own accumulation [25–27].

The uptake of MGBG into various cell types including, L1210 mouse ascites, HeLa, rabbit reticulocytes, non-mammalian erythrocytes [28], Ehrlich ascites [23, 27, 29] and cultured human lymphocytic leukaemia [27], have been documented and characterized to varying extents. In general, such systems appear to accumulate MGBG by an active transport process, which, interestingly, may result in intracellular concentrations up to 1000-fold greater than those found extracellularly [29].

Recently, we have shown that rat lung slices accumulate, by an apparently identical uptake system, the oligoamines, putrescine, spermidine, spermine and cadaverine [30], as well as the structurally related pulmonary toxin paraquat [31, 32]. The pulmonary putrescine uptake process has been shown to be temperature- and energy-sensitive [31, 33], insensitive to ouabain (100 $\mu$M) [31, 32] and different from the pulmonary uptake of monoamines such as 5-hydroxytryptamine [32, 34]. Moreover, studies examining the structural requirement for inhibition of pulmonary putrescine uptake revealed that MGBG and its congeners were amongst the most effective inhibitors of this uptake system [33].

In the present studies, we have characterized the accumulation of MGBG into rat lung slices and examined its relationship to the oligoamine uptake system. [$^{14}$C]-MGBG is accumulated by rat lung slices by a similar process to that described for putrescine and related compounds [30–33]. Pulmonary MGBG accumulation was shown to be energy-dependent, ouabain-insensitive and sodium-independent.

## MATERIALS AND METHODS

### *Materials*

Methylglyoxal-bis[$^{14}$C]guanylhydrazone dihydrochloride monohydrate (12 mCi/mmole), [1,4-$^{14}$C]-putrescine dihydrochloride (109 mCi/mmole), [$^{14}$C]spermidine trihydrochloride (115 mCi/mmole), [methyl-$^{14}$C] paraquat chloride (111 mCi/mmole) and [U$^{14}$C]-glucose (2–4 mCi/mmole) were purchased from Amersham International plc (Amersham, U.K.). Aquasol scintillation cocktail was obtained from New England Nuclear (Edinburgh, U.K.). Methylglyoxal-bis(guanylhydrazone)dihydrochloride monohydrate was purchased from the Aldrich Chemical Company (Gillingham, U.K.). Putrescine dihydrochloride, and spermidine trihydrochloride were purchased from the Sigma Chemical Company (Poole, U.K.). Analytical grade paraquat dichloride was kindly supplied by the Plant Protection Division (ICI plc). Halothane (Fluothane) was supplied by the Pharmaceutical Division (ICI plc). All other reagents were of analytical quality of the highest grade available.

*Animals.* Male, Alderley Park Wistar derived specific pathogen free rats (body weights 180–300 g) were used throughout.

### *Methods*

The accumulation of [$^{14}$C]-MGBG, paraquat, putrescine and spermidine were determined essentially by the method of Smith and Wyatt [31].

*Preparation of tissue slices.* Rats were anaesthetized with halothane and the tissues removed after the cessation of respiratory movements. Lung tissues were perfused with a modified Krebs–Ringer phosphate medium [33], whereas other tissues were extensively washed, prior to slicing. Tissue slices 0.5 mm thick were prepared using a McIlwain tissue chopper.

*Uptake of compounds into tissue slices.* Freshly prepared weighed tissue slices (20–50 mg) were incubated in 3.0 mls of a modified Krebs–Ringer phosphate medium (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) (pH 7.4), containing either 0.1 $\mu$Ci [$^{14}$C]-labelled putrescine, spermidine, paraquat or 0.03 $\mu$Ci [$^{14}$C]-MGBG made up to the desired concentration by the addition of the unlabelled salt and the appropriate unlabelled inhibitor where necessary. Where appropriate the sodium chloride (130 mM) content of normal KRP was substituted by sucrose (260 mM), lithium chloride (130 mM) or sodium isethionate (130 mM). Incubations were carried out at the appropriate temperature under air, in a shaking water bath at 60–70 cpm and the accumulation of the labelled compounds measured at pre-determined time intervals as previously described [31, 33]. The use of the labelled oligoamines or paraquat to determine their accumulation has been examined and shown to be a valid method over the time courses employed in these studies [30, 31]. The validity of employing [$^{14}$C]-MGBG to measure uptake was assumed as this compound has been shown not to be metabolized *in vivo* [35]. Rates of accumulation, where appropriate, were calculated using a weighted linear regression analysis including a zero time diffusion factor based on the water content of the lung as determined using $^3H_2O$ [33].

*Measurement of glucose oxidation using* [U$^{14}$C]-*glucose.* Weighed lung slices (0.5 mm thick) were incubated in 3 ml KRP medium containing 1 $\mu$Ci [U$^{14}$C]-glucose in respirometer flasks at 37°. $^{14}CO_2$ production was measured using 200 $\mu$l KOH (20% w/v), placed in the centre well with a 1 in. square filter paper to facilitate absorption. Glucose oxidation was terminated by the administration of 1 ml TCA (10% solution) injected through the self-sealing stoppers, slices were then further incubated for 30 min to facilitate the absorption of evolved $^{14}CO_2$. After incubation, the contents of the centre well were removed and radioactivity determined by liquid scintillation spectrometry.

## RESULTS

### *The accumulation of MGBG into lung slices*

The accumulation of [$^{14}$C]-MGBG (1–100 $\mu$M) by rat lung slices obeyed saturation kinetics (Fig. 1) with an apparent $K_m$ of 6.6 ± 1.7 $\mu$M and $V_{max}$ 75.3 ± 5.4 nmoles/g wet wt lung/hr (mean ± S.E.M., N = 3), as calculated from Eadie–Hofstee plots. Pulmonary uptake of MGBG (10 $\mu$M) was shown to be linear over a period of 1 hr and to be both temperature- and energy-dependent. The rate of [$^{14}$C]-MGBG accumulation into rat lung slices was very markedly decreased at 4° compared to slices incubated at 37° (Fig. 2). Transfer of slices incubated at 37° for 20 min to 4° resulted in a virtually complete inhibition of the rate of MGBG uptake. When slices, originally incubated at 4° for 20 min, were transferred to 37° the rates of MGBG accumulation observed were similar to those obtained for slices continuously incubated at 37° (Fig. 2). MGBG uptake into lung slices was markedly inhibited by the metabolic inhibitor, KCN (1 mM), but not by the $Na^+/K^+$ ATPase inhibitor, ouabain (100 $\mu$M) (Table 1). The changes in the rates of [$^{14}$C]-MGBG accumulation with temperature and the sensitivity of the process to KCN

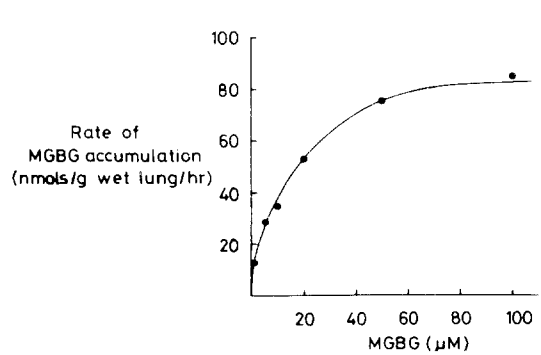


Fig. 1. Pulmonary accumulation of MGBG. Slices of rat lung were incubated in KRP medium containing [$^{14}$C]-MGBG (1–100 $\mu$M) at 37° for 5, 15 and 30 min for a substrate concentration of 1 $\mu$M and at 15, 30 and 60 min for all other concentrations. The rates of accumulation were then calculated using a weighted linear regression analysis as described in Materials and Methods. The results shown are from one experiment typical of three.

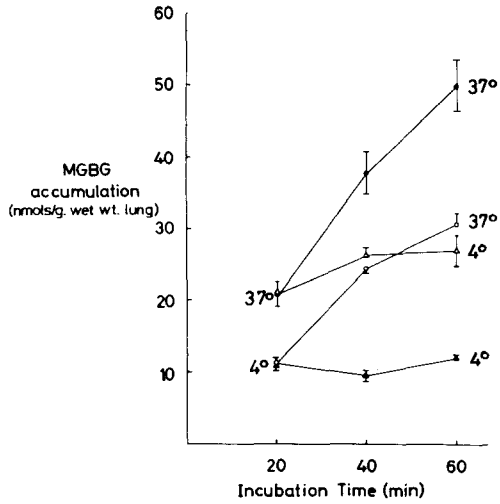


Fig. 2. The incluence of temperature on the accumulation of MGBG by rat lung slices. Slices of rat lung were incubated in KRP medium containing [$^{14}$C]-MGBG (10 $\mu$M) at 37° (●—●), 4° (▲—▲), 37° for 20 min then 4° (△—△) or at 4° for 20 min then at 37° (○—○). The results are expressed as the mean ± S.E.M. (N ≥ 3), where N refers to the number of animals.

but not ouabain are similar to those reported for the oligoamine, putrescine [31–33].

The herbicide, paraquat, is also accumulated by rat lung slices by a process similar to that described for the oligoamines [31, 32]. Pulmonary paraquat accumulation, like that of putrescine, was shown to be not only ouabain-insensitive but to be enhanced in sucrose-substituted sodium chloride deficient medium [31, 32, 34]. The similarity of the pulmonary accumulation of MGBG and paraquat was suggested by the observation that substitution of the sodium chloride of the KRP incubation medium with sucrose (KRP sucrose) resulted in an enhancement of the pulmonary uptake of both compounds (Fig. 3). However, substitution of the sodium chloride of the incubation medium with equimolar concentrations of either lithium chloride or sodium isethionate, thereby more closely mimicking the ionic nature of the original medium, did not result in any significant alteration in the accumulation of either paraquat or MGBG (Fig. 3). This observation suggests that the enhanced uptake noted in KRP sucrose may be due to an ionic imbalance between intracellular and extracellular environments rather than a specific deficiency in either $Na^+$ or $Cl^-$.

Table 1. The effects of KCN and ouabain on the accumulation of MGBG (10 $\mu$M) by slices of rat lung

| | Accumulation of MGBG (nmoles/g wet wt. lung) Time (min) | | |
|---|---|---|---|
| Incubation | 20 | 40 | 60 |
| Control | 26.8 ± 1.3 | 44.6 ± 4.2 | 65.0 ± 2.7 |
| + KCN (1 mM) | 14.8 ± 0.7 | 18.0 ± 1.0 | 21.7 ± 1.1 |
| + KCN (1 mM) after 20 min | — | 34.0 ± 2.3 | 35.8 ± 0.6 |
| + Ouabain (100 $\mu$M) | 25.0 ± 1.3 | 49.4 ± 4.0 | 57.8 ± 3.6 |
| + Ouabain (100 $\mu$M) after 20 min | — | 47.4 ± 3.7 | 58.2 ± 1.7 |

Lung slices were incubated at 37° in KRP medium containing [$^{14}$C]-MGBG (10 $\mu$M). The accumulation of MGBG was measured at 20, 40 and 60 min. Ouabain and KCN were added either initially or after 20 min. Results are expressed as the mean ± SEM of 6–8 determinations.

Table 2. The influence of various pre-treatments on subsequent MGBG and oligoamine accumulation and glucose oxidation

| | Substrate accumulation % of control | | | Glucose oxidation ($^{14}CO_2$ production from [U$^{14}$C]-glucose) % of control |
|---|---|---|---|---|
| Pre-treatment | MGBG (5 $\mu$M) | Putrescine (10 $\mu$ M) | Spermidine (10 $\mu$M) | |
| MGBG (100 $\mu$M) | 64.3 ± 3.1* | 34.7 ± 4.0* | 35.6 ± 3.5* | 95.8 ± 8.0 |
| Putrescine (100 $\mu$M) | 96.7 ± 11.9 | 75.4 ± 10.0* | 83.0 ± 5.2* | 90.9 ± 6.8 |
| Spermidine (100 $\mu$M) | 82.8 ± 8.2 | 65.8 ± 11.1* | 75.0 ± 7.5 | 88.9 ± 14.9 |

Slices were pre-incubated for 60 min at 37° in KRP containing 100 $\mu$M MGBG, putrescine or spermidine. Slices were then washed in fresh KRP before being incubated for a further 60 min in the presence of [$^{14}$C]-substrate. Controls were identically treated but were pre-incubated in KRP alone. Control values for MGBG, putrescine and spermidine were 38.1.4 ± 4.3, 219.3 ± 26.9 and 184.8 ± 13.3 nmols/g wet wt lung/60 min respectively. $^{14}CO_2$ production from [U$^{14}$C]-glucose by lung slices was measured over 120 min in the presence of the oligoamines or MGBG. Control incubations were carried out in the absence of the oligoamines or MGBG and yielded values of 13442 ± 1815 dpm/100 mg wet wt lung/120 min. Results are expressed as the mean ± S.E.M. of at least 3 determinations.

* Significantly different from control (paired $t$ test $P < 0.05$)

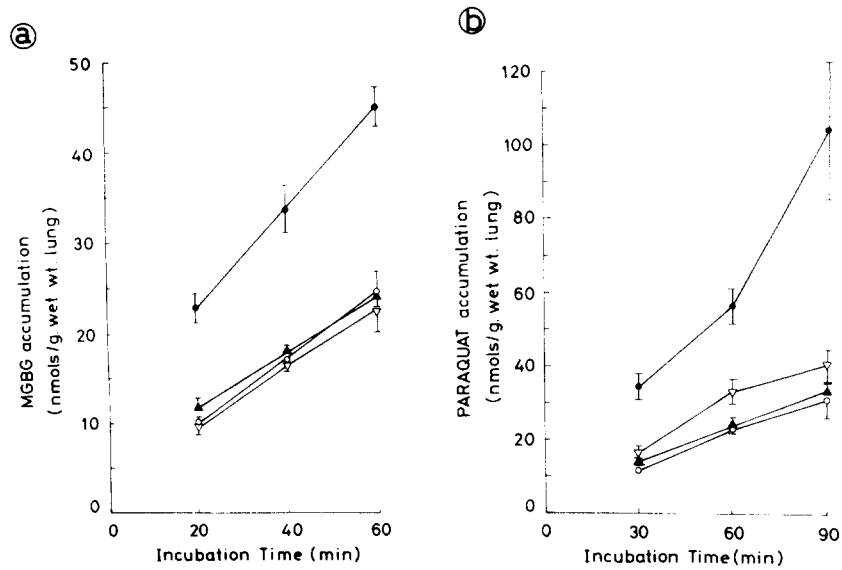


Fig. 3. The influence of NaCl deficient media on the pulmonary accumulation of (a) MGBG (5 $\mu$M) and (b) paraquat (10 $\mu$M). Slices of rat lung were incubated at 37° in KRP medium containing sodium chloride (130 mM) (▲—▲) or substituted by sucrose (260 mM) (●—●), lithium chloride (130 mM) (○—○) or sodium isethionate (130 mM) (▽—▽) in the presence of (a) [$^{14}$C]-MGBG (5 $\mu$M) or (b) [$^{14}$C]-paraquat (10 $\mu$M). Results are expressed as the mean ± S.E.M. ($N \geq 3$).

Table 3. The accumulation of MGBG (5 $\mu$M) and spermidine (5 $\mu$M) by various rat tissue slices

| | | | Accumulation of substrate (nmoles/g wet wt tissue) | | | |
|---|---|---|---|---|---|---|
| | | | MGBG | | Spermidine | |
| | | | Incubation time (min) | | | |
| Tissue | Temp °C | ±KCN 1 mM | 30 | 60 | 30 | 60 |
| Lung | 37 | − | 15.4 ± 1.6 | 28.6 ± 2.4 | 33.7 ± 2.1 | 55.6 ± 3.1 |
| | 37 | + | 10.3 ± 1.1 | 12.8 ± 0.9 | 5.5 ± 0.9 | 6.8 ± 1.2 |
| | 4 | − | 6.5 ± 0.7 | 7.2 ± 0.7 | 4.3 ± 0.5 | 4.9 ± 0.4 |
| Liver | 37 | − | 9.4 ± 0.6 | 17.2 ± 1.4 | 6.4 ± 0.5 | 7.9 ± 0.8 |
| | 37 | + | 13.2 ± 2.0 | 17.8 ± 1.0 | 6.9 ± 1.5 | 10.5 ± 0.6 |
| | 4 | − | 4.1 ± 0.6 | 6.8 ± 0.2 | 4.2 ± 0.1 | 4.7 ± 0.2 |
| Heart | 37 | − | 9.2 ± 0.6 | 10.5 ± 0.7 | 6.4 ± 0.5 | 6.8 ± 0.6 |
| (Ventricular | 37 | + | 8.3 ± 0.7 | 9.6 ± 1.2 | 6.2 ± 0.4 | 7.9 ± 0.4 |
| Tissue) | 4 | − | 8.1 ± 1.3 | 9.0 ± 1.4 | 5.2 ± 0.7 | 5.0 ± 0.5 |
| Kidney | 37 | − | 14.6 ± 0.6 | 16.9 ± 2.6 | 5.2 ± 0.7 | 6.7 ± 0.6 |
| (Cortex) | 37 | + | 9.7 ± 0.3 | 10.2 ± 1.4 | 4.2 ± 1.1 | 7.1 ± 1.0 |
| | 4 | − | 5.1 ± 0.6 | 6.9 ± 0.8 | 2.8 ± 0.2 | 3.8 ± 0.1 |
| Spleen | 37 | − | 6.6 ± 0.5 | 9.4 ± 1.2 | 4.4 ± 0.5 | 7.9 ± 0.9 |
| | 37 | + | 6.9 ± 0.6 | 9.3 ± 1.0 | 5.4 ± 0.7 | 6.8 ± 1.2 |
| | 4 | − | 7.4 ± 0.8 | 7.0 ± 0.8 | 3.9 ± 0.2 | 4.1 ± 0.2 |
| Brain | 37 | − | 8.5 ± 0.9 | 12.1 ± 1.1 | 7.2 ± 1.5 | 10.2 ± 2.2 |
| (Cortex) | 37 | + | 6.1 ± 0.1 | 6.1 ± 0.5 | 4.5 ± 0.5 | 4.2 ± 1.4 |
| | 4 | − | 3.4 ± 0.8 | 4.4 ± 0.6 | 3.9 ± 0.4 | 5.5 ± 0.8 |
| Seminal | 37 | − | 10.0 ± 0.7 | 18.7 ± 3.0 | 11.9 ± 1.9 | 34.1 ± 7.2 |
| Vesicles | 37 | + | 8.5 ± 0.5 | 10.9 ± 0.6 | 7.4 ± 1.7 | 9.5 ± 1.3 |
| | 4 | − | 8.6 ± 1.4 | 9.8 ± 2.0 | 6.8 ± 1.2 | 6.2 ± 0.4 |
| Submaxillary | 37 | − | 14.8 ± 1.0 | 22.1 ± 4.7 | 10.3 ± 1.7 | 12.5 ± 1.1 |
| Gland | 37 | + | 13.1 ± 1.5 | 17.7 ± 3.1 | 7.0 ± 0.8 | 10.8 ± 1.8 |
| | 4 | − | 5.3 ± 0.6 | 7.2 ± 1.1 | 3.5 ± 0.2 | 4.4 ± 0.5 |

Slices of various rat tissues were incubated at 37° in the presence or absence of KCN (1 mM) or at 4° in KRP medium containing either 5 $\mu$M [$^{14}$C]-spermidine or [$^{14}$C]-MGBG. Slices were incubated for 30 or 60 min. Results are expressed as the mean ± S.E.M. of at least 3 determinations.

As MGBG has been reported to utilize the oligoamine accumulation process in various systems [23, 25, 26] and to be an effective inhibitor of pulmonary putrescine accumulation [33], the influence of these oligoamines and MGBG on each other's pulmonary accumulation was studied.

The ability of various concentrations of MGBG to inhibit putrescine (2.5–20 $\mu$M) uptake into rat lung slices was investigated. MGBG (1 $\mu$M) appeared to inhibit putrescine uptake in a competitive manner. However, when higher concentrations of MGBG were employed the nature of this inhibition was unclear (data not shown).

MGBG was more effective in inhibiting the pulmonary accumulation of putrescine or spermidine than in either of the reverse situations (Fig. 4). Studies carried out at 4° to correct for non-specific binding suggested that MGBG displayed a higher degree of binding in proportion to the total amount associated with lung tissue than either putrescine or spermidine. Pre-incubation of slices with spermidine or putrescine (100 $\mu$M) for 60 min did not significantly reduce the subsequent accumulation of MGBG (5 $\mu$M) by such slices (Table 2). However, pre-incubation with MGBG (100 $\mu$M) did appear to inhibit both subsequent [$^{14}$C] oligoamine and [$^{14}$C]-MGBG uptake. Neither incubation of the slices with 100 $\mu$M MGBG nor the oligoamines affected their ability to oxidize glucose (Table 2) so implying that changes in the viability of the slices was not responsible for the above observations.

*The relationship between the* in vitro *MGBG and oligoamine accumulation systems in various rat tissues*

The accumulation of MGBG into various tissue slices was investigated with respect to their ability to accumulate oligoamines. The ability and nature of various tissue slices to accumulate MGBG appeared to correlate well with their ability to accumulate spermidine (Table 3) and putrescine (data not shown), whose uptake was similar to that observed in previous studies [30, 31, Kenneally *et al.* unpublished data]. Slices of lung, seminal vesicles and brain cortex accumulated MGBG by a time-dependent, KCN and temperature-sensitive process. In contrast to these findings, slices of liver, kidney cortex, spleen, heart and submaxillary gland accumulated MGBG or spermidine by processes which were either KCN- or temperature-insensitive.

## DISCUSSION

The ability of MGBG to influence the oligoamine uptake process in a variety of cell types is well documented [23, 27–29, 36]. More recently, MGBG has been shown to be an effective inhibitor of pulmonary putrescine uptake [33], a system believed to be responsible for the accumulation of other oligoamines and the pulmonary toxin paraquat [30–32, 34]. In order to elucidate the relevance of the pulmonary oligoamine uptake system to MGBG uptake, the general properties of various pulmonary accumulation systems were compared (Table 4). The pulmonary accumulation processes for MGBG, paraquat, putrescine and spermidine as well as the

Table 4. Comparison of the properties of various pulmonary accumulation systems

| Substrate | Saturation kinetics | Temperature sensitive | KCN ($10^{-3}$ M) sensitive | Ouabain ($10^{-3}$ M) sensitive | Enhancement in sodium substituted medium | | Proposed cells responsible for accumulation | Reference |
|---|---|---|---|---|---|---|---|---|
| | | | | | Substituted by sucrose | Substituted by lithium | | |
| Putrescine | + | + | + | –* | + | Nd | Types I & II | 30–33 |
| Spermidine | + + | + + | + + | –* | Nd | Nd | Types I & II | 30 |
| Paraquat | + | + | + | –* | + | – | Types I & II | 31,34, 39,40 |
| MGBG | + | + | + | –* | + | – | Types I & II | This study |
| 5-hydroxytryptamine | + | + | + | + | – | – | Pulmonary Endothelial Cells | 34, 39, 41, 42 |

The properties of the pulmonary accumulation of MGBG were compared to those documented for various substrates. All studies were carried out using lung slices except those for 5-hydroxytryptamine were both lung slices and perfused lung models were employed. The relevance of a particular property to an accumulation system is indicated by a + or –, where + indicates that that particular property was relevant. Nd indicates not determined.

* Unpublished data.

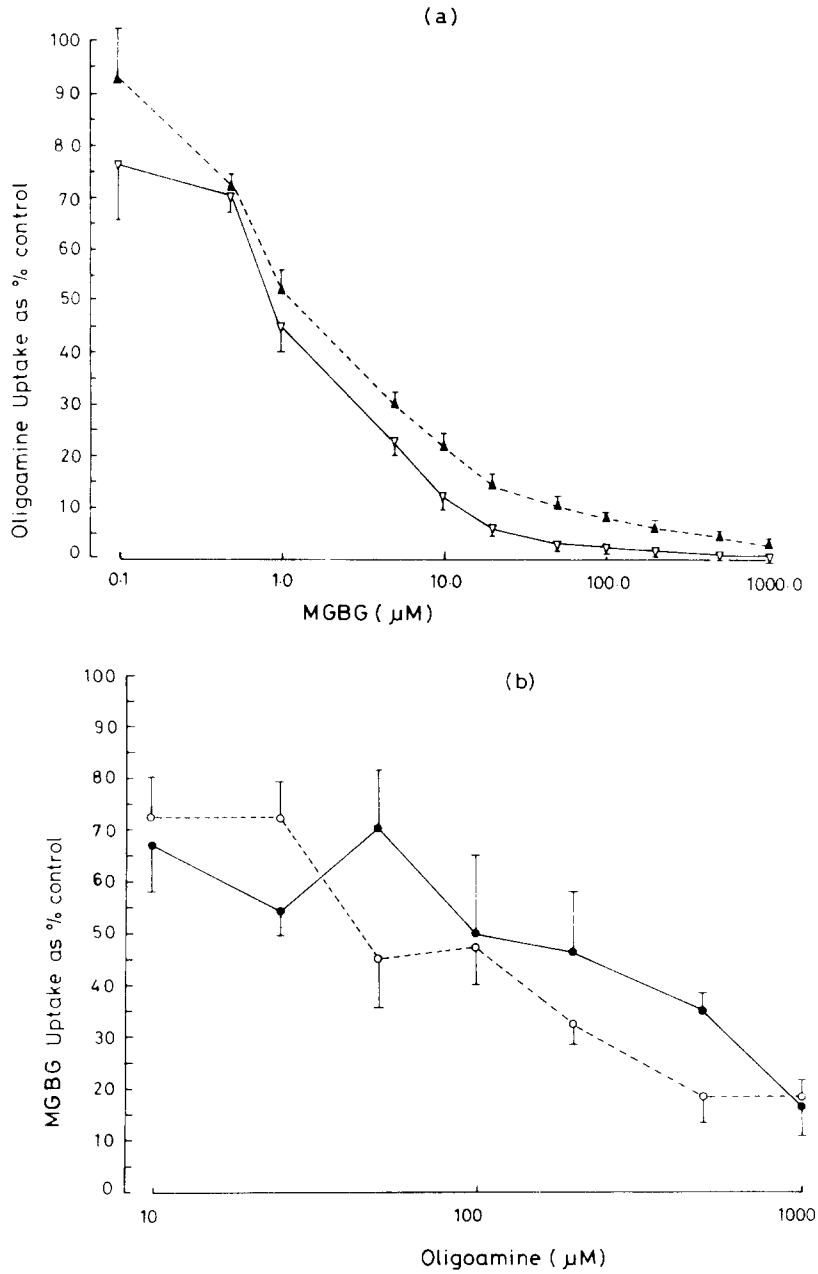


Fig. 4. The ability of (a) MGBG to inhibit oligoamine accumulation and (b) the oligoamines to inhibit MGBG accumulation by rat lung slices. Slices of rat lung were incubated for 60 min at 37° in KRP containing either (a) 5 μM [$^{14}$C] putrescine (▽—▽) or 5 μM [$^{14}$C]-spermidine (▲—▲) in the presence of various concentrations of MGBG, or (b) 5 μM [$^{14}$C]-MGBG in the presence of various concentrations of putrescine (○—○) or spermidine (●—●). Values were corrected for the non-energy-dependent components of accumulation using control incubations carried out at either 4° or in the presence of KCN (1 mM) both of which gave similar results. Results are expressed as a mean percentage ± S.E.M. (N = 3–6) of the appropriate corrected control values. Corrected control values were for MGBG, putrescine and spermidine, 18.8 ± 3.4, 128.0 ± 20.5 and 105.7 ± 13.6 nmoles/g wet wt lung/hr, respectively. Accumulation at 4° for MGBG in the presence of the oligoamines was 9.3 ± 0.3 nmoles/g wet wt lung/hr and for spermidine and putrescine in the presence of MGBG, 7.1 ± 0.4 and 4.4 ± 0.1 nmoles/g wet wt lung/hr respectively.

monoamine 5-hydroxytryptamine (5HT) obeyed saturation kinetics and were KCN- and temperature-sensitive. However, 5HT accumulation appears to differ from that of MGBG, paraquat and the oligoamines, being sensitive to ouabain (1 mM) and showing no enhancement in sucrose substituted sodium chloride-deficient medium. 5HT is also believed to be accumulated primarily into capillary endothelial cells whereas paraquat and the oligamines, appear to be accumulated into alveolar epithelial type I and

type II cells (Table 4). The similarity of the MGBG system to the paraquat or oligoamine system and its difference from the 5HT system, together with the ability of the oligoamines to inhibit MGBG pulmonary uptake (Fig. 4), suggest that the pulmonary uptake of MGBG may be primarily associated with epithelial type I and type II cells.

The oligoamines, spermidine and putrescine, and MGBG were found to mutually inhibit each other's accumulation by rat lung slices (Fig. 4). The relative abilities of these substrates to do this appeared to reflect the apparent relative kinetic parameters of these substrates for their uptake by rat lung slices. Hence, MGBG ($K_m$ 6.6 $\mu$M; $V_{max}$ 75.3 nmoles/g wet wt lung/hr) was found to be more effective in inhibiting both putrescine ($K_m$ 15 $\mu$M; $V_{max}$ 704 nmoles/g wet wt lung/hr [33]) or spermidine ($K_m$ 10.9 $\mu$M; $V_{max}$ 768 nmoles/g wet wt. lung/hr [31]), than in either of the reverse situations (Fig. 4). Several possibilities may account for the mutual inhibition observed for these substrates besides simple competition for a common uptake site. One possibility is that uptake was reduced as a result of a loss in slice viability. However, as the ability of slices to oxidize glucose was not significantly altered in the presence of 100 $\mu$M MGBG or the oligoamines (Table 2), this possibility seems unlikely. In addition putrescine has been documented not to affect other biochemical indices of viability such as oxygen consumption [37], fatty acid synthesis [38] and the maintenance of lung NADPH levels [39]. Another possible explanation may involve the influence of endogenous levels of the oligoamines on subsequent MGBG or oligoamine uptake. Pre-incubation of slices with the oligoamines did not influence subsequent MGBG accumulation (Table 2), suggesting that intracellular endogenous oligoamine levels were not a significant factor for pulmonary uptake in these studies. MGBG pre-incubation appeared to influence both subsequent MGBG and oligoamine accumulation. This observation, however, may be due to residual extracellular MGBG competing with subsequent [$^{14}$C]-substrate uptake. Similar explanations may account for the slight inhibitions noted for the oligoamines following pre-incubations with either spermidine or putrescine.

The nature and ability of various rat tissue slices to accumulate MGBG correlated well with their ability to accumulate spermidine (Table 3) and putrescine (unpublished results). Slices of rat lung accumulated both MGBG and spermidine more effectively than other tissues studied, although the actual amounts of oligoamine accumulated were somewhat less than those predicted from previous studies [30]. Lung, seminal vesicles and brain cortex slices accumulated both MGBG and spermidine by time, temperature and KCN-sensitive processes, whereas slices of liver, heart, spleen, kidney cortex and submaxillary gland displayed either KCN- or temperature-insensitivity in the accumulation of one or both of these substrates. These observations are consistent with the suggestion that lung, brain cortex and seminal vesicles accumulate oligoamines by an energy-dependent process which is similar or identical to that responsible for the *in vitro* accumulation of MGBG.

In summary, these studies have shown that rat lung slices accumulate MGBG by an energy-dependent but ouabain-insensitive process resembling that previously described for the herbicide paraquat and the oligoamines [31–33]. The nature of various tissue slices to accumulate both MGBG and oligoamines were very similar, suggesting that the major process by which MGBG is accumulated appears to be that normally utilized by the endogenous oligoamines.

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