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Alteration of fate of vasoactive autacoids in pulmonary circulation following monocrotaline-induced lung vascular injury in rats

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1 To learn how pulmonary vascular injury alters the ability of the lung to metabolize vasoactive autacoids, lung vascular lesions were produced in rats by a single subcutaneous injection of monocrotaline (90 mg kg^{-1}), and the blood pressure responses to angiotensin I (AI), angiotensin II (AII), bradykinin, prostaglandin E_2 (PGE_2) and substance P were examined. Vasoactive agents were given intravenously or intra-arterially.

2 On histological examination of the lung at 3 weeks after monocrotaline treatment, degeneration or necrotization of endothelial cells was evident.

3 The conversion of AI to AII was only slightly depressed by monocrotaline treatment. On the other hand, the depressor response to intravenously injected bradykinin was enhanced in monocrotaline-treated rats. When the rats were pretreated with indomethacin the depressor response to intravenous bradykinin was the same for both control and monocrotaline-treated groups which suggests that endogenous prostaglandins are involved in the enhancement of the response to bradykinin.

4 In monocrotaline-treated rats the depressor response to intravenous PGE_2 was significantly enhanced depending on the period following the treatment, while that to the intra-arterial injection did not differ from control.

5 The data suggest that monocrotaline-induced lung injury impairs the metabolism of PGE_2 during pulmonary circulation but has little effect on the conversion of AI to AII and the degradation of bradykinin in rats.

Introduction

The significance of the lung as a metabolic organ for endogenous active substances has become widely recognized during the last two decades (Vane 1969, Bakhle & Vane, 1974; Said, 1982). Pulmonary circulation can efficiently inactivate most of the 5-hydroxytryptamine, bradykinin, eicosanoids except prostacyclin, and other substances released into venous blood, while it converts angiotensin I (AI) to active angiotensin II (AII) (Said, 1982). Therefore the lung has an important role in the prevention of the systemic effects of many substances related to inflammation that have vasodepressor activity.

If pulmonary diseases impair the metabolic function of the lung, the altered fate of active substances may influence systemic haemodynamics and fluid-electrolyte balance. Thus pulmonary diseases may cause secondary effects through these endogenous substances in the whole body. However, little is known about the effect of lung injuries on the metabolism of autacoids in pulmonary circulation.

A pyrrolizidine alkaloid, monocrotaline, has a potent pulmonary toxicity causing endothelial damage and medial hypertrophy (Hayashi & Lalich, 1967; Meyrick *et al.*, 1980; Ghodsi & Will, 1981; Hilliker *et al.*, 1982; Molteni *et al.*, 1984). For this reason this substance has been used to induce experimental lung injury or pulmonary hypertension (Meyrick *et al.*, 1980; Hilliker *et al.*, 1982; Ghodsi & Will, 1981). In this and the following paper we aimed to clarify how pulmonary vascular lesions alter the metabolic function of rat lung. In this paper we evaluated the metabolic functions of the lung from the blood pressure response to vasoactive substances

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Table 1 Changes in body weight and the ratio of lung weight to body weight of rats after a subcutaneous injection of saline (control) or monocrotaline (90 mg kg^{-1})

Time after injection	*Body weight (g)*		*Lung/body weight (× 1000)*	
	Control	*Monocrotaline*	*Control*	*Monocrotaline*
1 week	367 ± 8	320 ± 5*	4.07 ± 0.14	4.75 ± 0.18*
3 weeks	389 ± 9	335 ± 6*	4.40 ± 0.14	6.30 ± 0.24*
5 weeks	447 ± 13	341 ± 18*	3.40 ± 0.19	8.34 ± 0.43*

* Significantly different from control ($P < 0.05$) $n = 8$–10.

entering the pulmonary circulation, and examined the effects of monocrotaline-treatment on these functions.

Methods

Animals

Healthy male Wistar rats were given single subcutaneous injections of monocrotaline 90 mg kg^{-1} in 0.9% saline solution or the same volume of vehicle at the age of 9 weeks. The rats were fed on normal chow and water *ad libitum* for 1 to 5 weeks. The body weights were checked throughout the experimental periods and the lung weights were measured after the blood pressure experiments. In different groups of rats the weight ratio of right ventricle of the heart to left ventricle plus septum was measured at 3 weeks after monocrotaline treatment.

Histological examination

Three weeks after the injection of monocrotaline or saline, rats were killed by a blow on the neck and bled. The lungs were excised and immersed in formalin. Paraffin sections of 4 μm thickness from the middle region of the left and right lungs were stained with haematoxylin and eosin, and were examined under light microscopy.

Blood pressure experiments

One, 3 or 5 weeks after the monocrotaline-treatment, rats were anaesthetized with pentobarbitone sodium (60 mg kg^{-1}, i.p.) and the trachea was cannulated. The arterial pressure was monitored via a cannula positioned in the left femoral artery and connected to a blood pressure transducer (Nihon Kohden MPU-0.5). A cannula for intra-arterial injection was inserted into the aortic arch from the right carotid artery, and another cannula was inserted for intravenous injection into the inferior vena cava from the left femoral vein. The tubing for injection had an internal volume of 0.3 ml. Each drug, dissolved in a volume of 0.05 ml per 100 g body weight, was at first put in the tubing and then flushed into the circulation by additional 0.4 ml saline in 2 s so that the whole amount of the drug went into the body in 2 s.

The metabolism of autacoids was evaluated from the difference in responses to intravenous injection and intra-arterial injection of each substance as described by Armstrong *et al.* (1978). The conversion of AI to AII was evaluated from the ratio of the responses to AI and AII administered intravenously.

Drugs

Drugs used were: AI, AII, bradykinin, substance P from Peptide Research Foundation; PGE_2 from Funakoshi Pharmaceuticals; indomethacin from Sigma; captopril from Sankyo and monocrotaline from Wako Pure Chemicals. AI, AII, bradykinin, captopril, substance P and monocrotaline were dissolved in 0.9% saline solution. PGE_2 and indomethacin were dissolved in ethanol in a concentration of 1 mg ml^{-1} and 100 mg per ml, respectively, and then diluted in saline.

Statistics

Data are expressed as mean ± s.e. Statistical difference was evaluated by Student's t-test. Significance was considered at the level of $P < 0.05$.

Results

Toxicity of monocrotaline to lungs

Following monocrotaline injection the body weight virtually stopped increasing, and therefore the body weights of monocrotaline-treated rats were significantly lower than those of control rats. By contrast, the lung weights were heavier in the treated rats so that the ratio of lung weight to body weight was significantly higher in monocrotaline-treated rats than control rats (Table 1). Three weeks after monocrotaline injection the weight of the right ventricle and that of the left ventricle plus septum were measured. The ratio of right ventricle to left ventricle plus septum was 0.338 ± 0.029 ($n = 11$) in monocrotaline-treated rats, which was significantly higher than the ratio in control rats (0.246 ± 0.014,

$n = 11$, $P < 0.02$) suggesting a rise in pulmonary arterial pressure. Therefore the monocrotaline-induced toxic effects observed in the present study appear to be similar to those described in other papers (Huxtable *et al.*, 1978; Ghodsi & Will, 1981; Hilliker *et al.*, 1982; Altiere, *et al.*, 1986). Four or five weeks after monocrotaline injection more than half of the treated animals died.

On microscopic examination of the lungs excised at 3 weeks after monocrotaline treatment, the tunica intima of pulmonary arteries and arterioles showed necrosis, cytoplasmic vacuolation and nuclear swelling of the endothelial cells. Oedema and lymphocytic infiltration in the subendothelial space was also observed. Some endothelial cells fell off the intimal layer. In addition, the tunica media exhibited atrophy of smooth muscle cells, interstitial oedema and lymphocytic infiltration. Similar lesions were also observed in pulmonary veins. From these results a single subcutaneous injection of monocrotaline ($90\,mg\,kg^{-1}$) proved to be satisfactory for producing severe lung vascular injury, as reported previously (Ghodsi & Will, 1981; Hilliker *et al.*, 1982; Kay *et al.*, 1982; Molteni *et al.*, 1984).

Pressor responses to angiotensins

Mean systemic arterial pressure under anaesthesia with pentobarbitone was 110 ± 3 mmHg ($n = 24$) in monocrotaline-treated rats compared with 123 ± 3 mmHg ($n = 24$) in control rats ($P < 0.05$), at 3 weeks after treatment. AI and AII were injected alternately via the intravenous route at equivalent doses on a molar basis. Injections were repeated at an interval of 15 min from the lowest dose to the maximum dose. Figure 1 shows the dose-pressor response relationship for AI and AII in control (a) and monocrotaline-treated (b) rats at 3 weeks after the treatment. AII at each dose induced the same magnitude of response in control and monocrotaline-treated rats. AI-induced responses, which were inhibited by captopril ($0.1\,mg\,kg^{-1}$, i.v.) by more than 85%, were the same in both groups. When the response to AI was compared with that to AII in each group, the response to AI was significantly smaller at a dose of $0.1\,\mu g\,kg^{-1}$ in control, and at the dose of 0.03 and $0.1\,\mu g\,kg^{-1}$ in monocrotaline-treated group. Therefore it is possible to say that the conversion of AI to AII at a low dose was slightly depressed in the monocrotaline-treated group. However, the ratio of conversion of AI to AII, which was calculated from the responses to AI and AII at the overall dose range, was $82.4 \pm 12.0\%$ in control rats and was not significantly different from that in monocrotaline-treated rats ($70.9 \pm 9.4\%$). Similar results were obtained at 1 or 5 weeks after the treatment (Figure 5). Thus, we can

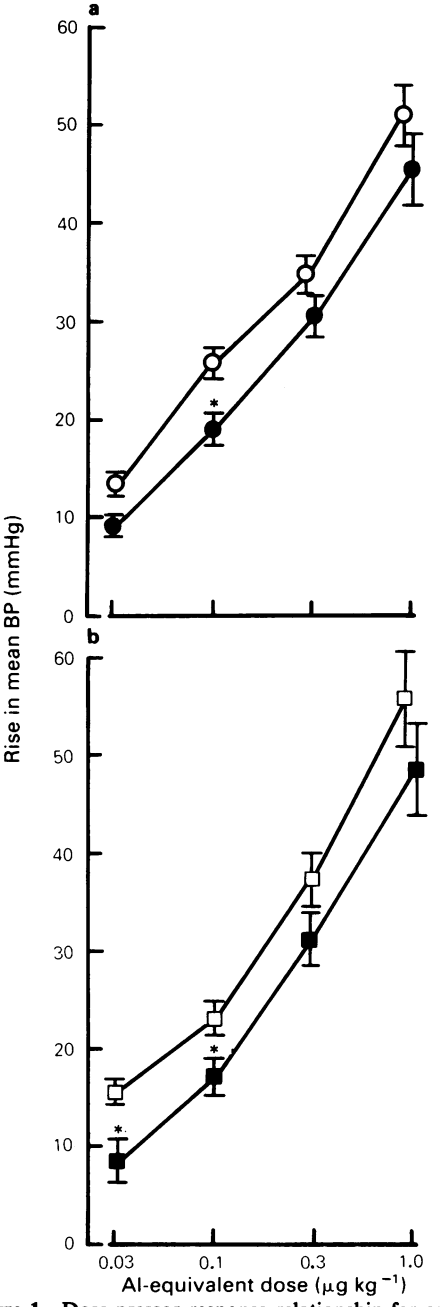

Figure 1 Dose-pressor response relationship for angiotensin I (AI) and angiotensin II (AII) in rats at 3 weeks after an injection of saline (Control: a) and monocrotaline (b). AI and AII were given alternately via intravenous route at an interval of 15 min from the lowest dose. In (a) (●) indicates AI and (○) AII; in (b) (■) for AI and (□) for AII. Each point represents a mean of the peak response to each substance with s.e. indicated by vertical bars. $n = 7$. $^*P < 0.05$ (vs. AII).

conclude that monocrotaline-induced pulmonary injury had little effect on the conversion of AI to AII.

Depressor response to bradykinin

In the control group, bradykinin at 0.1 $\mu g kg^{-1}$ or more given in the aortic arch induced a transient decrease of blood pressure while more than 1 $\mu g kg^{-1}$ was required to induce the depressor response when injected intravenously (Figure 2). Dose-response curves for intravenous and intra-arterial injections were parallel. Captopril (0.1 $mg kg^{-1}$, i.v.) significantly augmented the amplitude and prolonged the duration of the response to bradykinin via both routes. However, the enhancement by captopril was greater for the response caused by intravenous injection than that by intra-arterial injection; that is, captopril decreased the dose of intravenous bradykinin needed to induce a depressor response of 40 mmHg from 10 $\mu g kg^{-1}$ to 0.3 $\mu g kg^{-1}$ and that of intra-arterial bradykinin from 1 $\mu g kg^{-1}$ to 0.2 $\mu g kg^{-1}$. As a result, after captopril treatment the response to intravenous bradykinin was not significantly different from that to intra-arterial bradykinin (data not shown). These observations suggest that most of the intravenously injected bradykinin was inactivated by kininase II (angiotensin converting enzyme) during passage through the pulmonary circulation.

Figure 2 also shows the responses to bradykinin in rats at 3 weeks after treatment with monocrotaline. The depressor responses to intra-arterial bradykinin were slightly depressed in monocrotaline-treated rats. In contrast, the responses to intravenous bradykinin were enhanced in the treated rats at doses of 1 and 3 $\mu g kg^{-1}$. One possible explanation of the latter change is an inhibition of the degradation of bradykinin during pulmonary circulation after monocrotaline-induced lung injury. However, this is unlikely because the data on angiotensin effects showed that angiotensin converting enzyme (kininase II) activity was little affected even by monocrotaline treatment (Figure 1; Huxtable *et al.*, 1978). Bradykinin is known to increase the products of arachidonate cascade by stimulating phospholipase A_2 (Juan, 1977). Therefore endogenous eicosanoids might be involved in the enhanced response to intravenous bradykinin. To test this possibility the response to bradykinin was examined after inhibition of cyclo-oxygenase by indomethacin. Figure 3a shows the responses to bradykinin in rats pretreated with indomethacin (2.5 $mg kg^{-1}$, i.v.). Indomethacin pretreatment reduced the response to bradykinin, especially the response to intravenous bradykinin in monocrotaline-treated rats. After pretreatment with indomethacin the response to intravenous bradykinin in monocrotaline-treated rats was the same as that in control rats while the response to intra-arterial bradykinin was still smaller in monocrotaline-treated rats than in control rats. Figure 3b shows the indomethacin-sensitive component in the blood pressure response. This component was greater in the monocrotaline-treated group than in the control group when bradykinin was given intravenously while the component was the same between the two groups when bradykinin was given into the aortic arch.

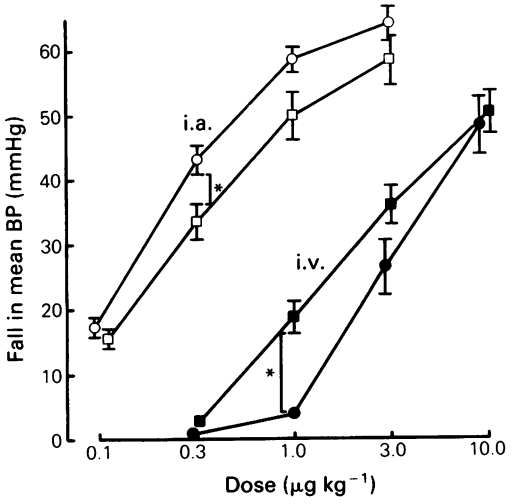

Figure 2 Dose-depressor response relationship for bradykinin in rats at 3 weeks after an injection of saline (Control; circles) and monocrotaline (squares). Bradykinin was given alternately via intravenous (i.v.; filled symbols) and intra-arterial (i.a; open symbols) routes. Each point represents a mean of the peak response with s.e. shown by vertical bars. $n = 8$. $^*P < 0.05$ (vs. control).

Depressor response to prostaglandin E_2

Figure 4a shows the blood pressure response to PGE_2 given intra-arterially or intravenously in control and monocrotaline-treated rats. In control rats the threshold dose of intravenous PGE_2 to decrease the blood pressure was 3 $\mu g kg^{-1}$, while that of intra-arterial PGE_2 was less than 0.3 $\mu g kg^{-1}$. Furthermore the response to PGE_2 via the intravenous route was significantly smaller than that via the intra-arterial route over the entire dose-range. When monocrotaline-treated rats received PGE_2 intravenously, the threshold dose decreased to 0.3 $\mu g kg^{-1}$ and the response was enhanced at each dose, compared to control rats. However, the response to PGE_2 given intra-arterially was not modified by monocrotaline treatment. Figure 4b shows the ratio of the effect of intravenous PGE_2 to

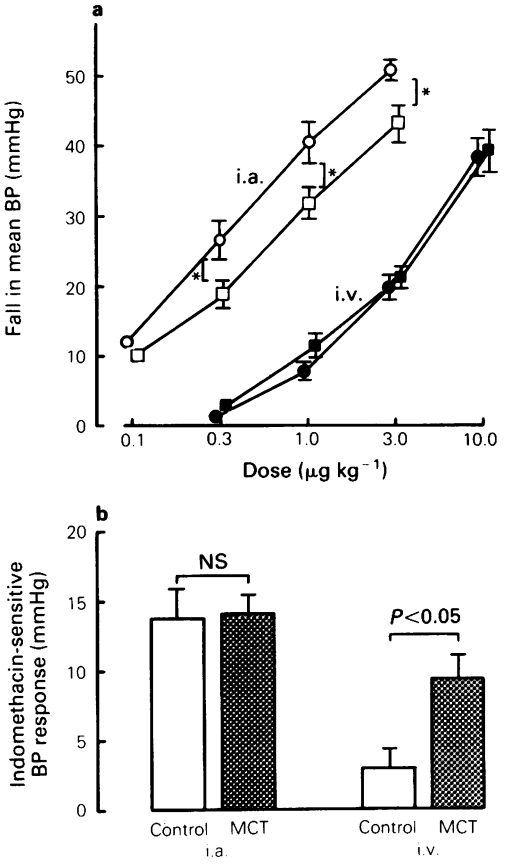

Figure 3 (a) Depressor response to bradykinin after treatment with indomethacin (2.5 mg kg^{-1}, i.v.) in rats at 3 weeks after an injection of saline (control; circles) and monocrotaline (squares). Ten min after indomethacin, bradykinin was given alternately via intravenous (i.v.; filled symbols) and intra-arterial (i.a.; open symbols) routes. Each point represents the mean of peak response with s.e. shown by vertical bar. $n = 8$. *$P < 0.05$ (vs. control). (b) Indomethacin-sensitive component of depressor response to bradykinin. This component was calculated by subtracting the magnitude of response to bradykinin (3 μg kg^{-1}, i.v. or i.a.) after indomethacin-treatment from that before indomethacin. Notice that the responses before indomethacin are not shown in (a). MCT, monocrotaline-treated; i.v., intravenous injections; i.a., intra-arterial injection. NS, not significant.

that of intra-arterial PGE_2 in each group. The data suggest that the degradation of PGE_2 during pulmonary circulation was depressed by monocrotaline-induced injury. The enhancement of the response to intravenously injected PGE_2 progressed with time after monocrotaline-treatment as shown in Figure 5.

Depressor response to substance P

Substance P has been reported not to be inactivated during passage through lungs (Bakhle & Vane, 1974). Figure 6 shows the blood pressure change due to intravenous and intra-arterial substance P in control and monocrotaline-treated rats. The response to intravenous substance P did not differ from that to intra-arterial substance P in control rats. Monocrotaline-treatment did not modify the response to substance P given either intravenously or intra-arterially.

Discussion

In this study we evaluated the metabolic function of the lung from the difference in blood pressure

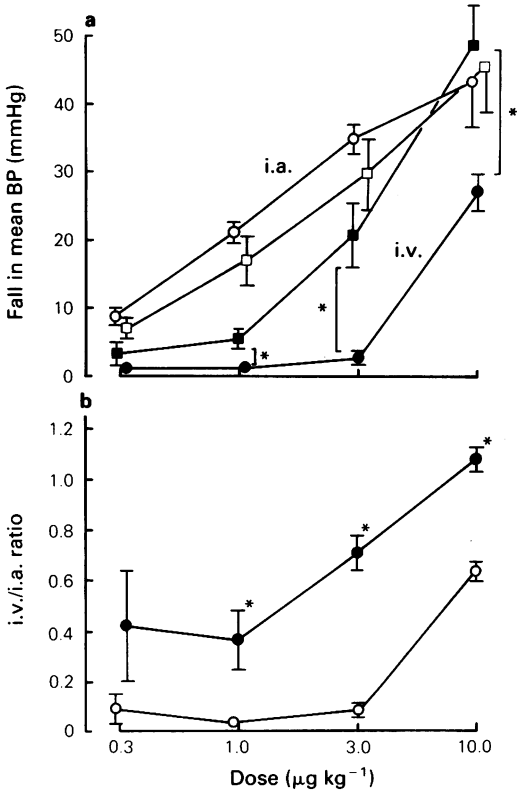

Figure 4 (a) Depressor response to prostaglandin E_2 (PGE_2) in rats at 3 weeks after an injection of saline (control; circles) and monocrotaline (squares). PGE_2 was given alternately via intravenous (i.v.; closed symbols) and intra-arterial (i.a.; open symbols) routes. Each point represents the mean of peak response with s.e. shown by vertical bars. $n = 8$. *$P < 0.05$ (vs. control). (b) Ratio of responses to intravenous PGE_2 to those to intra-arterial PGE_2; (○) control; (●) monocrotaline. *$P < 0.05$ (vs. control).

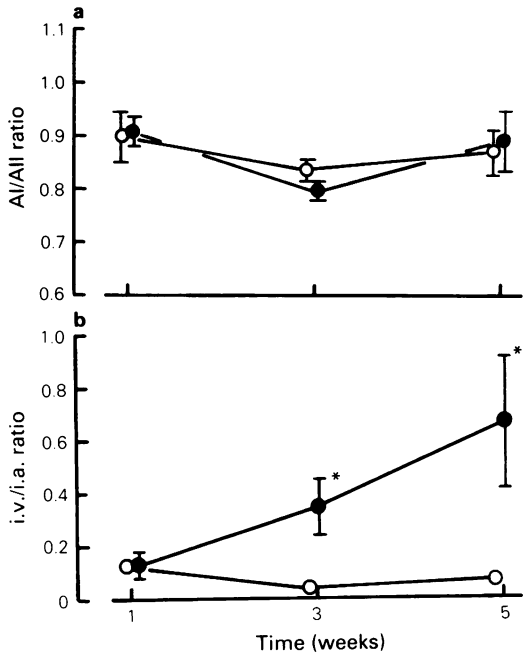

Figure 5 (a) Changes in the conversion of angiotensin I (AI) to AII as evaluated from the pressor responses to AI and AII at 1, 3 and 5 weeks after an injection of saline (control; ○) or monocrotaline (●). The ratio was calculated from the pressor effects of AI and AII at AI-equivalent doses of 0.3 and 1.0 $\mu g\,kg^{-1}$. (b) Changes in the ratio of the depressor effect of intravenous prostaglandin E_2 (PGE_2) to that of intra-arterial PGE_2 at 1, 3 and 5 weeks after an injection of saline (○) or monocrotaline (●). The ratio was calculated from the response to the doses of 1.0 and 3.0 $\mu g\,kg^{-1}$ PGE_2. *$P < 0.05$ (vs. control).

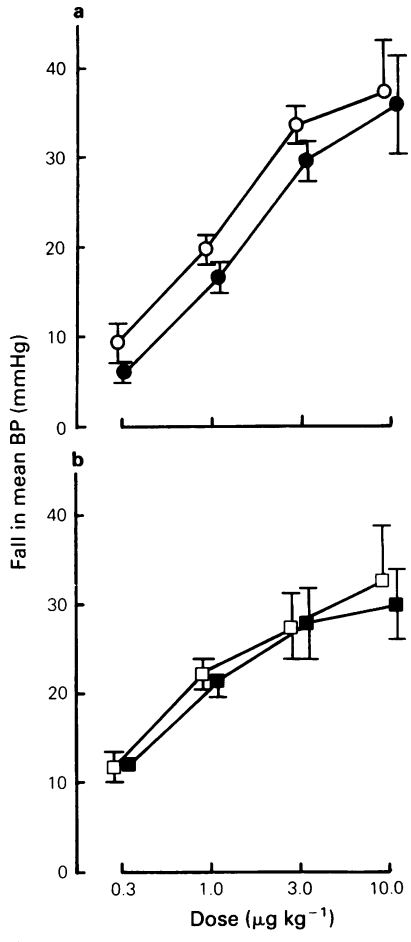

Figure 6 Depressor response to substance P in rats at 3 weeks after an injection of saline (a) and monocrotaline (b). Substance P was given alternately via intravenous (closed symbols) and intra-arterial (open symbols) routes. Each point represents the mean of peak responses with s.e. shown by vertical bar. $n = 6$.

responses to vasoactive substances given before and after pulmonary circulation. This provides an approximate estimate compared with the chemical determinations of metabolites. However, we think that the present data provide useful information about the influence of the lung on the effects of autacoids on systemic haemodynamics. Substance P, which is considered to be resistant to the metabolism in lungs (Bakhle & Vane, 1974), produced identical responses whether it was injected intravenously or intra-arterially. This suggests that the difference in the rate of distribution of drugs via intravenous and intra-arterial routes does not result in a large difference in the responses of the blood pressure.

In normal rats the intravenous injection of PGE_2 scarcely caused a reduction of blood pressure at a dose below 3 $\mu g\,kg^{-1}$ while the intra-arterial injection caused a considerable decrease even at 0.3 $\mu g\,kg^{-1}$. This means that almost all the PGE_2 injected intravenously at a dose below 3 $\mu g\,kg^{-1}$ was destroyed during a transit through the normal lung. In contrast, in monocrotaline-treated rats the depressor response to intravenous injection of PGE_2 was significantly enhanced whereas that to the intra-arterial injection was not affected by the treatment. Therefore it can be said that the pulmonary injury caused by monocrotaline resulted in decreased degradation of PGE_2 during pulmonary circulation. Similarly a decrease of the inactivation of PGE_2 was reported when lungs were injured by α-naphtyl thiourea (Bakhle, 1982), bleomycin (Chandler & Giri, 1983; Chandler *et al.*, 1985) or oxygen (Klein *et al.*, 1978; Toivonen *et al.*, 1981). Therefore it is possible

that the decreased degradation of PGE_2 is not specific for monocrotaline but common to many types of pulmonary injuries.

In spite of the severe lesions of pulmonary endothelium after monocrotaline treatment, the ratio of the pressor response to AI compared with that to AII was virtually the same as that in control rats, suggesting that the conversion of AI to AII was little impaired by monocrotaline. On the other hand, the response to intravenous bradykinin was significantly enhanced in monocrotaline-treated rats. These data might at first sight appear contradictory since the same enzyme is responsible for both the conversion of AI to AII and the degradation of bradykinin. After treatment with indomethacin, however, the response to intravenous bradykinin was the same between two groups and consequently the indomethacin-sensitive component of blood pressure response (i.v.) was greater in monocrotaline-treated rats than in control rats. Since the indomethacin-sensitive component probably represents the one involving endogenous prostaglandins, an increase of this component after monocrotaline-treatment suggests that the effect of prostaglandins formed as a result of stimulation of phospholipase A_2 by bradykinin was enhanced in monocrotaline-treated rats because of reduced degradation of prostaglandins during pulmonary circulation. This explanation agrees with the data of the enhanced response to PGE_2 in monocrotaline-treated rats. Lung can produce various kinds of metabolites from arachidonic acid which affect haemodynamics (Gryglewski *et al.*, 1978; Reeves *et al.*, 1985; Sirois *et al.*, 1985). Further studies are required to clarify whether the pulmonary injury induced by monocrotaline alters the amount of eicosanoids produced in lungs.

In view of the results suggesting the impairment of prostaglandin metabolism in the lung, it is surprising that the conversion of AI to AII and the inactivation of bradykinin appear unaltered after injury of pulmonary endothelium because the enzyme responsible for the metabolism of these substances is believed to be located on the surface of pulmonary endothelium (Caldwell *et al.*, 1976; Ryan *et al.*, 1976). Several groups investigated the change of pulmonary converting enzyme activity after lung injury induced by monocrotaline (Huxtable *et al.*, 1978; Kay *et al.*, 1982; Molteni *et al.*, 1984) or bleomycin (Lazlo *et al.*, 1981, 1986). However, the data are inconsistent since Kay *et al.* (1982) and Lazo *et al.* (1981, 1986) found a decrease of converting enzyme activity in calf, rabbit and rat lungs, while Molteni *et al.* (1984) reported an initial increase of pulmonary enzyme activity in rats followed by a sustained decrease and Huxtable *et al.* (1978) did not detect any change in the enzyme activity in perfused lungs from monocrotaline-treated rats. The inconsistency among these reports may be related to the difference of experimental conditions, i.e., animal species tested, the dose of the toxic substance, the route of administration, the period of observation, etc. One difference between previous studies and our study is that we measured the conversion of AI to AII in intact animals but other investigators measured the enzyme activity in serum or isolated tissues.

One possible reason for this inconsistency is that if converting enzyme exists abundantly in pulmonary endothelium, the enzyme activity present in surviving endothelium after monocrotaline treatment may be sufficient to metabolize the substrates at physiological concentrations. A second possible explanation is the different routes of metabolism for prostaglandins and peptides. AI and bradykinin are metabolized on the surface of cells by ectoenzymes (Caldwell *et al.*, 1976; Ryan *et al.*, 1976). In the case of PGE_2, it must be taken up into cells to be metabolized by an energy-dependent process (Anderson & Eling, 1976; Bito *et al.*, 1977). It is possible that this uptake process is more susceptible to monocrotaline toxicity than the enzymatic activity. A third possibility is that the site of metabolism of PGE_2 may be different from that of AI. It is generally accepted that most prostaglandins are degraded in lungs. However, it still remains to be determined which cells in lungs are responsible for the inactivation. The papers by Ody *et al.* (1979) and Ali *et al.* (1980) suggested that cultured pulmonary endothelial cells do not metabolize some prostaglandins. Alternatively, angiotensin converting enzyme may be present in other sites than vascular endothelium. In the accompanying paper we tested these possibilities using isolated pulmonary artery strips (Ito *et al.*, 1988).

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