Monocrotaline pyrrole-induced changes in angiotensin-converting enzyme activity of cultured pulmonary artery endothelial cells

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1 Changes in the structural and functional integrity of endothelium have been recognized as relatively early features of delayed and progressive pulmonary vascular injury caused by the pyrrolizidine alkaloid, monocrotaline (MCT). Although a number of investigators have evaluated angiotensin-converting enzyme (ACE) activity in the lungs of rats treated with MCT, the exact nature of changes in activity of this enzyme and the role they may play in MCT pneumotoxicity remain controversial.

2 We examined the direct effects of monocrotaline pyrrole (MCTP), a toxic metabolite of MCT, on cultured endothelial cell ACE activity. Post-confluent monolayers of porcine or bovine pulmonary artery endothelial cells (PECs or BECs, respectively) were treated with a single administration of MCTP at time 0; then they were examined for their ability to degrade the synthetic peptide, [³H]-benzoyl-Phe-Ala-Pro.

3 In PECs, which are relatively insensitive to the direct cytolytic effects of MCTP, monolayer ACE activity was unchanged initially but gradually decreased within 4 days after treatment with a high concentration of MCTP (150 µM). This decrease was transient, and PEC monolayer ACE activity returned to the control value by 10 days post treatment.

4 BEC monolayer ACE activity was also unchanged initially but rapidly declined within 4 days after MCTP treatment and remained depressed throughout the post treatment period. BECs were quite sensitive to the cytolytic effects of MCTP and the decline in ACE activity occurred coincident with the decrease in monolayer cellularity and appearance of marked cytotoxicity.

5 We conclude that high concentrations of MCTP decrease endothelial ACE activity. The decline in ACE activity is delayed and the magnitude and duration of the decrease corresponds to the degree of MCTP-induced cytotoxicity. This suggests that altered endothelial ACE activity is unlikely to be a direct effect of MCTP on the enzyme but may reflect the delayed cell injury which results from exposure to this compound.

Keywords: Monocrotaline; monocrotaline pyrrole; angiotensin-converting enzyme; cultured endothelium; pulmonary hypertension; response to injury

Introduction

The pyrrolizidine alkaloid, monocrotaline (MCT), causes delayed and progressive pulmonary vascular injury in rats and other sensitive species (Schoental & Head, 1955; Valdivia *et al.*, 1967; Hayashi & Lulich, 1967; Peckham *et al.*, 1974), resulting in the development of pulmonary hypertension and right ventricular hypertrophy (Chesney *et al.*, 1974; Meyrick & Reid, 1979; Sugita *et al.*, 1983). The pulmonary vascular endothelium has been suggested as a likely cellular target for initial injury, and changes in endothelial structural and functional integrity have been noted relatively early in the course of MCT pneumotoxicity *in vivo* (Hilliker *et al.*, 1982; Bruner *et al.*, 1983; Reindel *et al.*, 1990). It has been suggested that persistent changes in endothelial cell function may play a role in the development and/or maintenance of MCT-induced pulmonary hypertension (Reindel *et al.*, 1990; Roth & Reindel, 1990; Reindel & Roth, 1991b).

The pulmonary vascular endothelium carries out a number of complex functions which are important in the maintenance of vascular homeostasis and in regulating the vascular response to circulating mediators (for a review of this subject, see Fajardo, 1989). One of these functions is the cleavage of circulating, inactive angiotensin I (AI) to the potent vasoconstrictor, angiotensin II (AII), by the action of the exopeptidase known as angiotensin-converting enzyme (ACE) (Yang *et al.*, 1970; Erdos, 1975). It has been suggested that pulmonary vascular disease might be associated with changes in

pulmonary activity of this enzyme (Gillis & Catravas, 1982; Dobuler *et al.*, 1982; Catravas *et al.*, 1988).

A number of investigators have evaluated ACE in lungs of rats treated with MCT in an effort to correlate changes in enzyme activity with the development or progression of MCT-induced cardiopulmonary changes. The results of these studies have been disparate. Some investigators report that there is a decrease in lung ACE activity (Kay *et al.*, 1982; Keane *et al.*, 1982), whereas others contend that apparent changes in lung ACE activity are due to a dilution of this activity by the increased lung mass seen in this model (Huxtable *et al.*, 1978; LaFranconi & Huxtable, 1983); transient increases in ACE activity have also been reported (Molteni *et al.*, 1984). From these results of work done *in vivo*, it is difficult to draw definitive conclusions about ACE activity in lung vasculature injured by MCT.

Monocrotaline pyrrole (MCTP) is a putative, toxic metabolite of MCT. Injection of a relatively low dose of chemically synthesized MCTP into rats results in a spectrum of pulmonary changes which are comparable to those produced by MCT, including changes in endothelial cells (Bruner *et al.*, 1983). MCTP has also been shown to be toxic to cultured bovine and porcine pulmonary artery endothelial cells (BECs and PECs, respectively) (Reindel & Roth, 1991; Reindel *et al.*, 1991). This cultured endothelial cell model makes it possible to investigate the direct effects of MCTP treatment on endothelial cell function. The purpose of this study was to determine whether MCTP affects ACE activity in cultured

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endothelium and to characterize the development of any changes that occur.

Methods

Preparation of endothelial cells

Lines of PECs and BECs were derived from segments of pulmonary artery by modifications of the methods of Jaffe *et al.* (1987) and Booyse *et al.* (1975), as described by Reindel & Roth (1991). PECs and BECs exhibited characteristic cobblestone morphology in culture and were further characterized as described in Reindel *et al.* (1991).

BECs or PECs were maintained in Medium 199 (M199; GIBCO, Grand Island, NY, U.S.A.) containing 10% fetal bovine serum (FBS; Intergen, Purchase, NY, U.S.A.) and 1% penicillin (100 units ml⁻¹)-streptomycin (100 µg ml⁻¹)-fungizone (0.25 µg ml⁻¹) (antibiotic-antimycotic; GIBCO) or Opti-MEM (GIBCO) containing 3% FBS and 1% antibiotic-antimycotic, respectively. Cells were passed by enzymatic dissociation with 0.025% trypsin-0.27 mM EDTA (GIBCO) at a ratio of 1:3 or 1:4. Cells used in these studies were between passages 4 and 10.

Preparation of MCTP

MCTP was prepared from MCT (Transworld Chemical, Washington, DC, U.S.A.) via an N-oxide intermediate by the method of Mattocks (1968). MCTP isolated from this synthesis procedure has Ehrlich activity (Mattocks & White, 1971) and a structure compatible with MCTP as determined by mass spectrometry and nuclear magnetic resonance (Brunner *et al.*, 1986). MCTP was dissolved in N,N-dimethylformamide (DMF; Sigma Chemical Co., St. Louis, MO, U.S.A.) at a concentration of 20 mg kg⁻¹, and all dilutions of MCTP were made with DMF. A 2.5 µl volume of MCTP solutions or DMF vehicle (0 µM MCTP) per millilitre of medium was used to achieve the nominal concentrations of MCTP (0, 1.5, 15 or 150 µM) used in the study.

Assay of angiotensin-converting enzyme activity

PECs and BECs were plated into 12-well tissue culture clusters (25 mm diameter) and allowed to form confluent monolayers. Monolayers were not used to assay ACE activity until at least 10 days after plating because ACE activity of cultured endothelial cells is not restored after dissociation and replating until cells have been maintained in a confluent state for this length of time (DelVecchio & Smith, 1981). Mature monolayers were treated with a single administration of MCTP (0, 1.5, 15 or 150 µM) on day 0. ACE activity of monolayers were analysed at 8 h and 2, 4 and 7 days (also at 10 days, for PEC monolayers) post treatment using the standard radioassay protocol provided by Ventrex Laboratories, Inc. (Portland, ME, U.S.A.). The assay involves cleavage of the synthetic peptide [³H]-benzoyl-Phe-Ala-Pro by endothelial ACE to form [³H]-benzoyl-phenylalanine and the dipeptide, alanyl-proline. The tritiated product, soluble in organic scintillant, was extracted, separated and counted in a liquid scintillation counter. At the times indicated post treatment, monolayers were washed thoroughly with ACE assay buffer (0.05 M HEPES, 0.1 M NaCl; pH 7.5, 37°C). At a time 0, 2 ml of buffered substrate ([³H]-benzoyl-Phe-Ala-Pro: specific activity 20 Ci mmol⁻¹, diluted to 10⁵ c.p.m. 100 µl⁻¹ in assay buffer) were added to each well in a shaking water bath at 37°C. At 10 min intervals, 50 µl aliquots of the reaction mixture were removed to each of 2 test tubes containing 1 ml of 0.1 N HCl to stop the reaction; 1 ml of Ventrex scintillation cocktail No. 2 was added to each tube. The tubes were mixed for 30 s, then spun in a centrifuge for 3 min at 500 g. An aliquot (500 µl) of the upper counting phase was transferred to a 2 ml microvial (Ventrex) for measurement of rad-

ioactivity; 50 µl aliquots of the reaction buffer were also counted in aqueous scintillant to determine total c.p.m. per well. Monolayer ACE activity was determined in the presence or absence of the specific ACE inhibitor, captopril (10⁻⁶ M) to ensure that conversion of substrate was due to the action of ACE. In addition, wells without cells were used as a negative control. ACE activity was calculated by a simplified form of the integrated first-order rate equation as derived by Ryan (1988). A unit of ACE activity is defined as that quantity of enzyme required to hydrolyse substrate at an initial rate of 1% min⁻¹ at 37°C.

Effects of MCTP on monolayer cellularity

After determination of ACE activity as described above, monolayers were washed three times with calcium- and magnesium-free Hank's balanced salt solution to remove non-adherent cells. Adherent cells were enzymatically removed from the plate surface with 0.025% trypsin-0.27 mM EDTA solution (GIBCO) and counted in a Coulter counter (Model ZM).

Statistical analysis

ACE activity data are presented as means of 5 (PECs) or 3 (BECs) separate experiments, each consisting of duplicate wells at each concentration of MCTP. The standard error of difference (Steel & Torrie, 1980) for each data set is presented. Data were analysed with a blocked analysis of variance (Crunch Statistical Package, Version 3.12; Crunch Software Corporation, Oakland, CA, U.S.A.). Individual comparisons between treatment groups (MCTP and DMF vehicle) were

Figure 1 Cellularity of PEC (a) and BEC (b) monolayers exposed to a single administration of 1.5 (●), 15 (▽) or 150 (▼) µM monocrotaline pyrrole (MCTP) or N,N-dimethylformamide (DMF) vehicle (○) on day 0. Values represent the means of replicate studies ($n = 5$ for PECs; $n = 3$ for BECs). *Significantly different from vehicle control ($P < 0.05$).

made by Tukey's omega test ($P < 0.05$) (Steel & Torrie, 1980).

Cell numbers are presented as means $\pm$ s.e.mean. These data were analysed by a completely random analysis of variance, and individual comparisons were made by Tukey's omega test ($P < 0.05$).

Results

Effects of MCTP on cellularity

The effects of MCTP on cellularity of PEC and BEC monolayers are shown in Figures 1a and b respectively. Cell monolayers were treated once with MCTP at zero time. Whereas PEC and BEC cell numbers continued to increase slightly with time in wells treated with vehicle or the lowest concentration of MCTP, they declined in monolayers treated with the higher doses of MCTP, beginning at day 4 post treatment. When examined by phase contrast microscopy, all monolayers appeared to remain intact and free of obvious gaps throughout the post treatment period. PECs and BECs treated with higher concentrations of MCTP (15 or 150 μ M) demonstrated distinct morphological changes as monolayer cellularity decreased. These changes have been described previously (Reindel & Roth, 1991; Reindel *et al.*, 1991) and include cell enlargement, vacuole formation and nuclear changes in BECs. A milder enlargement with a shift toward a more spindle-like shape occurred in PECs.

Figure 2 Angiotensin-converting enzyme (ACE) activity of monolayers of PECs (a) and BECs (b) exposed to a single administration of 1.5 (●), 15 (▽) or 150 (▼) μ M monocrotaline pyrrole (MCTP) or N,N-dimethylformamide (DMF) vehicle (○) at time 0. One unit of ACE activity is defined as that quantity of enzyme required to hydrolyze substrate at an initial rate of 1% min^{-1} at 37°C. Values represent the means of replicate studies ($n = 5$ for PECs; $n = 3$ for BECs). Standard error of difference = 3.89 PECs; 7.55 BECs. *Significantly different from vehicle control ($P < 0.05$).

Changes in ACE activity

Changes in ACE activity of mature monolayers of PECs and BECs after a single exposure to MCTP are shown in Figure 2. ACE activity of PEC monolayers (Figure 2a) treated with 150 μ M MCTP decreased gradually, reaching a nadir at 4 days post treatment then returning to control levels by 10 days post treatment. BEC monolayers also showed a concentration-dependent decline in ACE activity with MCTP treatment, but the decrease was much more marked and persistent (Figure 2b). In monolayers treated with the highest concentration of MCTP, ACE activity was significantly decreased by 2 days post treatment, and after 4 days these monolayers demonstrated virtually no ACE activity. In BECs, the intermediate MCTP concentration also led to reduced ACE activity after day 2. In both PECs and BECs, cells treated with 1.5 μ M MCTP exhibited levels of ACE activity similar to those seen in controls.

Cellular ACE activity

Cellular ACE activity after MCTP treatment is expressed in Figure 3 as a function of PEC and BEC number (i.e., units of activity per million cells). Although the total activities of vehicle-treated PEC and BEC monolayers were similar (see Figure 2), activities normalised to cell number were quite different. BECs (Figure 3b) had about twice the ACE activity per million cells than PECs (Figure 3a). The delayed, transient decrease in ACE activity of PECs treated with 150 μ M MCTP was still evident, but cellular ACE activity returned to the control value by 7 days and was increased by 10 days

Figure 3 Angiotensin-converting enzyme (ACE) activity of mature monolayers of PECs (a) and BECs (b) expressed as a function of cell number. Cell monolayers were exposed to a single administration of 1.5 (●), 15 (▽) or 150 (▼) μ M monocrotaline pyrrole (MCTP) or N,N-dimethylformamide (DMF) vehicle (○) at time 0. Values represent the means of replicate studies ($n = 5$ for PECs; $n = 3$ for BECs). Standard error of difference = 2.44 PECs; 9.58 BECs. *Significantly different from vehicle control ($P < 0.05$).

post treatment. Cells treated with 15 μ M MCTP showed no change in activity until day 10, at which time cellular ACE activity was elevated. In BECs, cellular ACE activity was significantly depressed by 4 days post treatment in cells treated with the highest concentration of MCTP. However, decreases in cellular ACE activity were not evident in BECs treated with 15 μ M MCTP. PECs and BECs treated with 1.5 μ M MCTP exhibited similar ACE activity to vehicle-treated controls.

Discussion

A number of conditions which result in pulmonary injury or alterations in pulmonary blood flow have been associated with changes in angiotensin-converting enzyme activity in lung or serum. A rapid, transient decrease in lung ACE and a corresponding increase in serum ACE as is seen following administration of α -naphthylthiourea (ANTU) or paraquat (Hollinger *et al.*, 1980a,b) may reflect direct damage to the vascular endothelium. In other cases, changes in ACE activity appear to be compensatory and occur coincident with or subsequent to the development of pulmonary hypertension (Keane *et al.*, 1982; Jederlinic *et al.*, 1988; Oparil *et al.*, 1988).

The latter scenario has been described for MCT pneumotoxicity in the rat. Kay and coworkers reported that MCT treatment results in vascular remodelling and consequent pulmonary hypertension by 10–14 days post treatment with an accompanying decrease in lung ACE activity per mg protein and decreased conversion of AI to AII beginning by day 10 (Kay *et al.*, 1982; Keane & Kay, 1984). Molteni *et al.* (1984) reported a transient increase in pulmonary ACE activity after 1 week of MCT treatment followed by a persistent decline in activity during weeks 2 to 6 of treatment. Kay and Keane maintained that the alterations in ACE activity seen with MCT treatment occur in response to the pulmonary hypertensive conditions and may be a protective mechanism to limit this physiological change (Kay *et al.*, 1982; Keane *et al.*, 1982). This view is held by Molteni and coworkers as well, but they further suggest that the transient increase in ACE activity which they find in their model precedes vascular remodelling and reflects endothelial injury and dysfunction (Molteni *et al.*, 1984).

In a separate study, Shale *et al.* (1986) found that lung ACE (reported as nmol min⁻¹/lung and nmol min⁻¹ mg⁻¹ protein) decreased when MCT was given in an oral dosing regimen which did not produce pulmonary hypertension or right ventricular hypertrophy. Although this supports the idea that ACE does not play a causative role in the development of these sequelae, it also suggests that the decrease in ACE activity seen with MCT treatment is not solely a response to increased pulmonary vascular pressure. Rather, it suggests an early, direct effect of MCT on the lung. The increased pulmonary tissue mass due to inflammation and hyperplasia after MCT treatment may in fact dilute what is really an unchanged endothelial ACE content at this stage in the injury. This conclusion is in agreement with the findings of Huxtable and co-workers, who maintain that decreases in lung ACE activity in MCT-treated animals can be attributed to dilution by an increased total lung mass (Huxtable *et al.*, 1978; LaFranconi & Huxtable, 1983).

Our objective in performing this study was to determine whether MCTP would directly alter ACE activity in an endothelial cell system *in vitro*. Although we found that MCTP does decrease endothelial ACE activity *in vitro*, our results raise a number of interesting points with respect to changes in ACE activity after cell injury. In both PECs and BECs, decreases in ACE activity did not occur immediately after administration of MCTP; rather, changes were delayed for several days. This suggests that the decrease in ACE activity was not due to a direct interaction of MCTP with the enzyme or to other changes which may occur at the cell

surface at the time of treatment. MCTP is quite reactive and much of it probably binds to cells rapidly after administration *in vitro*. However, the inactivation of ACE does not appear to be an early or immediate change effected by MCTP, even at relatively high concentrations. Ryan & Catravas (1990) suggest that, in addition to enzyme dysfunction, changes in ACE activity may occur as a result of changes in the microenvironment of the enzyme. Some of the delayed, cytotoxic changes which occur as a consequence of exposure to hypoxia or MCTP treatment could result in subtle alterations of the endothelial cell surface which are incompatible with ACE function.

There are clear species differences in the response of pulmonary artery endothelial cells to treatment with MCTP (Reindel *et al.*, 1991). While BECs are quite sensitive to the cytolytic effects of MCTP, PECs appear to be relatively resistant. However, both cell types show distinct changes in morphology after MCTP treatment. The differences noted with respect to ACE activity of BEC and PEC monolayers after MCTP treatment are also pronounced, and these seem to parallel the morphological and cytotoxic changes. For example, the transient nature of the decrease in PECs corresponds with the decline in cell numbers, and the more dramatic and persistent fall in ACE activity in BECs reflects the more marked cytotoxic response of this cell type.

Recent work with ACE inhibitors and AII receptor antagonists suggests that AII may be important in the pathophysiology of the vascular response to injury (Zakheim *et al.*, 1975; Grotendorst *et al.*, 1982; Molteni *et al.*, 1985; Schweiger *et al.*, 1987; Powell *et al.*, 1990; Clozel *et al.*, 1991). Bell & Madri (1990) propose that blockade of ACE activity may decrease local generation of AII and reduce vascular wall injury by enhancing wound closure, increasing the antithrombotic tendency of the area and decreasing the rate of smooth muscle cell infiltration. ACE activity declined substantially in our cells after treatment with MCTP only after there was evidence of cytotoxicity; changes in enzyme activity may have occurred in response to these alterations and might function to limit the local damage. In PECs, in which the cellular damage was less pronounced, the changes in ACE activity were also quite subtle and transient. These cells eventually had increased ACE activity on a cellular basis by 10 days post treatment, which may reflect more persistent alterations in the cells' response to injury.

It is important to point out that the most dramatic changes in ACE activity in both PECs and BECs occurred only at the highest concentrations of MCTP. Whether the pulmonary vascular endothelium experiences such an exposure *in vivo* after a pulmonary hypertension-producing dose of MCT or MCTP is open to question (Reindel *et al.*, 1990). Our results indicate clearly that direct inhibition of endothelial cell ACE does not happen at MCTP exposures that probably occur *in vivo*. It appears likely that changes in ACE activity seen *in vivo* occur indirectly from altered lung mass or through a response of the endothelium to injury (e.g., modified gene expression), altered vascular surface area and/or changes in blood flow or pressure.

In summary, ACE activity in pulmonary artery endothelium is altered after exposure to relatively high concentrations of MCTP *in vitro*, and this alteration is clearly not secondary to hypoxia, increased pressure, or other complicating haemodynamic factors that may be present *in vivo*. The decline in ACE activity is delayed, and the magnitude and duration of the decrease corresponds to the degree of MCTP-induced cytotoxicity. These changes apparently are not due to a direct action of MCTP on the enzyme but may occur as a response to the delayed cell injury which results from exposure to this compound.

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References

- BELL, L. & MADRI, J.A. (1990). Influence of the angiotensin system on endothelial and smooth muscle cell migration. *Am. J. Pathol.*, **137**, 7–12.
- BOOYSE, F.M., SEDLAK, B.J. & RAFELSON, M.E. (1975). Culture of arterial endothelial cells: characterization and growth of bovine aortic cells. *Thromb. Diath. Haemorrh.*, **35**, 825–839.
- BRUNER, L.H., CARPENTER, L.J., HAMLOW, P. & ROTH, R.A. (1986). Effect of a mixed function oxidase inducer and inhibitor on monocrotaline pyrrole pneumotoxicity. *Toxicol. Appl. Pharmacol.*, **85**, 416–427.
- BRUNER, L.H., HILLIKER, K.S. & ROTH, R.A. (1983). Pulmonary hypertension and ECG changes from monocrotaline pyrrole in the rat. *Am. J. Physiol.*, **245**, H300–H306.
- CATRAVAS, J.D., BURCH, S.E., SPURLOCK, B. & MILLS, L.R. (1988). Early effects of ionizing radiation on pulmonary endothelial angiotensin converting enzyme and 5'-nucleotidase, *in vivo*. *Toxicol. Appl. Pharmacol.*, **94**, 342–355.
- CHESNEY, C.F., ALLEN, J.R. & HSU, I.C. (1974). Right ventricular hypertrophy in monocrotaline treated rats. *Exp. Mol. Pathol.*, **20**, 257–268.
- CLOZEL, J.P., HESS, P., MICHAEL, C., SCHIETINGER, K. & BAUMGARTNER, H.R. (1991). Inhibition of converting enzyme and neointima formation after vascular injury in rabbits and guinea pigs. *Hypertension*, **18** (Suppl II), II-55–II-59.
- DELVECCHIO, P.J. & SMITH, J.R. (1981). Expression of angiotensin-converting enzyme activity in cultured pulmonary artery endothelial cells. *J. Cell. Physiol.*, **108**, 337–345.
- DOBULER, K.J., CATRAVAS, J.D. & GILLIS, C.N. (1982). Early detection of oxygen-induced lung injury in conscious rabbits: reduced activity of angiotensin converting enzyme and removal of 5-hydroxytryptamine. *Am. Rev. Respir. Dis.*, **126**, 534–539.
- ERDOS, E.G. (1975). Angiotensin converting enzyme. *Circ. Res.*, **36**, 247–255.
- FAJARDO, L.F. (1989). The complexity of endothelial cells: a review. *Am. J. Clin. Pathol.*, **92**, 241–250.
- GILLIS, C.N. & CATRAVAS, J.D. (1982). Altered removal of vasoactive substances in the injured lung: detection of lung microvascular injury. *Ann. NY Acad. Sci.*, **384**, 458–474.
- GROTENDORST, G.R., CHANG, T., SEPPE, H.E.J., KLEINMAN, H.K. & MARTIN, G.R. (1982). Platelet-derived growth factor is a chemoattractant for vascular smooth muscle cells. *J. Cell. Physiol.*, **113**, 261–266.
- HAYASHI, Y. & LALICH, J.J. (1967). Renal and pulmonary alterations induced in rats by a single injection of monocrotaline. *Proc. Soc. Exp. Biol. Med.*, **124**, 392–396.
- HILLIKER, K.S., BELL, T.G. & ROTH, R.A. (1982). Pneumotoxicity and thrombocytopenia after a single injection of monocrotaline. *Am. J. Physiol.*, **242**, H573–H579.
- HOLLINGER, M.A., GIRI, S.N., PATWELL, S., ZUCKERMAN, J.E., GORIN, A. & PARSONS, G. (1980a). Effect of acute lung injury on angiotensin converting enzyme in serum, lung lavage, and effusate. *Am. Rev. Respir. Dis.*, **121**, 373–376.
- HOLLINGER, M.A., PATWELL, S., ZUCKERMAN, J.E., GORIN, A.B., PARSONS, G. & GIRI, S.N. (1980b). Effect of paraquat on serum angiotensin converting enzyme. *Am. Rev. Respir. Dis.*, **121**, 795–798.
- HUXTABLE, R., CIARAMITARO, D. & EISENSTEIN, D. (1978). The effect of a pyrrolizidine alkaloid, monocrotaline, and a pyrrole, dehydrotetronecine, on the biochemical functions of the pulmonary endothelium. *Mol. Pharmacol.*, **14**, 1189–1203.
- JAFFE, E.A. (1987). Cell biology of endothelial cells. *Hum. Pathol.*, **18**, 234–239.
- JEDERLINIC, P., HILL, N.S., OU, L.C. & FANBURG, B.L. (1988). Lung angiotensin converting enzyme activity in rats with differing susceptibilities to chronic hypoxia. *Thorax*, **43**, 703–707.
- KAY, J.M., KEANE, P.M., SUYAMA, K.L. & GAUTHIER, D. (1982). Angiotensin converting enzyme activity and evolution of pulmonary vascular disease in rats with monocrotaline pulmonary hypertension. *Thorax*, **37**, 88–96.
- KEANE, P.M. & KAY, J.M. (1984). Lung angiotensin converting enzyme activity in monocrotaline pulmonary hypertension (letter). *Thorax*, **39**, 159–160.
- KEANE, P.M., KAY, J.M., SUYAMA, K.L., GAUTHIER, D. & ANDREW, K. (1982). Lung angiotensin converting enzyme activity in rats with pulmonary hypertension. *Thorax*, **37**, 198–204.
- LAFRANCONI, W.M. & HUXTABLE, R.J. (1983). Changes in angiotensin-converting enzyme activity in lungs damaged by the pyrrolizidine alkaloid monocrotaline. *Thorax*, **38**, 307–309.
- MATTOCKS, A.R. (1968). Toxicity of pyrrolizidine alkaloids. *Nature (Lond.)*, **217**, 723–728.
- MATTOCKS, A.R. & WHITE, I.N.H. (1971). The conversion of pyrrolizidine alkaloids to N-oxides and to dihydropyrrolizidine derivatives by rat liver microsomes *in vitro*. *Chem. Biol. Interact.*, **3**, 383–396.
- MEYRICK, B. & REID, L. (1979). Development of pulmonary arterial changes in rats fed *Crotalaria spectabilis*. *Am. J. Pathol.*, **94**, 37–50.
- MOLTENI, A., WARD, W.F., TS'AO, C.H., PORT, C.D. & SOLLIDAY, N.H. (1984). Monocrotaline-induced pulmonary endothelial dysfunction in rats. *Proc. Soc. Exp. Biol. Med.*, **176**, 88–94.
- MOLTENI, A., WARD, W.F., TS'AO, C.H., SOLLIDAY, N.H. & DUNNE, M. (1985). Monocrotaline-induced pulmonary fibrosis in rats: amelioration by captopril and penicillamine (42151). *Proc. Soc. Exp. Biol. Med.*, **180**, 112–120.
- OPARIL, S., NARKATES, A.J., JACKSON, R. & ANN, H.S. (1988). Altered angiotensin-converting enzyme in lung and extrapulmonary tissues of hypoxia-adapted rats. *J. Appl. Physiol.*, **65**, 218–227.
- PECKHAM, J.C., SANGSTER, L.T. & JONES, O.H. JR (1974). *Crotalaria spectabilis* poisoning in swine. *J. Am. Vet. Med. Assoc.*, **165**, 633–638.
- POWELL, J.S., MULLER, R.K.M., ROUGE, M., KUHN, H., HEFTI, F. & BAUMGARTNER, H.R. (1990). The proliferative response to vascular injury is suppressed by angiotensin-converting enzyme inhibition. *J. Cardiovasc. Pharmacol.*, **16** (Suppl. 4), S42–S49.
- REINDEL, J.F., GANEY, P.E., WAGNER, J.G., SLOCOMBE, R.F. & ROTH, R.A. (1990). Development of morphologic, hemodynamic, and biochemical changes in lungs of rats given monocrotaline pyrrole. *Toxicol. Appl. Pharmacol.*, **106**, 179–200.
- REINDEL, J.F., HOORN, C.M., WAGNER, J.G. & ROTH, R.A. (1991). Comparison of the responses of bovine and porcine pulmonary artery endothelial cells to monocrotaline pyrrole. *Am. J. Physiol. (Lung Cell. Mol. Physiol. 5)*, **261**, L406–L414.
- REINDEL, J.F. & ROTH, R.A. (1991). The effects of monocrotaline pyrrole on cultured pulmonary artery endothelial and smooth muscle cells. *Am. J. Pathol.*, **138**, 707–719.
- ROTH, R.A. & REINDEL, J.F. (1990). Lung vascular injury from monocrotaline pyrrole, a putative hepatic metabolite. In *Biological Reactive Intermediates*, IV. ed. Witmer, C.M., Snyder, R.R. & Jollow, D. pp. 477–487. New York, NY: Plenum Press.
- RYAN, J.W. (1988). Angiotensin-converting enzyme, dipeptidyl carboxypeptidase I, and its inhibitors. In *Methods in Enzymology*, Vol. 163. ed. diSabato, G. pp. 194–210. New York, NY: Academic Press.
- RYAN, J.W. & CATRAVAS, J.D. (1990). Angiotensin converting enzyme as an indicator of pulmonary microvascular function. In *Focus on Pulmonary Pharmacology and Toxicology*. ed. Hollinger, M.A. pp. 183–210. Boca Raton, FL: CRC Press, Inc.
- SCHOENTAL, R. & HEAD, M.A. (1955). Pathological changes in rats as a result of treatment with monocrotaline. *Br. J. Cancer*, **9**, 229–237.
- SCHWEIGERER, L., NEUFELD, G., FRIEDMAN, J., ABRAHAM, J.A., FIDDES, J.C. & GOSPODAROWICZ, D. (1987). Capillary endothelial cells express basic fibroblast growth factor, a mitogen that promotes their own growth. *Nature (Lond.)*, **325**, 257–259.
- SHALE, D.J., WISEMAN, M.S. & COOKSON, W.O.C.M. (1986). Effect of monocrotaline ingestion on the distribution of protein and angiotensin converting enzyme activity in the rat lung. *Thorax*, **41**, 914–918.
- STEEL, R.G.D. & TORRIE, J.H. (1980). *Principles and Procedures of Statistics: A Biometrical Approach*. p. 381. New York: McGraw-Hill, Inc.

- SUGITA, T., HYERS, T.M., DAUBER, I.M., WAGNER, W.W., MCMURTRY, I.F. & REEVES, J.T. (1983). Lung vessel leak precedes right ventricular hypertrophy in monocrotaline-treated rats. *J. Appl. Physiol.*, **54**, 371.
- VALDIVIA, E., LALICH, J.J., HAYASHI, H. & SONNAD, J. (1967). Alterations in pulmonary alveoli after a single injection of monocrotaline. *Arch. Pathol.*, **84**, 64–76.
- YANG, H.Y.T., ERDOS, E.G. & LEVIN, Y. (1970). A dipeptidyl carboxypeptidase that converts angiotensin I and inactivates bradykinin. *Biochem. Biophys. Acta*, **214**, 374–376.
- ZAKHEIM, R.M., MATTIOLI, L., MOLTENI, A., MULLIS, K.B. & BARTLEY, J. (1975). Prevention of pulmonary vascular changes of chronic alveolar hypoxia by inhibition of angiotensin-1-converting enzyme in the rat. *Lab. Invest.*, **33**, 57–61.

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