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Effect of ventilation on removal of [^{14}C]mescaline by perfused rabbit lung

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Many investigators have used isolated perfused lung preparations to study the pulmonary disposition of circulating drugs and endogenous vasoactive substances. In many of these studies [1–6], the perfused lungs were statically inflated, while in others [7–15] the lungs were mechanically ventilated. It is possible that under these different conditions there exist qualitative and/or quantitative differences in the pulmonary removal and metabolism of the perfused substances.

We tested this possibility using mescaline, which is removed from the circulation by statically inflated, perfused rabbit lung [16] and extensively metabolized to 3,4,5-trimethoxyphenylacetic acid (TMPA) by a semicarbazide-sensitive amine oxidase which is present in lung homogenates [17].

Methods

Left and right rabbit lungs were independently perfused at 10 ml/min via the first branches of the pulmonary artery in a non-recirculating (single-pass) system as previously described [5]. The Krebs-bicarbonate perfusion medium (pH 7.4) was aerated with 95% O_2, 5% CO_2. Lungs were ventilated with room air via a tracheal cannula at 51 strokes/min and 21 cc/stroke [18] using a Harvard Apparatus model 661 Small Animal Respirator. Perfusion pressure and tracheal pressure were monitored using Statham pressure transducers (models P 23 DC and P 23 Bc, respectively) and a Grass model 5 Polygraph.

The experimental protocol was as follows. After a 10-min stabilization period during which statically inflated lungs were perfused with mescaline-free perfusion medium, lungs were perfused with medium containing 0.1 μM [^{14}C]mescaline for 12.5 consecutive min. At the beginning of amine perfusion (taken as zero time) the lungs were inflated with 25 ml of room air. From 10 to 10.5 min (Period 1) a 0.5-min sample of effluent perfusion medium was collected from each lung. At 10.5 min control lungs were immediately reinflated with 25 ml of room air, while experimental lungs were mechanically ventilated until min 11.5. A second sample of effluent was collected between min 11.0 and 11.5 of the ventilatory period (Period 2). At 11.5 min, lungs were again (statically) reinflated to a volume of 25 ml. A third effluent sample was collected during this final period of static inflation at 12.0 min (Period 3).

Radiolabeled amine in 0.5-ml aliquots from collected samples was separated from its TMPA metabolite using Bio-Rex 70 cation exchange chromatography as previously described [19]. Radioactivity was measured after addition of 10 ml Aquasol in a Packard Tri-Carb model 3320 liquid scintillation spectrometer. From the radioactivity measurements the concentration of amine and metabolite in the inflow and outflow perfusion medium was calculated.

Per cent removal (% R) of perfused amine was calculated as:

$$\% R = \left[\frac{C_{a,i} - C_{a,o}}{C_{a,i}}\right] \times 100,$$

where $C_{a,i}$ and $C_{a,o}$ represent concentrations of [^{14}C]amine in the inflow and outflow perfusion medium respectively.

Per cent of effluent radioactivity as [^{14}C]mescaline metabolite (% M) was calculated as:

$$\% M = \frac{C_{m,o}}{C_{t,o}} \times 100,$$

where $C_{m,o}$ is the concentration of [^{14}C]metabolite in the outflow perfusion fluid and $C_{t,o}$ is the total molar concentration of [^{14}C]amine plus [^{14}C]metabolite in the effluent.

Results from independently perfused left and right lungs were similar; data from left lungs are presented in this paper. The results were analyzed statistically by a Student's t-test [20].

Mescaline HCl [8-^{14}C] (21 mCi/m-mole) and Aquasol were purchased from New England Nuclear Corp., Boston, MA. Bio-Rex 70 cation exchange resin (100–200 mesh) was purchased from Bio-Rad Laboratories, Richmond, CA.

Results

The effect of ventilation on removal of 0.1 μM [^{14}C]mescaline by isolated perfused rabbit lung is shown in Fig. 1. [^{14}C]mescaline removal by control lungs, which were reinflated to static volume instead of being ventilated, remained unchanged during the period of effluent collection (Fig. 1, control). In lungs which were ventilated, however, percentage removal increased from 58 per cent during static inflation (Period 1) to 83 per cent during the ventilatory period (Period 2). Amine removal remained elevated 1 min after the ventilatory period (i.e. during Period 3), although it had decreased from the peak attained during ventilation.

The per cent of effluent radioactivity appearing as [^{14}C]mescaline metabolite ([^{14}C]TMPA) is shown in Fig.

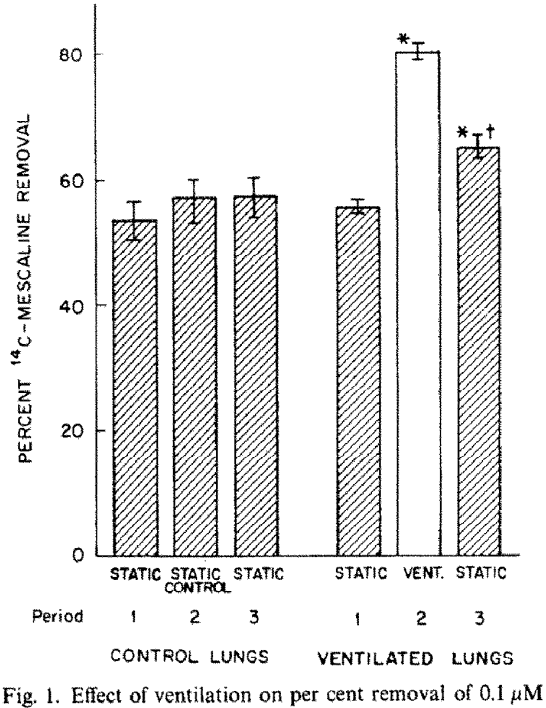

Fig. 1. Effect of ventilation on per cent removal of 0.1 μM [^{14}C]mescaline by perfused rabbit lung. Protocol was as described in Methods. Shaded bars represent removal determined during static inflation. Values are expressed as mean ± S. E. M. N = 4 for control, and 6 for ventilated groups. The asterisk (*) indicates significantly different from the Period 1 value of the same group; the dagger (†) indicates significantly different from the Period 2 value of same group, $P < 0.05$ (Student's t-test, paired analysis).

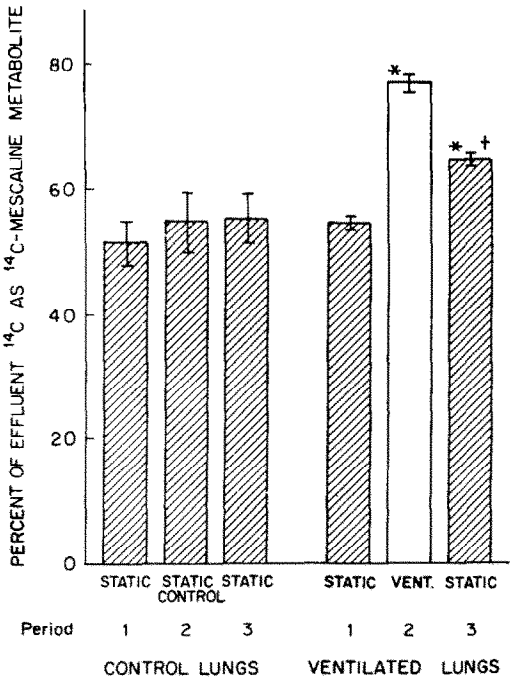

Fig. 2. Effect of ventilation on fraction of effluent radioactivity appearing as [^{14}C]mescaline metabolite. Shaded bars represent periods of static inflation. Values are expressed as mean ± S. E. M. N = 4 for control lungs, and 6 for ventilated lungs. The asterisk (*) indicates significantly different from the Period 1 value of the same group; the dagger (†) indicates significantly different from the Period 2 value of the same group, $P < 0.05$ (Student's t-test, paired analysis).

2. The rate of appearance of [^{14}C]mescaline metabolite was constant in lungs which were statically inflated (Fig. 2, control lungs). Ventilation, however, produced a significant increase in the percentage of effluent radioactivity appearing as [^{14}C]metabolite. This effect can be accounted for primarily by an increase in the concentration of [^{14}C]metabolite appearing in the effluent, which accompanied a decrease in effluent [^{14}C]mescaline during ventilation.

Perfusion pressure remained unchanged during experiments in which lungs were statically inflated (Table 1). Ventilation produced small but consistent decreases in mean perfusion pressure. Lung weight was not significantly altered after the 1-min ventilatory period (Table 1).

Discussion

This study shows that ventilation of isolated rabbit lungs increases the removal of perfused [^{14}C]mescaline. One min after the cessation of ventilation the magnitude of [^{14}C]mescaline removal had partially returned to its pre-ventilatory value. Results presented in Fig. 2 suggest that the observed ventilation-induced changes in mescaline removal are associated with elevated metabolism of the amine. The mechanism of this ventilatory effect is presently unknown. The fact that perfusion pressure and lung weight were not altered 1 min after the ventilatory period (Table 1) suggests that ventilation-induced alterations in amine removal are not associated with irreversible damage to the organ.

These results emphasize the importance of considering ventilatory conditions in the design of experiments with perfused lung and interpretation of results from such studies. The study also raises the possibility that altered ventilatory patterns resulting from pulmonary disease, shock and exercise may change the effectiveness of the lung in removing circulating drugs and other bioactive agents *in vivo*.

Table 1. Effect of ventilation on perfusion pressure and weight of perfused rabbit lungs*

	Control values: static during Period 2 (N = 4)	Experimental values: ventilated during Period 2 (N = 6)
Period	Mean perfusion pressure (mm Hg)	
1	4.9 ± 0.3	4.7 ± 0.4
2	4.9 ± 0.5	3.8 ± 0.4†
3	4.8 ± 0.4	4.9 ± 0.5‡
	Lung wt (g)	
	4.98 ± 0.35	5.28 ± 0.60

* Values represent $\bar{X}$ ± S. E. M. for left lungs perfused as described in Methods with Krebs medium at a flow of 10 ml/min.

† Significantly different from Period 1 value ($P < 0.05$, Student's t-test, paired analysis).

‡ Significantly different from Period 2 value ($P < 0.05$, Student's t-test, paired analysis).

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REFERENCES

1. P. J. Piper and J. R. Vane, *Nature, Lond.* **223,** 29 (1969).
2. Y. S. Bakhle, A. M. Reynard and J. R. Vane, *Nature, Lond.* **222,** 756 (1969).
3. V. A. Alabaster and Y. S. Bakhle, *Br. J. Pharmac.* **40,** 468 (1970).
4. L. A. Gruby, C. Rowlands, B. Q. Varley and J. H. Wyllie, *Br. J. Surg.* **58,** 525 (1971).
5. C. N. Gillis and Y. Iwasawa, *J. appl. Physiol.* **33,** 404 (1972).
6. A. A. Mathe, B. I. Levine and M. J. Antonucci, *J. Allergy clin. Immun.* **55,** 170 (1975).
7. B. Eiseman, L. Bryant and T. Waltuch, *J. thorac. cardiovasc. Surg.* **48,** 789 (1964).
8. D. Davila and P. S. Khairallah, *Archs int. Pharmacodyn. Thér.* **185,** 357 (1970).
9. B. L. Fanburg and J. B. Glazier, *J. appl. Physiol.* **35,** 325 (1973).
10. T. C. Orton, M. W. Anderson, R. D. Pickett, T. E. Eling and J. R. Fouts, *J. Pharmac. exp. Ther.* **186,** 482 (1973).
11. S. Berkov, *Circulation Res.* **35,** 256 (1974).
12. M. K. White, H. B. Hechtman and D. Shepro, *Microvasc. Res.* **9,** 230 (1975).
13. J. W. Ryan, R. S. Niemeyer and U. Ryan, *Prostaglandins* **10,** 101 (1975).
14. D. M. Turner, A. K. Armitage, R. H. Bryant and C. T. Dollery, *Xenobiotica* **5,** 539 (1975).
15. E. Bingham, R. Niemeier and W. Dalbey, *Fedn Proc.* **35,** 81 (1976).
16. R. A. Roth, J. A. Roth and C. N. Gillis, *J. Pharmac. exp. Ther.* **200,** 394 (1977).

17. L. J. Riceberg, M. Simon, H. Van Vunakis and R. H. Abeles, *Biochem. Pharmac.* **24**, 119 (1975).
18. P. L. Altman and D. S. Ditmer (Eds.), *Biology Data Book*, p. 220. Fedn. Am. Assoc. Socs. exp. Biol., Washington, D.C. (1964).
19. J. A. Roth and C. N. Gillis, *J. Pharmac. exp. Ther.* **194**, 537 (1975).
20. G. W. Snedecor and G. C. Cochran, *Statistical Methods*, pp. 92–4. Iowa State University Press, Ames, Iowa (1967).

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Catecholamine hypersensitivity of adenylate cyclase after chemical denervation in rat heart

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Trendelenburg [1] in his classical review on supersensitivity to sympathetic amines discussed several mechanisms which could lead to hypersensitivity, among them relationship between supersensitivity and norepinephrine content, deformation of the receptor and release of norepinephrine from nerve endings. Further investigations on these mechanisms carried out in different tissues and animals confirmed the importance of these factors [2–4]. In these investigations the method of chemical denervation with reserpine and 6-hydroxydopamine was widely used during acute and chronic treatments.

The hormonal sensitivity of adenylate cyclase and its close connection with beta-receptor made a good tool of this membrane bound enzyme to investigate receptor sensitivity [5]. Several studies were made concerning the supersensitivity of the adenylate cyclase toward sympathetic amines not only in brain and heart slices [6–8] of rabbit, rat and guinea pig, but also in heart cell membrane preparations from molluscs [9].

The aim of the present work was to compare the effects of reserpine and 6-hydroxydopamine treatment on the catecholamine sensitivity of the adenylate cyclase rat heart particulate cell fractions.

Materials and methods. All fine chemicals were from Sigma (St. Louis), labelled compounds were from New England Corp. Boston.

Wistar rats were used for the experiments. 2.5 mg/kg reserpine i.p., were injected 24 hr or 100 mg/kg 6-hydroxydopamine i.p. 2 hr before excising the heart quickly under ether anesthesia. Controls were treated with saline. Ventricles were homogenized in 9 vol. 0.05 M Tris–HCl pH 7.4 buffer containing 0.25 M sucrose using a Potter apparatus at 4°. The homogenate was sedimented at 10000 *g* at 4° for 20 min, washed twice and sedimented again at 10000 *g* with the same volumes of buffer-sucrose. Finally the pellet was suspended in 9 vol. of buffer omitting sucrose. Protein was determined according to Lowry *et al.* [10].

Adenylate cyclase assay. The reaction mixture contained 50 mM Tris pH 7.4 buffer, 4 mM theophylline, 2 mM ATP/1 μCi [^{3}H]ATP sp. act. 26 Ci (mM), 2 mM cAMP, 2 mM $MgCl_2$, 6 mg/ml albumine, 1 mM phosphoenolpyruvate and 0.1 mg pyruvate kinase and 100 μl enzyme solution containing 200 μg protein. Incubation was carried out in a final vol. of 300 μl at 37° for 5 and 10 min, c-AMP production was linear during the incubation time. The reaction was stopped by boiling the samples at 100° for 3 min in the presence of a 100 μl recovery mixture (10 mM ATP and 1 mM c-AMP) or by the addition of 200 μl of equal parts of 5% $ZnSO_4$ and 5N $Ba(OH)_2$, 50 μl of the supernatants was applied to Whatman No. 3 MM for paper chromatography as described previously [11]. Radioactivity was measured in a Nuclear-Chicago scintillation spectrometer.

Results and discussion. Figure 1 demonstrates adenylate cyclase activity in control and reserpine treated rat ventricles in the presence and absence of 10^{-6} and 10^{-5} M noradrenaline. The noradrenaline sensitivity with respect to basal activity is increased in the reserpine treated rats, basal activity is significantly lower with respect to the control. Figure 2 shows the changes in adenylate cyclase activity in response to isoproterenol 10^{-5} M after 6-hydroxydopamine treatment. There is a significant increase in hormonal stimulation after 6-hydroxydopamine treatment and a decrease in basal activity as well. The decreased basal activity of adenylate cyclase after chemical depletion of catecholamines is observed only in particulate cell fractions whereas in slices no changes in basal activity were observed [3, 12]. Catecholamine concentrations were chosen to give maximal stimulation. No significant differences in noradrenaline K_a values were reported after chemical denervation in brain slices by Vetulani *et al.* [16].

These results seem to emphasize the role of the receptor in short-term supersensitivity since it develops relatively early after denervation, even before the catecholamine content of the heart entirely disappeared. After a single injection of 100 mg/kg 6-OHDA the catecholamine content of the rat heart fell from 0.902 μg/g to 0.042 μg/g w/w within 2 hr [4, 9, 15]. Adenylate cyclase shows supersensitivity not only after *in vitro* noradrenaline stimulation but also after

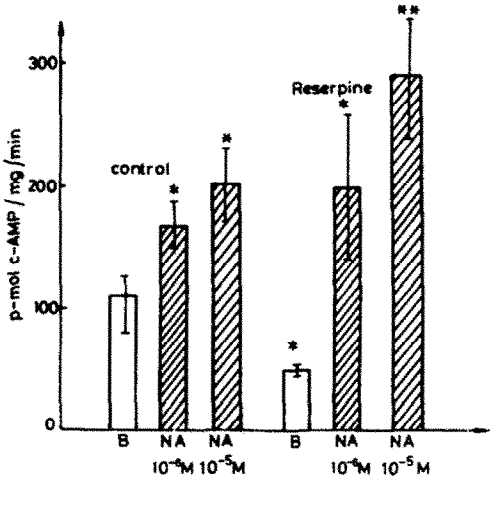

Fig. 1. Adenylate cyclase activity from rat heart ventricle particulate preparations before and after reserpine treatment. B = basal activity, NA = noradrenaline, $n = 5$.