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PULMONARY ASCORBIC ACID LOSS INDUCED BY CATECHOLAMINES

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Abstract—Phenylephrine, norepinephrine, epinephrine or isoprenaline (5 μmoles/kg) was administered to anesthetized mice. All of these treatments, except isoprenaline, caused a loss of about 35 per cent of the ascorbic acid content of lung tissue and an increase of about 20 per cent in lung weight within 15 min. Increases in lung weight were interpreted as being due to the development of pulmonary edema. In some experiments, either phenoxybenzamine or propranolol was given prior to catecholamine administration. These experiments showed that both the loss of lung ascorbic acid and the development of pulmonary edema depended on α-receptor activity and was potentiated by β-receptor activity. The results suggest an association between the loss of lung ascorbate and the development of catecholamine-induced pulmonary edema in mice.

IN PREVIOUS experiments,[1] we have shown that both hyperbaric stress and administration of a large dose of norepinephrine can decrease the content of ascorbic acid in rat lung. Also, a prior dose of reserpine could prevent the ascorbate loss caused by hyperbaric stress. These results suggested that part of the ascorbate store in lung was under sympathetic control. The reason for the disappearance of pulmonary ascorbic acid is not known, but may be explained by an alteration in ascorbic acid transport or metabolism following stimulation of α- or β-receptors.

This paper presents changes in the level of ascorbic acid in mouse lung when several adrenergic agonists have been given alone, or in the presence of α- or β-blocking agents. Since large doses of epinephrine are known to cause pulmonary edema in several mammalian species[2,3] and since edema might lower the ascorbic acid content per wet weight of lung, changes in lung weight are also examined in the present study.

METHODS

Animals. Male mice 20–30 g (Queensland University Central Animal Breeding House strain) were used in all experiments, and were fed a commercial breeding ration.

Drug administration and dosage. After anesthesia with pentobarbitone (282 μmoles/kg, intraperitoneally), mice were given saline (0·9%, w/v), phenoxybenzamine (10 μmoles/kg) or propranolol (12 μmoles/kg) via the tail vein. They received a second intravenous injection of saline, phenylephrine, norepinephrine, epinephrine or isoprenaline (all 5 μmoles/kg) 10 min after the first injection. All injections had a volume of 2 ml/kg and were injected over a period of 5 sec. Fifteen min after the second injection, mice were exsanguinated by cutting the vena cava. This time was selected because preliminary experiments has shown that maximum changes in ascorbic acid

concentrations had occurred by then. One-sixth of the mice receiving epinephrine died within 15 min of its injection and were not used in the present study. No other treatments were lethal.

Extraction and chemical assay. Immediately after death, lungs were removed, extracted with cold metaphosphoric acid (5% w/v), and assayed for reduced ascorbic acid using the 2,4-dinitro-phenylhydrazine procedure of Roe and Kuether,[4] with the modification described by Bolin and Book.[5]

Lung weight ratios and statistics. Since there was a linear correlation between the lung to body weight ratio and the body weight of mice injected with saline (correlation coefficient, $-0{\cdot}58$; $P < 0{\cdot}001$), substitution into this equation of body weights of mice receiving drugs (lung to body weight ratio $= 1{\cdot}37 \times 10^{-4} \times$ body weight $+ 96{\cdot}6 \times 10^{-4}$) allowed prediction of the lung weights which would have been obtained had mice of similar weight received saline. The ratio of the observed lung weight to the lung weight predicted by the equation is called the lung weight ratio and is a measure of the change in lung weight due to drug administration. A lung ascorbic acid value, which was corrected for any change in lung weight, was calculated as the product of the ascorbic acid concentration and the lung weight ratio.

Statistical significance of data was tested with Student's *t*-test; standard errors were calculated from the error variance of the analyses of variance.

RESULTS

Lungs removed from mice treated with epinephrine had a translucent and engorged appearance. Norepinephrine produced similar but less severe changes. Mice receiving treatments other than epinephrine or norepinephrine had no obvious lung damage.

Compared to the saline treatment, administration of phenylephrine, norepinephrine or epinephrine decreased the lung ascorbic acid by 26, 33 and 40 per cent respectively (Table 1). In each case, the loss of ascorbic acid was prevented by a prior dose of phenoxybenzamine, while propranolol reduced but did not prevent the loss. Isoprenaline, phenoxybenzamine or propranolol produced no significant change in lung ascorbic acid.

Phenylephrine, norepinephrine or epinephrine increased the lung weight by 12, 27 and 48 per cent respectively (Table 2). Phenoxybenzamine prevented these increases in lung weight. Propranolol reduced but did not prevent the weight increase caused by epinephrine, and was without effect on the weight increase caused by phenylephrine or norepinephrine. Isoprenaline, phenoxybenzamine or propranolol caused no change in lung weight.

Corrected ascorbate levels showed decreases of 18, 16 and 16 per cent of saline treatment levels for phenylephrine, norepinephrine and epinephrine respectively (Table 3). Phenoxybenzamine and propranolol had effects similar to those shown in Table 1, except that propranolol prevented the decrease in ascorbate normally caused by epinephrine.

Intravenous injection of serotonin, histamine or dibutyryl-cAMP (all of 5 μmoles/kg) produced no significant change in either the weight or the ascorbic acid content of the lung.

TABLE 1. CHANGES IN THE LUNG ASCORBIC ACID CONCENTRATION OF ANESTHETIZED MICE TREATED WITH CATECHOLAMINES IN THE PRESENCE OF RECEPTOR BLOCKERS

	Ascorbic acid content* (mg/100 g fresh tissue)					
	Saline		Phenoxybenzamine		Propranolol	
	Mean ± S.E.	*t*-test	Mean ± S.E.	*t*-Test	Mean ± S.E.	*t*-Test
Saline	34·3 ± 0·9 (28)		33·1 ± 1·0 (20)	vs Saline-Saline NS	34·5 ± 1·0 (20)	vs Saline-Saline NS
Phenylephrine	25·5 ± 1·0 (20)	vs Saline-Saline $P < 0.001$	33·4 ± 1·3 (12)	vs Saline-Phenoxybenzamine NS	24·6 ± 1·3 (12)	vs Saline-Propranolol $P < 0.001$
Norepinephrine	23·0 ± 1·0 (20)	vs Saline-Saline $P < 0.001$	33·0 ± 1·3 (12)	vs Saline-Phenoxybenzamine NS	25·3 ± 1·3 (12)	vs Saline-Propranolol $P < 0.001$
Epinephrine	20·6 ± 1·0 (20)	vs Saline-Saline $P < 0.001$	30·9 ± 1·3 (12)	vs Saline-Phenoxybenzamine NS	30·1 ± 1·3 (12)	vs Saline-Propranolol $P < 0.05$
Isoprenaline	36·3 ± 1·6 (8)	vs Saline-Saline NS	33·8 ± 1·6 (8)	vs Saline-Phenoxybenzamine NS	33·2 ± 1·6	vs Saline-Propranolol NS

* The number of mice in each group is shown in parentheses. NS means $P > 0.05$.

TABLE 2. CHANGES IN THE LUNG WEIGHT RATIO OF ANESTHETIZED MICE TREATED WITH CATECHOLAMINES IN THE PRESENCE OF RECEPTOR BLOCKERS

	Lung weight ratio*					
	Saline		Phenoxybenzamine		Propranolol	
	Mean ± S.E.	*t*-Test	Mean ± S.E.	*t*-Test	Mean ± S.E.	*t*-Test
Saline	0·99 ± 0·02 (28)		1·02 ± 0·04 (20)	vs Saline-Saline NS	1·02 ± 0·04 (20)	vs Saline-Saline NS
Phenylephrine	1·12 ± 0·04 (20)	vs Saline-Saline $P < 0·001$	0·97 ± 0·04 (12)	vs Saline-Phenoxybenzamine NS	1·11 ± 0·04 (12)	vs Saline-Propranolol $P < 0·05$
Norepinephrine	1·27 ± 0·04 (20)	vs Saline-Saline $P < 0·001$	0·98 ± 0·04 (12)	vs Saline-Phenoxybenzamine NS	1·27 ± 0·04 (12)	vs Saline-Propranolol $P < 0·001$
Epinephrine	1·48 ± 0·04 (20)	vs Saline-Saline $P < 0·001$	1·04 ± 0·04 (12)	vs Saline-Phenoxybenzamine NS	1·18 ± 0·04 (12)	vs Saline-Propranolol $P < 0·001$
Isoprenaline	0·96 ± 0·04 (8)	vs Saline-Saline NS	0·96 ± 0·04 (8)	vs Saline-Phenoxybenzamine NS	0·99 ± 0·04 (8)	vs Saline-Propranolol NS

* A ratio > 1 indicates increased lung weight. Details of calculation procedure are given in Methods. The number of mice in each group is shown in parentheses. NS means $P > 0·05$.

TABLE 3. CHANGES IN THE CORRECTED LUNG ASCORBIC ACID CONCENTRATION OF ANESTHETIZED MICE TREATED WITH CATECHOLAMINES IN THE PRESENCE OF RECEPTOR BLOCKERS

	Ascorbic acid content* (mg/100 g fresh tissue)					
	Saline		Phenoxybenzamine		Propranolol	
	Mean ± S.E.	*t*-Test	Mean ± S.E.	*t*-Test	Mean ± S.E.	*t*-Test
Saline	33·7 ± 0·8 (28)		33·6 ± 0·9 (20)	vs Saline-Saline NS	35·8 ± 0·9 (20)	vs Saline-Saline NS
Phenylephrine	27·7 ± 0·9 (20)	vs Saline-Saline $P < 0·001$	32·6 ± 1·2 (12)	vs Saline-Phenoxybenzamine NS	26·9 ± 1·2 (20)	vs Saline-Propranolol $P < 0·001$
Norepinephrine	28·5 ± 0·9 (20)	vs Saline-Saline $P < 0·001$	32·1 ± 1·2 (12)	vs Saline-Phenoxybenzamine NS	31·0 ± 1·2 (12)	vs Saline-Propranolol $P < 0·01$
Epinephrine	28·4 ± 0·9 (20)	vs Saline-Saline $P < 0·001$	32·0 ± 1·2 (12)	vs Saline-Phenoxybenzamine NS	34·5 ± 1·2 (12)	vs Saline-Propranolol NS
Isoprenaline	34·0 ± 1·5 (8)	vs Saline-Saline NS	32·6 ± 1·5 (8)	vs Saline-Phenoxybenzamine NS	33·0 ± 1·5 (8)	vs Saline-Propranolol NS

* Ascorbic acid contents are corrected for the presence of edema fluid. For more details, see Methods. The number of mice in each group is shown in parentheses. NS means $P > 0·05$.

DISCUSSION

In the present experiments, loss of lung ascorbic acid from lung tissue is produced by phenylephrine, norepinephrine and epinephrine. These results for mice agree with the observation that norepinephrine decreased the ascorbate content of rat lung.[1] The ascorbic acid assay used in the present experiments was chosen because it combined the specificity of both the reduction of ascorbic acid in acid solution by 2,6-dichlorophenol-indophenol and the formation of the hydrazone derivative of ascorbate.

Inseparable from the decreases in lung ascorbic acid concentration are the concomitant increases in lung weight. As values for blood ascorbate are small compared to lung levels,[6] the influx of blood or edema fluid into lung could be expected to lower the lung ascorbic acid concentration. Procedures for the quantitation of pulmonary edema have been evaluated by Visscher *et al.*[3] They concluded that the lung to body weight ratio was one of the most reliable practical methods. In the present experiments, it was found that the lung to body weight ratio of control mice was not constant over the range of body weights used, but decreased as the body weight increased. By using the linear correlation between these two parameters, it was possible to derive a lung weight ratio which was a better measure of increased lung weight than the lung to body weight ratio. Lung ratios greater than unity were regarded as a measure of lung edema rather than pulmonary hyperemia, since mice were bled before removal of the lungs.

In order to assess the importance of edema in lowering the lung ascorbic acid concentration, corrected ascorbate contents were calculated as the product of the measured ascorbic acid concentration and the corresponding lung weight ratio. This correction assumes that the edema fluid contains no ascorbic acid. Regardless of how precise this assumption is, the correction based on it provides a maximum estimate of the decrease in lung ascorbic acid concentration caused by changes in wet weight due to edema. The corrected results show that measured decreases in lung ascorbate content are greater than can be explained by a dilution of lung ascorbic acid by edema fluid.

The ability of phenoxybenzamine to block both the lung ascorbate loss and the lung edema caused by phenylephrine, norepinephrine and epinephrine shows that these changes depend on α-receptor activity. In contrast, propranolol partially blocks increases in lung weight and decreases in ascorbic acid levels produced by norepinephrine and epinephrine. These results could be explained by propranolol possessing some α-blocking activity, but this does not agree with the result that propranolol has little effect on the ascorbate loss and increased lung weight produced by phenylephrine, an α-stimulant. A better explanation would be that β-receptors act synergistically with α-receptors in causing ascorbate loss and increased lung weight. This is supported by the fact that propranolol is more effective in preventing the pulmonary edema and ascorbate loss as the β-character of the amine causing these changes increases.[7,8] The activity of β-receptors alone cannot cause ascorbate loss and edema, since isoprenaline, a β-stimulant, is unable to cause these changes.

The failure of phenoxybenzamine and propranolol to separate the loss of lung ascorbic acid from the development of pulmonary edema, both caused by various sympathetic agonists, suggests that these two phenomena may be related. Woo and Hedley-Whyte[9] have reported that in dog lung there is a 4-fold increase in the

number of intravascular leucocytes and intra-alveolar macrophages following edema induced by over inflation of the lung. It was suggested that a likely role for the macrophages was to scavenge components of surfactant. Since ascorbic acid may have a role in phagocytosis,[10] the decreased ascorbate content of lungs following catecholamine-induced edema may be due to increased activity of leucocytes or macrophages. Another possibility for the loss of ascorbate during lung edema might be due to an inhibition of the active uptake of ascorbic acid following anoxia. This occurs in rat adrenal *in vitro* after removal of oxygen from the incubation medium.[11] Alternatively, lung ascorbate may be lost directly as a result of stimulation of α-receptors in lung. The concomitant formation of edema due to a hemodynamic change initiated by stimulation of α-receptors elsewhere could be coincidental. The relationship between ascorbic acid and α-receptors is supported by the fact that in guinea pigs ascorbate is necessary to maintain the response of blood vessels to the vascular stress produced by hemorrhage.[12] Also, as ascorbic acid is known to be a cofactor in the biosynthesis of norepinephrine,[13] it may be expended following any increase in sympathetic nerve activity in lung.

Any of these explanations might account for the catecholamine-induced loss of lung ascorbic acid seen in the present experiments, but evidence currently available does not favour any particular alternative.

REFERENCES

1. R. J. Willis and C. C. Kratzing, *Am. J. Physiol.* **222,** 1391 (1972).
2. G. R. Cameron, *Br. med J.* **1,** 964 (1948).
3. M. B. Visscher, F. J. Haddy and G. Stephens, *Pharmac. Rev.* **8,** 389 (1956).
4. J. H. Roe and C. A. Kuether, *J. biol. Chem.* **147,** 399 (1966).
5. D. W. Bolin and L. Book, *Science, N.Y.* **106,** 451 (1947).
6. R. P. Maickel, *Analyt. Biochem.* **1,** 498 (1960).
7. R. P. Ahlquist, *Am. J. Physiol.* **153,** 586 (1948).
8. R. P. Ahlquist, in *Handbook of Psysiology* (Eds. W. F. Hamilton and P. Dow), p. 2457. Am. Physiol. Soc., Washington, D.C. (1965).
9. S. W. Woo and J. Hedley-Whyte, *J. appl. Physiol.* **33,** 14 (1972).
10. L. DeChatelet, C. McCall and M. Cooper, *Fedn. Proc.* **30,** 1275 (1971).
11. A. F. DeNicola, M. Clayman and R. M. Johnstone, *Endocrinology* **82,** 436 (1968).
12. R. E. Lee, *Ann. N.Y. Acad. Sci.* **92,** 295 (1961).
13. E. Y. Levin, B. Lovenberg and S. Kaufman, *J. biol. Chem.* **235,** 2080 (1960).