Biochemical Pharmacology, Vol. 31, No. 7, pp. 1375–1379, 1982.
Printed in Great Britain.

0006-2952/82/071375-05 $03.00/0

EFFECT OF HYPOXIA ON THE CONVERSION OF ANGIOTENSIN I TO II IN THE ISOLATED PERFUSED RAT LUNG*

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(*Received* 17 *July* 1981; *accepted* 17 *September* 1981)

Abstract—Acute hypoxia in the intact animal and in cultured endothelial cells has been shown to be associated with a decrease in conversion of angiotensin I (AI) to angiotensin II (AII). Alterations in capillary surface and in contact time resulting from hemodynamic changes have been shown to influence the rate of pulmonary AI conversion. The dependency of AI conversion on hemodynamics complicates the interpretation of experiments showing changes in AI conversion in intact animals. We studied the effect of acute hypoxia on AI conversion in the isolated rat lung perfused at constant flow without recirculation of perfusate. Three levels of oxygenation were produced by ventilating lungs and equilibrating perfusate with a range of hypoxic gas mixtures. AI (1 μg) was injected into the pulmonary artery, and the effluent was collected for measurement of AI and AII. Instead of the expected hypoxic inhibition, percent conversion of AI to AII increased slightly but significantly from 69.3 ± 3.1 (mean ± S.E.M.) at normal oxygenation to 74.4 ± 3.0 at moderate hypoxia ($P < 0.005$, paired t) and to 73.5 ± 3.9 at severe hypoxia ($P < 0.01$, paired t). Decreasing mean transit time of substrate through the lung (by increasing perfusate flow rate from 5 to 20 ml/min) resulted in a significant decrease in conversion of AI from 88.7 ± 2.9 to 73.4 ± 2.1% ($P < 0.001$, paired t). These data confirm the effect of contact time on the rate of AI conversion in the lungs. The isolated rat lung preparation does not exhibit the phenomenon of hypoxia-induced inhibition of AI conversion. The authors speculate that hypoxia-induced inhibition of AI conversion *in vivo* may be secondary to the effects of hypoxia on hemodynamics.

Previous studies in intact dogs by Leuenberger *et al.* [1] and by Stalcup *et al.* [2] have shown that acute hypoxia inhibits the conversion of exogenous angiotensin I (AI) to angiotensin II (AII) and the inactivation of exogenous bradykinin by pulmonary endothelium. The phenomenon occurs in endothelial cells in culture but cannot be shown when cell membrane-free angiotensin converting enzyme is made hypoxic *in vitro*, suggesting that it may be dependent on a critical interaction between the endothelial cell membrane and membrane-bound enzyme [3]. The ability of lungs to convert exogenous angiotensin I is influenced by hemodynamic factors. Both mean transit time and the effective perfused area of the capillary bed have been shown to modify the quantity of AI converted in a single passage through lungs [4]. The dependency is analogous to the relationship between substrate flow and product formation in affinity columns. The interpretation of changes in pulmonary conversion of exogenous AI is thus made difficult when significant hemodynamic effects occur concomitantly [5].

Catravas and Gillis studied the pulmonary metabolism of benzoyl-phenylalanyl-alanyl-proline (BPAP) by angiotensin converting enzyme in single passage studies during acute hypoxia in intact dogs. They concluded that hypoxia-induced alterations in angiotensin converting enzyme activity *in vivo* seemed to be secondary to the effects of hypoxia on pulmonary hemodynamics [6].

The present experiments were undertaken to study the characteristics of angiotensin I conversion in isolated perfused rat lungs in which the perfusate did not recirculate. We subjected the preparation to a wide range of hypoxic challenges under constant hemodynamics conditions. We examined the effect of changing transit time under conditions of constant oxygenation.

MATERIALS AND METHODS

The isolated perfused lung was prepared by a modification of the method of Junod [7]. Sprague–

* This work was supported in part by USPHS Grant HL-22544, by Grant-in-Aid 76-777 of the American Heart Association, and by the Otho S. A. Sprague Memorial Fund. Dr. Oparil was an Established Investigator of the American Heart Association during the time that this work was performed.

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Dawley rats (Southern Animal Farms, Prattville, AL) of either sex (350 g in weight) were anesthetized using sodium pentobarbital (100 mg/kg, i.p.). Tracheas were cannulated and connected to a model 680 Harvard rodent respirator (Harvard Apparatus Co., Millis, MA). Lungs were washed free of blood *in vivo* by gentle manual infusion of normal saline at 37° into the interior vena cava while allowing blood to run off from the sectioned abdominal aorta. A polyvinyl chloride catheter (0.045 in. internal diameter) was placed through the right ventricle into the pulmonary artery and secured with ligatures around the pulmonary artery and the ventricles. The washed lungs were excised and suspended in a water-jacketed, humidified chamber that was maintained at 38°. The lungs were ventilated initially with a gas mixture containing 16% O_2, 6% CO_2 and balance N_2 using the Harvard respirator at a tidal volume of 2 ml and frequency of 60/min. Subsequently, as indicated below, acute hypoxia was induced by ventilating lungs and equilibrating reservoir with a range of hypoxic gas mixtures. The pulmonary artery was perfused at constant flow (initially 10 ml/min) using a Holter pump (model 911 Extracorporeal Medical Specialities, King of Prussia, PA). The perfusate consisted of Krebs bicarbonate buffer solution containing 1% human plasma and 5 mmoles of glucose that had been partially equilibrated with the same gas mixture using a Travenol model 5MO321 membrane oxygenator (Travenol Laboratories, Morton Grove, IL). The pH of the perfusate ranged from 7.40 to 7.50, and the PO_2 ranged from 123 to 135 mm Hg. Perfusate temperature was kept at 38°. The entire pulmonary venous effluent was dripped through a glass funnel and collected so that substances introduced into the pulmonary artery passed through the pulmonary capillary bed only once.

Pulmonary artery pressure was monitored continuously using a Statham P23 Db transducer connected to a lateral tap of the perfusion line close to the point of insertion into the pulmonary artery. The bottom of the lung was used as the hydrostatic level of reference. Airway inflation pressures were monitored using a similar approach. Criteria suggested by Fisher were used to identify the development of alveolar pulmonary edema. Thus, when a progressive rise of perfusion and airway pressures occurring over 10–15 min. was identified, the lungs were discarded [8]. In addition, at the conclusion of each experiment, lungs were weighed before and after drying to a constant weight in an oven. The median wet/dry ratio of eleven control lungs washed free of blood with heparinized normal saline, but not pump perfused, was 3.14 (range 2.88 to 3.36). Accordingly, experimental lungs subsequently found to have a wet/dry ratio greater than 4.0 were discarded. The median wet/dry ratio of thirty-eight lungs used in these experiments was 3.70 (range 3.45 to 3.84). To assess the effects of hypoxia on AI conversion, three levels of oxygenation were compared: "normal oxygenation", "moderate" and "severe hypoxia". During "normal oxygenation" the PO_2 of the perfusion solution was 128 ± 6 mm Hg. With "moderate hypoxia" the lungs were perfused at a PO_2 of 53 ± 2 mm Hg; with "several hypoxia" the perfusion PO_2 was 36 ± 2 mm Hg. The order of exposure to these three levels of oxygenation was random but each lung studied was exposed to all three levels of oxygenation, usually with two exposures to each level.

The pH, PO_2 and PCO_2 of the perfusate, sampled at the pulmonary artery port, were measured using a standard electrode assembly (Instrumentation Laboratories model 326-10, Lexington, MA). AI (I-Asp, 5 Ile angiotensin I synthesized in our laboratory) [9] was injected into the pulmonary artery as a 1 nmole bolus dissolved in 0.2 ml perfusate. The pulmonary effluent was collected continuously for a period of 1 min in a chilled test tube containing dimercaprol, 8-OH-quinoline and EDTA as previously described [9] and later subjected to radioimmunoassay for AI [10] and AII [11]. Preliminary studies using [^{125}I]AI (New England Nuclear Corp., Boston, MA) had shown that virtually all labeled material administered into the pulmonary artery passed through the lung within 30 sec.

To assess the specificity of the conversion process in this system, SQ14,225 (2-D-methyl-3-mercaptopropanoyl-L-proline, captopril), a highly specific competitive inhibitor of angiotensin converting enzyme, was injected into the pulmonary artery as a 2 mg (six animals) or a 5 mg (six animals) bolus dissolved in 0.2 ml of perfusate 1 min prior to the injection of a final 1 nmole bolus of AI. The pulmonary effluent was collected and subjected to radioimmunoassay for AI and AII as previously described.

The expression $(AII \div (AI + AII)) \cdot 100$, in which AII is the concentration of AII in the pulmonary effluent and AI is the concentration of AI in the pulmonary effluent, was used to express percent conversion of AI to AII in a single passage through the pulmonary circulation. Angiotensin II production (pmoles/min) was calculated as AII · volume of effluent collected in 1 min. Control measurements of injectate just as it entered the lung revealed no detectable AII. Percent recovery of immunoreactive material (AI + AII) was calculated for each experiment.

The speed of the Holter pump was altered to study the effect of changes in perfusion rate on mean transit time of [^{125}I]AI and the conversion of AI to AII through the pulmonary circulation. A series of six lungs prepared as previously described under "normal oxygenation" conditions were perfused at flow rates of 20, 10 and 5 ml/min for periods of 10 min. The order of the perfusion rates was chosen at random, and each lung was perfused at all three rates, usually with two exposures to each perfusion rate. During the first period of perfusion AI was injected as a 1 nmole bolus, and the pulmonary effluent was collected for 1 min as previously described except that, at the 5 ml/min flow rate, effluent was collected for 1.5 min to ensure completeness of collection. During the second period of perfusion [^{125}I]AI (20 pmoles, or 2.0×10^4 cpm) was injected into the pulmonary artery as a bolus dissolved in a 0.2 ml of perfusate. Since the average volume of distribution between injection and sampling sites was 2–3 ml, we calculate the average substrate concentration to be of the order of 1×10^{-9} M. This concentration is well below (approxi-

mately 0.01%) the K_m of the enzyme *in vitro* for this substrate and allows direct comparison of the current data with those of Catravas and Gillis [6] and our previous observations in dogs [5]. The pulmonary effluent was collected continuously for a period of 40 sec in test tubes mounted on a turntable-collector run at a speed such that each tube received effluent collected over a 2-sec period. The collection tubes were then placed in a gamma counter (model 1285 Searle, Des Plaines, IL) to quantify the labeled material. Percent recovery of labeled material was calculated. Time–concentration curves for total radioactivity were plotted for each experimental run; mean transit times were calculated from the plots according to formulas from indicator dilution theory. Corrections for delays in transit through tubing were made according to Milnor and Jose [12]. In previous experiments, we had shown that the volume of distribution and the transit time of total radioactivity (AI + AII) were not different from those of a non-diffusible indicator [5].

The statistical significance of differences between PO_2, pH, perfusate and airway pressures, percent conversion and AII production rates at different levels of oxygenation and different perfusion rates was assessed using the paired *t*-test [13]. Linear regression analysis of the relationship between perfusate oxygen tension and percent conversion of AI to AII was performed using a Hewlett Packard model 9815A programmable computer (Hewlett Packard, Atlanta GA). The relationship between mean transit time and percent conversion was analyzed using the function percent conversion = $a\ (1-e^{bt})$ where t is mean transit time [14].

RESULTS

Conversion rates, expressed either as percent conversion of AI to AII or as AII production rate, increased slightly in the presence of hypoxia (Fig. 1). Mean (±S.E.M.) percent conversion increased from 69.3 ± 3.1 under normoxic conditions to 74.4 ± 3.0 during moderate hypoxia ($P < 0.005$) and to 73.5 ± 3.9 during severe hypoxia ($P < 0.01$). AII production was significantly increased ($P < 0.05$) above baseline levels only under conditions of severe hypoxia. Linear regression analysis showed no significant relationship between oxygen tension and

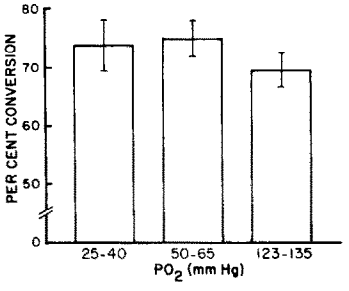

Fig. 1. Effect of altering perfusate oxygen tension on percent conversion of AI to AII in fifteen lungs. Exposure to both moderate and severe hypoxia was associated with a significant increase in conversion of AI as compared to the normotensive control ($P < 0.005$ and 0.01, respectively, using Student's paired *t*-test). Bars indicate mean values and brackets standard errors.

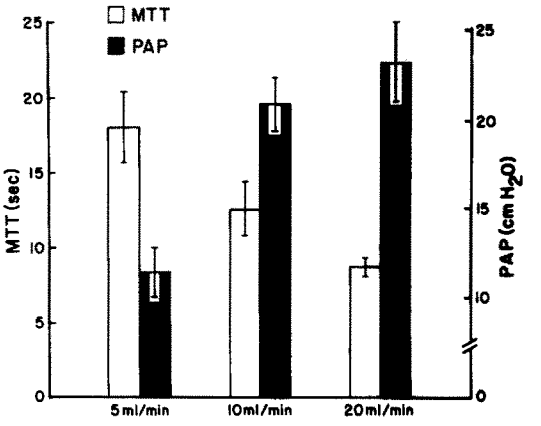

Fig. 2. Effects of altering perfusate flow rate on mean transit time (MTT) and pulmonary arterial pressure (PAP) in six lungs. Increments in perfusion rate were associated with significant decrements in MTT ($P < 0.001$ for flow rates of 10 and 20 ml/min vs baseline value for 5 ml/min using Student's paired *t*-test). Bars indicate mean values and brackets standard errors.

percent conversion of AI to AII ($r = 0.01$) or generation rate of AII ($r = 0.0189$).

Following pretreatment with 2 mg SQ14,255, mean percent conversion decreased from 69.3 ± 3.1 to 13.1 ± 1.08 ($P < 0.001$), and following 5 mg SQ14,225, to 8.65 ± 2.41% ($P < 0.001$), confirming that AII generation in this system was due to an action of angiotensin converting enzyme.

The recovery of 1 nmole boluses of unlabeled immunoreactive AI and AII from the pulmonary effluent was 79.1 ± 2.6% under normoxic conditions and was not altered significantly by hypoxia. This was slightly lower than the percent recovery of tracer doses of [^{125}I]AI (94.6 ± 3.2) introduced into the

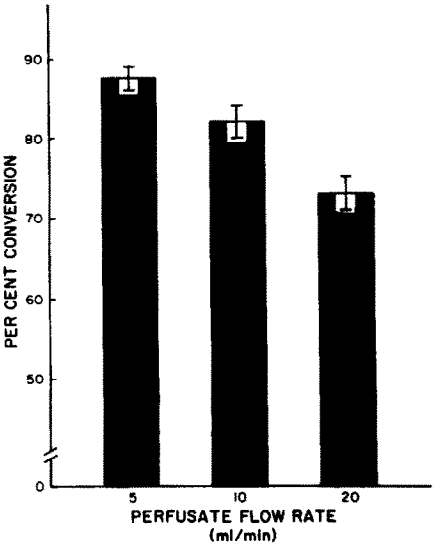

Fig. 3. Effect of altering perfusate flow rate on percent conversion of AI to AII in six lungs. Mean percent conversion decreased significantly when flow rate was increased from a baseline of 5 ml/min to 10 ml/min ($P < 0.005$) and to 20 ml/min ($P < 0.001$). P values refer to Student's paired *t*-test. Bars indicate mean values and brackets standard errors.

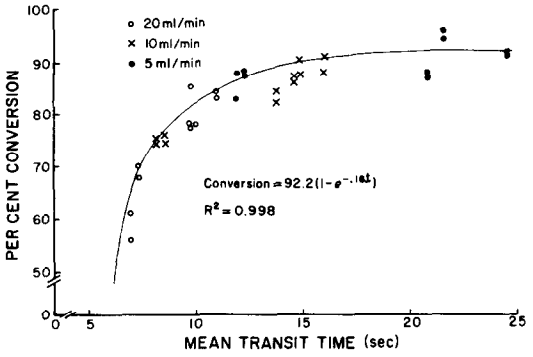

Fig. 4. Relationship between mean transit time and percent conversion analyzed using the function percent conversion $= a\ (1-e^{bt})$ where t is the mean transit time. This model relates mean transit time to percent conversion and provides an estimate of the rate constant, b (sec^{-1}), and the asymptotic upper limit of percent conversion, a.

pulmonary artery under similar conditions. The difference is probably due to metabolism of unlabeled AI and AII into nonimmunoreactive fragments.

Mean pulmonary artery pressure (21.90 ± 1.77 cm H_2O) and airway inflation pressure (14.29 ± 1.47 cm H_2O) were not changed significantly by hypoxia. The administration of 1 nmole AI into the pulmonary artery under "normal oxygenation" conditions was associated with an increase in perfusate pressure from 21.90 ± 1.77 cm H_2O to 25.98 ± 2.15 cm H_2O) ($P < 0.001$). Under conditions of "moderate hypoxia", a similar increase was observed (pre-AI = 22.17 ± 1.50 cm H_2O; post-A = 25.57 ± 1.77 cm H_2O; $P < 0.005$). During "severe hypoxia" no change was seen following administration of AI.

The effects of altering perfusion rate from 20 to 10 to 5 ml/min by changing the speed of the Holter pump on mean transit times and on perfusion pressures are shown in Fig. 2. Mean transit times decreased from 18.16 ± 2.42 sec at a flow rate of 5 ml/min to 12.64 ± 1.83 sec at 10 ml/min ($P < 0.001$) and to 8.83 ± 0.58 sec at 20 ml/min ($P < 0.001$).

The effects of altering perfusate flow rate on percent conversion of AI to AII are summarized in Fig. 3. Mean (±S.E.M.) percent conversion decreased from 88.7 ± 2.9 at a perfusate flow rate of 5 ml/min to 81.8 ± 1.9 at 10 ml/min ($P < 0.005$) and to 73.4 ± 21. ($P < 0.001$) at 20 ml/min. The relationships between conversion rate, mean transit time and perfusate flow rate for all animals studied are represented in Fig. 4. There was an exponential increase in conversion rates of AI with increases in mean transit time from approximately 6 to 12 sec, with apparent saturation at this level. Further increases in transit time did not result in corresponding increments in conversion.

DISCUSSION

Using a bioassay technique based on systemic blood pressure response to intravenous bolus injections of AI, Leuenberger *et al.* [1] demonstrated a striking (40%) decrease in apparent intrapulmonary conversion of AI to AII during acute hypoxia in the intact anesthetized dog. These investigators attributed the decrease in conversion to a direct effect of hypoxia on endothelial cell function or conformation. Subsequent evidence supportive of this hypothesis was obtained by the same group when they demonstrated decreased arteriovenous differences in bradykinin concentration across the dog lung during hypoxia [2] and decreased conversion of AI to AII by hypoxic endothelial cells in culture [3].

Earlier studies using isolated perfused dog lungs [4] had shown that changes in hemodynamic factors such as intravascular pressures and mean transit times had a significant effect on the quantity of AI converted into AII in a single passage. Presumably these factors operated by altering either the size of the endothelial surface being perfused and, hence, effective enzyme availability or the contact time between enzyme and substrate.

Using direct radiochemical and radioimmunoassay techniques for quantification of AI and AII, we were able to confirm the finding of hypoxia-induced decrease in conversion of AI to AII in anesthetized dogs [5]. However, because of associated significant decrease in mean transit time, we were unable to determine to what extent hypoxia-induced suppression of AI conversion was due to altered hemodynamic factors or was the result of a direct effect on endothelial cell function.

Catravas and Gillis [6] concluded from their studies on pulmonary metabolism of [^{3}H]BPAP by angiotensin converting enzyme that acute hypoxia probably exerted its inhibitory effects on conversion indirectly, i.e. through its effects on pulmonary hemodynamics. The direct mechanism described by Stalcup *et al.* [3] was questioned: if present *in vivo* its role remained to be determined.

In order to examine further the mechanism by which acute hypoxia alters intrapulmonary conversion of AI to AII, we performed the present experiments. In the isolated rat lung preparation the pulmonary artery was pump perfused at a constant flow rate without recirculation of the perfusate. This permitted maintaining a steady mean transit time through the capillary bed and at the same time eliminated the complicating hemodynamic effects associated with vasoconstriction caused by recirculation of generated AII.

The isolated perfused rat lung preparation exhibited a capacity to convert AI to AII in a single passage which was comparable to that found in intact animals: approximately 80% of inflowing substrate was hydrolyzed in one passage [9]. In addition, and in keeping with the evidence of Fanburg and Glasier [4], the isolated lung preparation showed dependency of AI conversion rates (or AII generation rates) on mean transit times (Fig. 4). This mean transit time dependence occurred in spite of the fact that increases in pump flow rate caused changes in perfusion pressures which would be expected to recruit unperfused capillaries and to produce effects on AI conversion opposite to those induced by the corresponding changes in mean transit time (Fig. 2). The apparent relative insensitivity of AI conversion in the isolated rat lung preparation to changes in perfusion pressure differs from the behaviour of the

isolated dog lung preparation [4]. The most likely explanation for this difference lies in differences in linear dimensions of lung between rat and dog. At the lowest perfusion pressures used in our experiments, none of the rat lung capillaries would be expected to be in zone 1 [15]. However, unlike other experiments [2, 3], the isolated rat lung preparation did not exhibit attenuation of AI conversion during hypoxia. Instead, a slight but significant increase in AI conversion was demonstrated. Given the lack of significant changes in pulmonary artery pressure with hypoxia, it is difficult to ascribe the increase in AI conversion to passive capillary recruitment. However, it is possible that the type of capillary recruitment described by Wagner and Latham in hypoxic dogs [16] may have been operative.

The possibility exists that the phenomenon of hypoxia-induced depression of pulmonary conversion of AI is not normally exhibited by rat endothelium. Alternatively, our inability to demonstrate the phenomenon of hypoxia-induced depression of AI conversion in the isolated rat lung could be explained by consistent selective loss of specific endothelial cell functions. It is conceivable that hypoxic inhibition of converting enzyme activity is the manifestation of a delicate property of the endothelial cell membrane which becomes impaired by circumstances of the experimental preparation.

Given the current analytical requirements for AI and AII determinations, it would be extremely difficult to perform quantitative experiments using multiple blood samples to verify this conjecture in intact rats. Interestingly, the overall ability of the endothelial surface to convert substrate was well preserved or even enhanced during hypoxia. Conceivably, hypoxia-induced inhibition of conversion is a separate phenomenon from converting capacity. In cultured endothelial cells, hypoxia-induced inhibition of AI conversion is progressively attenuated when the converting enzyme is separated from cell membrane by analytical means. However, the ability to hydrolyze substrate is maintained [3].

The current study substantiates the influence of contact time on the conversion of AI as it passes through lungs. It reiterates the need to take into consideration changes in hemodynamic factors when interpreting AI conversion experiments in which these change significantly [5].

It seems that the available evidence, *in vivo*, favors the concept that hypoxia-induced inhibition of converting enzyme activity is primarily a manifestation of the effects of acute hypoxia on pulmonary hemodynamics. The *in vivo* significance of the direct effect of hypoxia on conversion of AI, demonstrated in cultured endothelial cells, remains to be investigated.

Acknowledgements—The authors wish to express their gratitude to Braxton C. Bowdoin and Mildred Daise for their technical assistance; to Mr. L. R. Smith for assistance in statistical analysis of the data; to Dr. Stanley B. Digerness for use of equipment used in the transit time experiments; to the Squibb Institute, New Brunswick, NJ, for supplying SQ14,225; and to Joan Chisolm, Karen Riley and Barbara Webb for typing the manuscript.

REFERENCES

1. P. J. Leuenberger, S. A. Stalcup, R. B. Mellins, L. M. Greenbaum and G. M. Turino, *Proc. Soc. exp. Biol. Med.* **158**, 586 (1978).
2. S. A. Stalcup, J. S. Lipset, P. M. Legant, P. J. Leuenberger and R. B. Mellins, *J. appl. Physiol: Respir. environ. Exercise Physiol.* **46**, 227 (1979).
3. S. A. Stalcup, J. S. Lipset, J-M. Woan, P. J. Leuenberger and R. B. Mellins, *J. clin. Invest.* **63**, 966 (1979).
4. B. L. Fanburg and J. B. Glasier, *J. appl. Physiol.* **35**, 325 (1973).
5. J. P. Szidon, N. Bairey and S. Oparil, *Circulation Res.* **46**, 221 (1980).
6. J. Catravas and C. N. Gillis, *J. Pharmac. exp. Ther.* **217**, 263 (1981).
7. A. F. Junod, *J. Pharmac. exp. Ther.* **183**, 341 (1972).
8. A. B. Fisher, C. Dodia and J. Linask, *Exp. Lung Res.* **1**, 3 (1980).
9. S. Oparil, G. W. Tregear, T. Koerner, B. A. Barnes and E. Haber, *Circulation Res.* **29**, 682 (1971).
10. E. Haber, T. Koerner, L. B. Page, B. Kilman and A. Purnode, *Endocrinology* **29**, 1349 (1969).
11. L. B. Page, E. Haber, A. Y. Kimura and A. Purnode, *J. clin. Endocr. Metab.* **29**, 200 (1969).
12. W. R. Milnor and A. D. Jose, *J. appl. Physiol.* **15**, 177 (1960).
13. G. W. Snedecor and W. G. Cochran, *Statistical Methods*, 6th Ed. Iowa State University Press, Ames, IA (1967).
14. A. Jacquez, *Compartmental Analysis in Biology and Medicine*, Chap. 1. Elsevier-North Holland, New York, (1971).
15. J. B. West, C. T. Dollery and A. Naimark, *J. appl. Physiol.* **19**, 713 (1964).
16. W. W. Wagner and L. P. Latham, *J. appl. Physiol.* **39**, 900 (1975).