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Reduction in Hypertension-Induced Protein Synthesis in the Rat Pulmonary Trunk after Treatment with Teprotide (SQ 20881) (41959)

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Abstract. Angiotensin II has been previously implicated as a mediator of vasoconstriction during the development of hypoxic pulmonary hypertension. The effect of angiotensin-converting enzyme inhibition with teprotide (SQ 20881) on development of pulmonary hypertension was determined by measurement of the drug's ability to modify hypertension-induced protein synthetic changes in the rat pulmonary trunk. Rats were injected with either SQ 20881 (2 mg/kg body wt every 8 hr) or saline vehicle during exposure to chronic hypoxia at 0.5 atm for either 3 or 7 days. Comparisons were made of tissue weight, absolute protein content, and *in vitro* synthesis of collagen and noncollagen protein of the pulmonary trunks of SQ-treated hypoxic, SQ-treated normoxic, saline-treated hypoxic, and saline-treated normoxic rats. Treatment of hypoxic rats with SQ 20881 was found to significantly decrease right ventricular pressure, tissue weight, absolute protein content, and *in vitro* protein synthesis after 7 days compared to saline-treated hypoxic rats. Neither right ventricular hypertrophy nor the development of polycythemia was decreased by SQ 20881 treatment.

The role of the renin–angiotensin system in the induction and maintenance of pulmonary hypertension has been studied both clinically and experimentally but remains equivocal. Angiotensin-converting enzyme (ACE), which directs the transformation of angiotensin I (AI) to the potent vasoconstrictor angiotensin II (AII), is located on the luminal membrane of vascular endothelium on both the arterial and venous sides of the pulmonary capillary bed (1). It has therefore been hypothesized that alveolar oxygen content may regulate the development of pulmonry hypertension by increasing ACE activity and thus increasing local AII-mediated vasoconstriction.

Berkov (2) has demonstrated that AII is necessary for hypoxia-induced vasoconstriction in the isolated perfused rat lung. However, analysis of serum and lung ACE activity during the onset and maintenance of hypoxia and monocrotaline-induced pulmonary hypertension provides a complex temporal pattern of ACE alterations. Serum ACE levels were increased after mice were exposed to hypoxia for 7 days (3), and have been reported to increase (4) or remain the same (5) after 3 weeks of hypoxic exposure. Lung ACE activity increased by 9 days in mice (3) but, appeared to be decreased in rats exposed to 3 weeks of hypobaric hypoxia (5). During the onset of monocrotaline-induced pulmonary hypertension lung ACE activity is elevated one week after initiation of treatment (6), but is dramatically reduced by 3 weeks (7). In addition, long-term treatment with converting enzyme inhibitors reduced right ventricular hypertrophy and pulmonary arterial wall thickness in rats exposed to chronic hypoxia (4, 8). Thus, evidence from those studies indicate that the angiotensin system may play an important role in the development and maintenance of pulmonary hypertension.

To date, most studies of the antihypertensive effects of ACE inhibition in pulmonary hypertension have involved physiological and morphological measurements. However, McKenzie and Klein (9, 10) have demonstrated dramatic changes in the ability of the rat pulmonary trunk to synthesize protein *in*

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vitro and *in vivo* after 3 or 7 days of hypoxic exposure. In the present study, the converting-enzyme inhibitor teprotide (SQ 20881) was used to determine whether inhibition of AII formation could diminish or block the development of hypoxia-induced pulmonary hypertension as measured by the level of hypertension-associated protein synthesis in the pulmonary trunk. Alterations in biosynthetic parameters of the pulmonary trunk were correlated with changes in physiological parameters produced by hypoxic exposure and teprotide treatment.

Materials and Methods. One hundred twenty-eight male Sprague–Dawley rats (Sasco, Omaha, Neb.) were used in these experiments. For each experiment rats were separated into the following groups (16 rats each): saline-normoxic (SC), saline-hypoxic (SH), SQ-normoxic (SQC), and SQ-hypoxic (SQH). Hypoxic groups were placed in 0.25-m^3 hypobaric chambers maintained at 0.5 atm with an airflow of 0.08-m^3/min and pair-fed with normoxic controls housed in an open chamber in the same room. A 12-hr light–dark cycle was maintained with lights on 0600–1800 hr. Chambers were opened for about 30 min every 8 hr to inject rats, replenish food and water and clean cages. The rats in groups SQC and SQH received sc injections of SQ 20881 (2 mg/kg body wt) in sterile physiological saline at 0800, 1600, and 2400 hr while rats in groups SC and SH received injections of vehicle only. This dosage schedule has been shown to effectively reduce pulmonary arteriolar medial hypertrophy during the development of hypoxia-induced pulmonary hypertension (4).

Hematocrit was measured using an IEC Microcapillary Centrifuge (Model MB) and Reader. Mean right ventricular pressure was measured as described previously (9). The heart was removed from each rat and fixed in 2% glutaraldehyde/0.1 *M* phosphate buffer for determination of right-ventricular hypertrophy (RVH) which was evaluated by expressing the weight of the free wall of the right ventricle (RV) as a ratio of the weight of the left ventricle and interventricular septum (LV + S) (11). The pulmonary trunk (PT) and left pulmonary artery (from the infundibulum to the hilum of the left lung) were removed in one piece and placed in cold (4°C) Krebs Ringer's bicarbonate buffer. Collagen and noncollagen protein assays have been described previously (9), but are summarized briefly. The vessels were stripped of loose fat and connective tissue, sized to 9 mm by removing material from the distal end, pooled in groups of three and sliced into 1- to 2-mm rings. Subsequent processing followed the method of Newman and Langner (12). Pooled trunks were incubated for 90 min at 37°C in 10 ml Krebs Ringer's bicarbonate buffer containing 10 μCi [^{14}C]glycine (New England Nuclear, Boston, Mass.) under 95% O_2/5% CO_2. Following incubation the pooled trunks were washed in two changes of cold (4°C) distilled water, blotted dry, and weighed. The pooled trunks were then frozen in liquid nitrogen and stored at −70°C for up to 2 weeks. Pooled trunks were subsequently homogenized in 2.0 ml of 0.25 *M* sucrose in a glass homogenizer and the homogenate was diluted to 5 ml with the same sucrose solution. Protein content of the homogenate was determined from a 0.1-ml aliquot by the Bio-Rad Protein Assay (BioRad, Richmond, Calif.). A 2.0-ml aliquot of the homogenate was then combined with 2.0 ml 10% TCA (final concentration 5% TCA). Centrifugation and washing of the pellet were performed three times and collagen was solubilized with hot TCA. A 0.2-ml aliquot of the collagen extract was combined with 10 ml of scintillation cocktail and counted in a Searle Model 6892 Liquid Scintillation Counter using standard ^{14}C windows and 10-min counts. The residual protein pellet was solubilized for 20 hr at 45°C in 2.0 ml Protosol and a 0.2-ml aliquot was taken for liquid scintillation counting (LSC). The data were analyzed statistically by analysis of variance and the Bonferroni modification of Student's *t* test for multiple comparisons (13). A P value ≤ 0.025 was considered statistically significant.

Results. There were no differences in mean hematocrit or mean right-ventricular pressure within hypoxic groups (SH vs SQH) or normoxic groups (SC vs SQC) at 3 or 7 days. Therefore, values for control groups were combined. After 3 days of hypoxic exposure, hematocrit was significantly increased in both groups of hypoxic rats (SH = 57.7 ± 1.2%, SQH = 56.7 ± 1.3%) compared to normoxic

groups (C = 48.4 ± 0.5%) and remained elevated at 7 days (SH = 58.2 ± 1.6%, SQH = 57.6 ± 1.4%).

Mean right-ventricular pressure (RVP) was significantly elevated in both hypoxic groups (SH = 26.0 ± 2.7 mm Hg, SQH = 21.0 ± 1.0 mm Hg) compared to normoxic controls (15.7 ± 1.8 mm Hg) at 3 days. RVP continued to be significantly elevated in SH (24.0 ± 1.9 mm Hg) compared to normoxic controls at Day 7. However, a significant reduction in RVP occurred by Day 7 in SQH group (18.5 ± 1.0 mm Hg) compared to SH.

Heart weight and the ratio of right-ventricular weight to the weight of the left-ventricle plus septum (RV/LV + S) showed variable results at Day 3 (Fig. 1). At 7 days, RV/LV + S remained significantly elevated in SQH compared to SH. This was apparently not due to an increase in RV weight, but to a decrease in LV + S in the SQH group. At this time, both hypoxic groups demonstrated

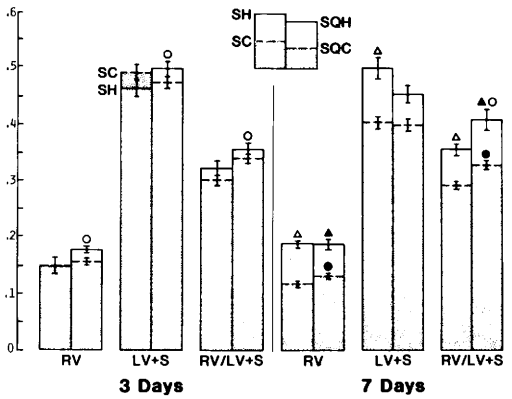

FIG. 1. Ventricle weight and right-ventricular hypertrophy of control rats and rats exposed to hypobaric hypoxia for 3 or 7 days. *Y*-axis represents ventricle weight in grams or the ratio of right-ventricular weight to the weight of the left ventricle and septum. RV = right-ventricle weight, LV + S = left ventricle + septum, right-ventricular hypertrophy = RV/LV + S, SH = saline hypoxic rats, SC = saline control rats. SQH = SQ 20881-treated hypoxic rats, and SQC = SQ 20881-treated control rats. After 3 days of hypoxia, SH rats demonstrated reduced LV + S compared to SC, but with overlapping standard error bars. ○, SQH significantly different from SH, $P \leq 0.025$; ●, SQC significantly different from SC, $P \leq 0.025$; △, SH significantly different from SC, $P \leq 0.025$; ▲, SQH significantly different from SQC, $P \leq 0.025$.

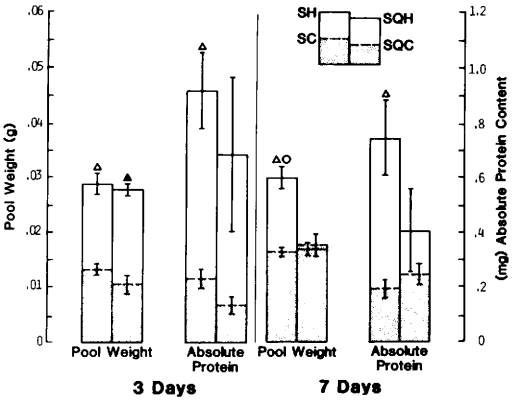

FIG. 2. Pool weight (g) and absolute protein content (mg) of control rats and rats exposed to hypobaric hypoxia for 3 or 7 days. See Fig. 1 legend for explanation of abbreviations and key to symbols.

significant increases in RV and RVH. When compared to values from SH rats, SQH rats did not demonstrate significant differences in the weight of the right ventricle (RV) or left ventricle + septum (LV + S) (Fig. 1).

Both pooled tissue weight and absolute protein content were significantly elevated in hypoxic vs normoxic control groups at Day 3 (Fig. 2) with one exception. Although having a higher ratio of means (hypoxic/normoxic) than the saline groups, absolute protein content was not significantly different in SQH vs SQC due to a large standard error in SQH (Fig. 2). This difference was significant when analyzed by a *t* test unmodified for multiple comparisons. Pool weight and absolute protein content remained significantly elevated in SH vs SC at Day 7. There was also a significant decrease in absolute protein content and pool weight in SQ-treated hypoxic rats as compared to saline-treated hypoxic rats at this time.

Changes in the *in vitro* synthesis of collagen (C) and noncollagen protein (NC) followed much the same pattern as pooled trunk weight and absolute protein content at 3 and 7 days (Fig. 3). At 3 days there were no significant differences between either C or NC in SQH vs SH. Collagen synthesis was significantly elevated in both hypoxic groups at 3 days compared to normoxic controls.

Synthesis of noncollagen protein was also elevated in hypoxic rats although the difference between SQH and SQC, as analyzed by

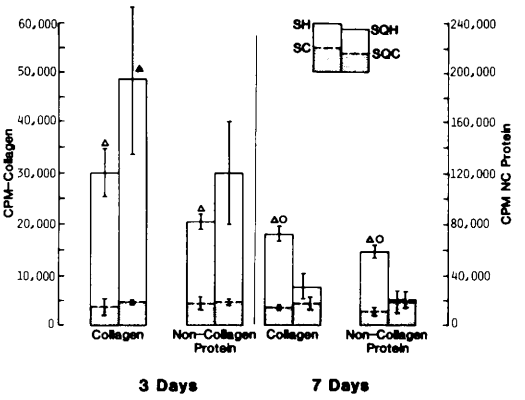

FIG. 3. [^{14}C]Glycine incorporation into collagen and noncollagen protein of control rats and rats exposed to hypobaric hypoxia for 3 or 7 days. See Fig. 1 legend for explanation of abbreviations and key to symbols.

t test for multiple comparisons, was not significant due to a large standard error. By 7 days, there was a dramatic decrease in *in vitro* synthesis of C and NC in SQH compared to SH as C and NC synthesis in SQH were reduced to near control levels (SQC).

Discussion. In previous studies from this laboratory, we have demonstrated that the development of pulmonary hypertension results in both hyperplastic and hypertrophic changes in the rat pulmonary trunk (10) and that the hypertrophic response included increased synthesis (9) and deposition (9, 10) of extracellular matrix protein, particularly collagen. Thus, the quantitative and temporal pattern of hypertension-induced changes in protein synthesis of the pulmonary trunk, in association with other markers for the development of pulmonary hypertension, provides a model system for studies of the regulation of pulmonary arterial pressure in the hypoxic rat. This model system has previously been used to determine the contribution of the sympathetic nervous system to the development of PH in the rat (14). In the present study of the role of angiotensin II in the development of hypoxia-induced pulmonary hypertension treatment with the angiotensin-converting enzyme inhibitor SQ 20881 resulted in decreased mean right-ventricular pressure (RVP) and protein synthesis in the pulmonary trunks of hypoxic rats after 7 days.

Right-ventricular pressure and right-ventricular hypertrophy were used to assess the development and/or regression of pulmonary hypertension in treated and untreated hypoxic rats. Previous studies have demonstrated almost identical patterns of development of RVP and RVH in hypoxic rats (9). In the present study, treatment with teprotide concurrent with hypoxic exposure produced a significant reduction in mean RVP after 7 days. This reduction corroborates the work of Kentera *et al.* (8) in which long-term treatment of rats with captopril during chronic hypoxic exposure resulted in a significant decrease in right-ventricular systolic and mean right-ventricular pressures. Although SQ 20881 treatment decreased RVP compared to untreated hypoxic rats in this study, there was no concurrent decrease in RVH. This result contradicts long-term treatment (3–4 weeks) with captopril or teprotide which resulted in significant decreases in RVH and a larger relative decrease in weight of the right ventricle (4, 8). This discrepancy is most likely due to the differences in duration of hypoxic exposure and treatment between this and the previous studies. Other factors indirectly related to SQ 20881 treatment may also have contributed to this discrepancy. After 3 days of hypoxic exposure, RV weight and RVH were significantly higher in treated vs untreated rats. Left-ventricular weight was also significantly elevated in SQH vs SH at this time. However, after 7 days RV weights had equalized while significant left-ventricular hypertrophy appeared in untreated rats. At this time LV weight of the treated hypoxic group was not significantly different from treated controls, resulting in a significantly larger RV/LV + S ratio in treated vs untreated hypoxic rats. In order to further understand the effect of SQ 20881 treatment on the development of RVH it may be necessary to completely dissociate hemodynamic from direct cardiac effects. At this point we are unable to explain the significant elevations in RV weight and RV/LV + S in normoxic treated vs untreated rats.

The significant inhibition of protein synthesis and deposition, as determined from analysis of changes in absolute PT protein content and *in vitro* incorporation of [^{14}C]-glycine into newly synthesized collagen and

noncollagen protein, was coincident with significantly decreased RVP in SQ 20881-treated hypoxic rats in the present study. Increased deposition of collagen in blood vessel walls has been routinely found to be a prominent feature of vascular hypertrophy in experimental (15, 16) studies of systemic hypertension as well as in pulmonary hypertension (9, 10, 17, 18) and appears to be positively correlated with increased blood pressure (8, 9, 19, 20, 21). The periods of 3 and 7 days of hypoxic exposure and SQ 20881 treatment used in the present study were chosen because they represent the peak periods for the hypertension-induced increases in collagen and noncollagen protein synthesis respectively in the rat pulmonary trunk (9). The pattern of changes in protein synthesis in SH vs SC reported here are almost identical to those previous studies thus indicating that the observed significant reduction in protein synthesis in the PT of treated rats was not due to variations in the model system. It is also possible that SQ 20881 treatment may have a direct inhibitory effect on vascular protein synthesis. This explanation, however, is unlikely since treatment did not result in any significant differences in tissue weight, absolute protein content or protein synthesis between normoxic groups at either 3 or 7 days. It appears likely, then, that SQ 20881 treatment reduced pressure-induced protein synthesis in the pulmonary trunk as a result of inhibiting conversion of AI to AII in the peripheral pulmonary vascular bed and thus reducing levels of AII available to induce local vasoconstriction.

Failure of SQ 20881 to alter RVP and protein synthesis after only 3 days of treatment may reflect a real lag-time in efficient inhibition of angiotensin-converting enzyme by the dosage of inhibitor used, perhaps due to increased converting enzyme levels (4, 22). Recent studies have reported decreased lung ACE levels after pulmonary hypertension has become well established in either monocrotaline or chronic hypobaric hypoxia models (5, 7). However, Szidon *et al.* (23) have demonstrated that decreased lung ACE activity during chronic hypoxia may reflect altered pulmonary hemodynamics rather than an absolute reduction in ACE activity. In addition, other studies suggest transient increases in lung ACE activity during the early development of hypoxia-induced PH in mice (3) or monocrotaline-induced PH in rats (6). It is also possible that other factors may regulate the development of PH during this early time period.

In conclusion, we have demonstrated the ability of converting-enzyme inhibition with SQ 20881 to significantly reduce RVP and protein synthetic capability in the rat pulmonary trunk during the early development of hypoxia-induced pulmonary hypertension. Furthermore, the results presented herein indicate that treatment with SQ 20881 did not directly inhibit protein synthesis but accomplished this through reduction of arterial pressure. Finally, the failure of ACE inhibition to prevent increased arterial pressure and associated increased protein synthesis at 3 days indicates that angiotensin II may play a role in the maintenance but not early induction of hypoxia-induced pulmonary hypertension.

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1. Ryan US, Ryan JW, Whitaker C, Chiv A. Localization of Angiotensin converting enzyme (kininase II). II. Immunocytochemistry and immunofluorescence. Tissue Cell **8**:125–145, 1976.
2. Berkov S. Hypoxic pulmonary vasoconstriction in the rat. The necessary role of angiotensin II. Circ Res **35**:256–261, 1974.
3. Molteni A, Zakheim RM, Mullis KB, Mattioli L. The effect of chronic alveolar hypoxia on lung and serum angiotensin I converting enzyme activity. Proc Soc Exp Biol Med **147**:263–265, 1974.
4. Zakheim RM, Mattioli L, Molteni A, Mullis KB, Bartley J. Prevention of pulmonary vascular changes of chronic alveolar hypoxia in inhibition of angiotensin I-converting enzyme in the rat. Lab Invest **33**:57–61, 1975.
5. Keane PM, Kay JM, Suyama KL, Gauthier D, Andrew K. Lung angiotensin converting enzyme activity in rats with pulmonary hypertension. Thorax **37**:198–204, 1982.
6. Molteni A, Ward WF, Ts'ao C-H, Port CD, Solliday NH. Monocrotaline-induced pulmonary endothelial dysfunction in rats. Proc Soc Exp Biol Med **176**:88–94, 1984.

7. Kay JM, Keane PM, Suyama KL, Gauthier D. Angiotensin converting enzyme activity and evolution of pulmonary vascular disease in rats with monocrotaline pulmonary hypertension. Thorax **37**:88–96, 1982.
8. Kentera D, Susic D, Cvetkovic A, Djordjivic G. Effects of SQ 14,225, an orally active inhibitor of angiotensin-converting enzyme, on hypoxic pulmonary hypertension and right ventricular hypertrophy in rats. Basic Res Cardiol **76**:344–351, 1981.
9. McKenzie JC, Klein RM. Protein synthesis in the rat pulmonary trunk during the early development of hypoxia-induced pulmonary hypertension. Blood Vessels **20**:283–294, 1983.
10. McKenzie JC, Clancy J Jr, Klein RM. Autoradiographic analysis of cell proliferation and protein synthesis in the pulmonary trunk of rats during the early development of hypoxia-induced pulmonary hypertension. Blood Vessels **21**:80–89, 1984.
11. Fulton RM, Hutchinson EC, Jones AM. Ventricular weight in cardiac hypertrophy. Brit Heart J **112**:413–420, 1952.
12. Newman RA, Langner RO. Comparison of TCA and collagenase in the isolation of tissue collagen. Anal Biochem **66**:175–184, 1975.
13. Miller RG. Simultaneous Statistical Inferences, McGraw-Hill, New York, p272, 1966.
14. McKenzie JC, Klein RM. The effect of neonatal guanethidine administration on hemodynamic and physical alterations in the adult rat pulmonary artery during the development of hypoxia-induced pulmonary hypertension. J Auton Nerv Syst **10**:199–203, 1984.
15. Wolinsky H. Response of the rat aortic wall to hypertension: Morphological and chemical studies. Circ Res **26**:507–522, 1970.
16. Folkow B. Cardiovascular structural adaptation; its role in the initiation and maintenance of primary hypertension. Clin Sci Mol Med **55**:35–225, 1978.
17. Meyrick B, Reid L. Hypoxia-induced structural changes in the media and adventitia of the rat hilar pulmonary artery and their regression. Amer J Pathol **100**:151–178, 1980.
18. Kameji R, Otsuka H, Hayashi Y. Increase of collagen synthesis in pulmonary arteries of monocrotaline-treated rats. Experientia **36**:441–442, 1980.
19. Iwatsuki K, Cardinale GJ, Spector S, Udenfriend S. Reduction of blood pressure and vascular collagen in hypertensive rats by β-aminopropionitrile. Proc Natl Acad Sci (USA) **74**:360–362, 1977.
20. Ooshima A, Midorikawa O. Increased lysyloxidase activity in blood vessels of hypertensive rats and effect of β-aminopropionitrile on arteriosclerosis. Jap Circul J **41**:1337–1340, 1977.
21. Sheridan PJ, Kozar LG, Benson SC. Increased lysyloxidase activity in aortas of hypertensive rats and effect of β-aminopropionitrile. Exp Mol Pathol **30**:315–324, 1979.
22. Boomsma F, deBruyn JHB, Derkx FHM, Schalekamp MADH. Opposite effects of captopril on angiotensin I-converting enzyme "activity" and "concentration"; Relation between enzyme inhibition and long-term blood pressure response. Clin Sci **60**:491–498, 1981.
23. Szidon P, Oparil S, Osikowicz G, Booyse FM. Effect of hypoxia on the conversion of angiotensin I to II in cultured porcine pulmonary endothelial cells. Biochem Pharmacol **32**:1201–1205, 1983.

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