Biochemical Pharmacology, Vol. 35, No. 4, pp. 714–716, 1986.
Printed in Great Britain.

0006-2952/86 $3.00 + 0.00

Polyamine content in rat lung during development of hypoxia-induced pulmonary hypertension*

(*Received* 30 *May* 1985; *accepted* 16 *August* 1985)

Pulmonary arterial hypertension, regardless of the specific etiology, leads to progressive medial arterial thickening which underlies sustained, structurally based increases in pulmonary vascular resistance [1]. The biochemical mechanisms which couple the initial insult to structural changes in the pulmonary circulation are not understood.

The polyamines, putrescine, spermidine and spermine, are recognized as important regulators of cell differentiation and proliferation [2]. Since cell differentiation and proliferation play central roles in the vascular remodeling accompanying pulmonary arterial hypertension [3–6], we have proposed that the polyamines may constitute an obligatory biochemical link between the initial insult and the hypertrophic and hyperplastic responses of the medial arterial smooth muscle. In support of this contention, we have shown that monocrotaline-induced pulmonary hypertension in rats is accompanied by early and prolonged increases in lung levels of ornithine decarboxylase (ODC, EC 4.1.1.17) [7] and *S*-adenosylmethionine decarboxylase (AdoMet-DC; EC 4.1.1.50) activities with attendant increases in lung levels of the polyamines [8]. Blockade of ODC with an enzyme-activated, highly selective inhibitor, α-difluoromethylornithine, inhibits the increase in lung polyamines and blunts the development of medial arterial thickening as well as the pulmonary hypertension [8, 9]. Although these observations support a role for polyamines in the monocrotaline model, if the polyamines are of general importance in the pathophysiology of pulmonary hypertension, they should play a role in other models as well. Accordingly, the present study tested the hypotheses that chronic normobaric hypoxia in rats elevates lung polyamine levels and that this change is temporally related to increases in pulmonary artery pressure and the evolution of right ventricular hypertrophy.

Methods

Male Sprague–Dawley rats weighing 150–200 g were randomly divided into two groups of twenty-four animals per group. One group served as control and was housed in wire hanging cages in a room with a 12-hr photoperiod. Food and water were provided *ad lib*. The second group was housed in a plexiglass chamber purged with nitrogen and compressed air to obtain an atmosphere of 10% O_2. Pressure inside the chamber was the same as the atmospheric pressure. A modified dehumidifier and fan system was built into the chamber to control the humidity and circulate the air. In addition, a cannister of barium hydroxide pellets was placed in-line with the circulator fan to absorb CO_2. Both O_2 and CO_2 levels were monitored continuously with the aid of appropriate electrodes and a chart recorder.

Animals were studied at 1, 4, 7 and 10 days after initiation of hypoxic exposure. Six animals from each group were killed at each time point, and total lung content of the polyamines (putrescine, spermidine, and spermine), mean pulmonary artery pressure, and the extent of right ventricular hypertrophy were determined as previously described [7, 8]. Briefly, animals were anesthetized with an intraperitoneal injection of sodium pentobarbital (60 mg/kg), tracheostomized, and ventilated with a Harvard small animal respirator. After a median sternotomy, a 20-g needle attached to a Statham pressure transducer was inserted through the right ventricular free wall and advanced into the main pulmonary artery. Pulmonary artery pressure was recorded on a Grass polygraph and mean pressure, averaged over 5 heart beats, was calculated as the diastolic pressure + 1/3 pulse pressure. After determination of pulmonary arterial pressure, the lungs and heart were removed, and the extent of right ventricular hyperterophy was assessed as the quotient of the weight of the right ventricular free wall and the weight of the left

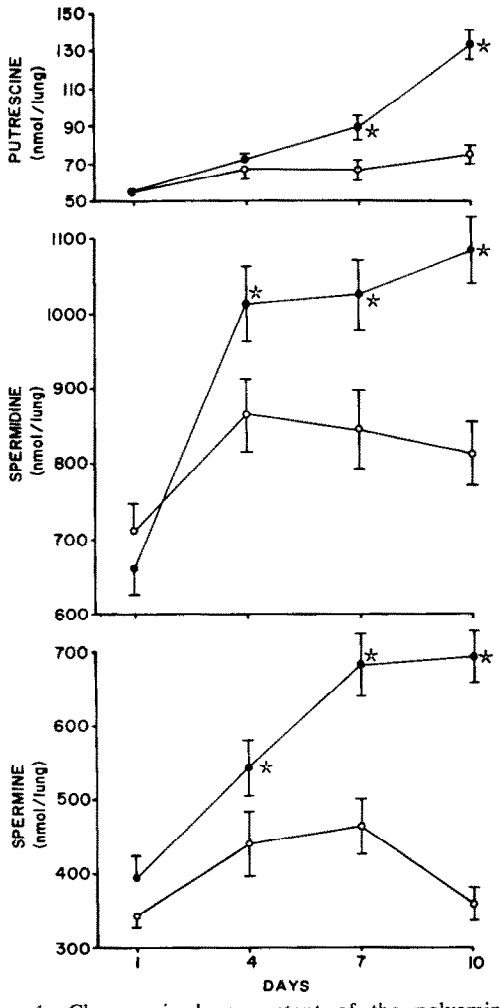

Fig. 1. Changes in lung content of the polyamines, putrescine, spermidine and spermine, as a function of time after exposure to normobaric hypoxia. Each point represents the mean ± S.E. (bars) of duplicate determinations of six rats. Key: (○—○) control, and (●—●) hypoxic. An asterisk (*) indicates significantly different from control at $P < 0.05$.

* Supported in part by grants from the Kentucky Affiliate of the American Heart Association, The Isabel Kircher Research Fund, and Grant HL-23709 from the National Heart, Lung and Blood Institute. R.J.A. is a Parker B. Francis Fellow of the Puritan-Bennett Foundation.

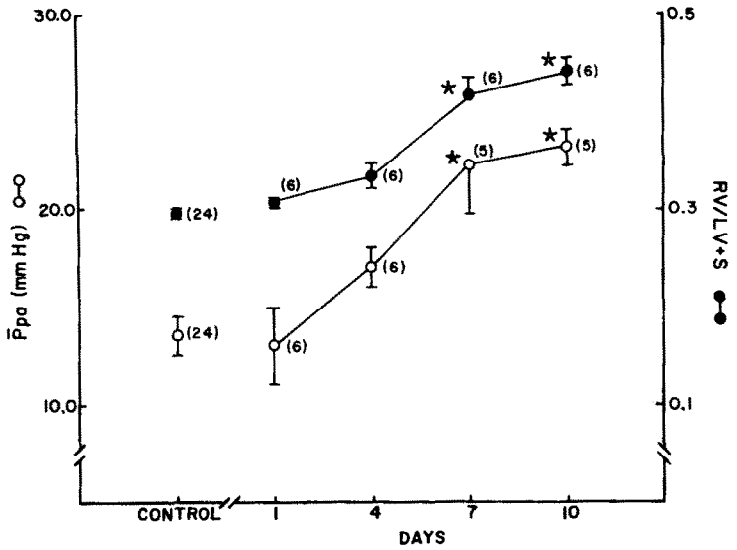

Fig. 2. Changes in mean pulmonary artery pressure and development of right ventricular hypertrophy expressed as a function of time after exposure to normobaric hypoxia. The numbers in parentheses equal the number of animals. An asterisk (*) indicates significantly different from control at $P < 0.05$.

ventricle plus septum (RV/LV+S). The total lung contents of putrescine, spermidine and spermine were determined by a high performance liquid chromatographic technique according to procedures described by Hacker *et al.* [10].

Results are expressed as the mean ± the standard error. A two-way analysis of variance combined with the Newman–Kuels test was used to detect significant time-dependent differences. Differences were considered significant when $P < 0.05$.

Results and discussion

The impact of chronic hypoxia on lung levels of putrescine, spermidine, and spermine is shown in Fig. 1. Polyamine levels in control lungs varied somewhat as a function of time; therefore, control values were not pooled for either statistical analysis or for graphical representation. The level of putrescine in lungs from hypoxic rats was significantly greater than time-matched controls at days 7 and 10 after initiation of hypoxia. Both spermidine and spermine levels were significantly greater than time-matched controls by day 4 and remained elevated throughout day 10.

As illustrated in Fig. 2, chronic hypoxia caused a progressive increase in mean pulmonary arterial pressure and right ventricular hypertrophy. Mean pulmonary arterial pressure in control animals did not vary as a function of time, thereby permitting these values to be pooled for subsequent analysis. In hypoxic rats, mean pulmonary arterial pressure tended to be elevated by day 4 and was significantly increased at days 7 and 10. Right ventricular hypertrophy, expressed as the ratio RV/LV+S, was apparent at days 7 and 10.

The major observations of this study are that chronic normobaric hypoxia in rats increased lung polyamine levels and that the increases in these organic cations coincided in time with the evolution of pulmonary hypertension and right ventricular hypertrophy. Since the polyamines are established regulators of cell growth and differentiation [2], these findings support the hypothesis that the polyamines couple the initial hypoxic stimulus to remodelling of the pulmonary vasculature.

The precise events underlying hypoxia-induced vascular remodeling are not completely understood. Differentiation of fibroblasts [6] or pericytes [3] as well as division of existing smooth muscle cells [5] have been suggested as possible mechanisms of the hyperplastic response of the medial arterial smooth muscle. It is interesting that polyamines are known to regulate differentiation of fibroblasts into adipocytes [11], thereby supporting the contention that fibroblast differentiation into new smooth muscle also may be a polyamine-dependent event. Based upon the established role of polyamines in other systems, they may be involved in pericyte differentiation and smooth muscle division as well.

Previous reports from our laboratories have suggested that monocrotaline-induced perivascular edema formation, pulmonary vascular hyperresponsiveness [12], medial arterial thickening [9], pulmonary hypertension and right ventricular hypertrophy [8] are polyamine dependent. We reasoned that, if polyamines are of general importance to the development of hypertensive pulmonary vascular disease, then they should play a role in models of pulmonary hypertension other than that induced by monocrotaline. The present study, by demonstrating a temporal relation between elevated lung polyamine levels and chronic hypoxia-induced pulmonary hypertension and right ventricular hypertrophy, supports the contention that polyamines may be of general importance in hypertensive pulmonary vascular disease. However, confirmation of this hypothesis awaits a thorough study of changes in ODC and AdoMet-DC activities during chronic hypoxia as well as the demonstration that blockade of polyamine synthesis inhibits medial arterial thickening and other major pathophysiologic sequelae of chronic hypoxia.

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Biochemical Pharmacology, Vol. 35, No. 4, pp. 716–718, 1986.
Printed in Great Britain.

0006-2952/86 $3.00 + 0.00

Stereoselective interactions of 2-[(2′,6′-dimethoxyphenoxyethyl)aminomethyl]-1,4-benzodioxane (WB-4101) with the calcium channel

(*Received* 17 *April* 1985; *accepted* 4 *September* 1985)

There have been numerous reports in the literature regarding the α-adrenergic blocking activity of various calcium channel blockers [1–4]. Several investigators have suggested that some of the clinical effects of verapamil, a calcium channel blocker, may be due to its α_1-adrenergic blocking effects [2–4]. Conversely, it appears that certain adrenergic blocking agents may have calcium channel blocking activity [5]. A study by Atlas and Adler [5] suggests that the low affinity binding sites of the ^{3}H-labeled α_1-adrenergic antagonist 2-[(2′,-dimethoxyphenoxyethyl)-aminomethyl]-1,4-benzodioxane ([^{3}H]WB-4101) may actually represent binding sites on the calcium channel.

To determine more directly whether WB-4101 interacts with binding sites closely linked to calcium channels, we examined its interaction with the receptor recently identified for the dihydropyridine calcium channel blockers [6–8]. We used enantiomeric pairs of WB-4101 to aid in differentiating specific from non-specific interactions. Our results suggest that, at higher concentrations than those needed for adrenergic blocking activity, WB-4101 interacts specifically with the dihydropyridine receptor and that these higher concentrations may be associated with calcium channel blocking properties.

Methods

Compounds. [^{3}H]Nitrendipine (71 Ci/mmole) was obtained from New England Nuclear (Boston, MA). The enantiomers of WB-4101 were synthesized by previously reported methods [9, 10].

Binding studies. Male Sprague–Dawley rats (200–300 g) were killed by cervical dislocation. After removal, the heart was rapidly perfused with 10 ml of ice-cold buffer (50 mM Tris–HCl, pH 7.4) to remove red cells. The perfused heart was stripped of great vessels and extraneous connective tissue and placed in ice-cold buffer to produce a concentration of 100 mg tissue/ml buffer. The tissue was homogenized on a Brinkmann Polytron homogenizer using three 5-sec bursts at 80% of the maximum speed. The resultant homogenate was centrifuged at 48,000 *g* for 15 min, and the supernatant fraction was discarded. This procedure was repeated twice. The pellet was resuspended in buffer to a final protein concentration of 0.1 mg/ml.

Incubations were carried out at 25° for 90 min. Saturation experiments were performed with concentrations of [^{3}H]-nitrendipine ranging from 0.02 to 1.0 nM. For displacement experiments, the concentration of [^{3}H]nitrendipine in the incubates was 0.05 nM. Concentrations of antagonists ranging from 1×10^{-7} M to 3.2×10^{-4} were present. Concentrations greater than 3.2×10^{-4} M were not employed because of limited quantities of the enantiomers. Incubations were terminated by rapid vacuum filtration over filters (Whatman GF/B) that were subsequently rinsed with 12 ml of ice-cold buffer. Bound radioactivity was determined by liquid scintillation counting of the filters. Counting efficiency ranged from 38 to 42%. Non-specific binding was determined as the amount of [^{3}H]nitrendipine bound in the presence of 10^{-6} M nifedipine and routinely accounted for 40–50% of total binding.

Pharmacologic studies. Male Sprague–Dawley rats (200–300 g) were killed by cervical dislocation. Longitudinal strips of the right ventricle (~5 mm in length, 2 mm in width) were obtained and placed in a 100 ml organ bath at 37° containing isotonic Krebs–Henseleit buffer (pH 7.4) and aerated with 95% O_2 and 5% CO_2. Muscle strips were mounted and attached to a Kistler–Morse model dSC-6 force displacement transducer. Contractions were induced by field stimulation at a rate of 2.0 Hz. After an equilibration period of 60 min at 1.5 g tension, the contraction signals were amplified and recorded (Gould model ES1000 Physiologic Recorder) at a tension of 1 g. Cumulative concentration–response curves for inhibition of isometric contractions by racemic WB-4101 were obtained. Because of limited quantities of the enantiomers, we could not carry out pharmacologic studies with these compounds.

Data analysis. The data from individual saturation experiments were expressed as amount bound and plotted against concentration of [^{3}H]nitrendipine. To obtain the K_d and B_{max} (maximum number of binding sites) of nitrendipine the data were fit by digital computer (FIT FUNCTION on the PROPHET SYSTEM) to the following equation:

$$\text{Amount bound} = \frac{B_{max} \times C}{K_d + C} + K_{ns} \times C$$

where C is the total concentration of nitrendipine in the incubation mixture and K_{ns} is the non-specific binding constant. Since the amount bound to the membrane particulates routinely accounted for less than 10% of the added amount of [^{3}H]nitrendipine, total approximated free concentrations.

The data from the displacement experiments were expressed as a percentage of specifically bound [^{3}H]nitrendipine and plotted against the log concentration of the displacing agent. The data ranging from 20 to 80% specifically bound [^{3}H]nitrendipine were fit by log-linear least squares regression analysis. The IC_{50} was obtained by calculating the concentration at which the specifically bound [^{3}H]nitrendipine was 50%. Data were handled in this way instead of with traditional 3–4 parameter logistic equations because maximum displacement of [^{3}H]nitrendipine was not obtained.