Biochemical Pharmacology, Vol. 42, No. 7, pp. R9–R12, 1991.
Printed in Great Britain.

0006-2952/91 $3.00 + 0.00

RAPID COMMUNICATION

INHIBITION OF HYPOXIC PULMONARY VASOCONSTRICTION BY DIPHENYLENEIODONIUM

Henry M. Thomas, III,* Roger C. Carson, Ethan D. Fried and Richard S. Novitch

Division of Pulmonary and Critical Care Medicine, Cornell University Medical College, Will Rogers Pulmonary Research Laboratory, 785 Mamaroneck Ave., White Plains, NY 10605, U.S.A.

(Accepted 24 July 1991)

In contrast to systemic arteries, pulmonary arteries constrict in response to hypoxia and thereby optimize gas exchange. Despite intensive investigation, it is unknown how a pulmonary vessel senses the local oxygen concentration and initiates smooth muscle contraction [1, 2]. Recently, Acker et al.[3] deduced from light absorbance spectra that NADPH oxidase was present in the carotid body; furthermore, in the carotid body, the NADPH oxidase inhibitor diphenyleneiodonium (DPI) blocked two effects of hypoxia: the change in the light absorbance spectrum [3] and, importantly, the neural discharge [4]. Thus, NADPH oxidase appeared to be a sensor protein for hypoxia, possibly transducing PO_2 by varying the production of oxygen free radicals [3, 4]. Similarly, NADPH oxidase could have a role in hypoxic pulmonary vasoconstriction, perhaps by a mechanism involving superoxide anion or hydogen peroxide [5, 6].

Thus, the possibility that NADPH oxidase acts as a physiological oxygen sensor was assessed in isolated, perfused rat lungs by examining the effect of DPI on hypoxic vasoconstriction. Our goals were: (1) to demonstrate the effect of DPI on hypoxic pulmonary vasoconstriction, and (2) to compare the effect of DPI on hypoxic vasoconstriction with the effect of DPI on vasoconstriction produced by other agents.

METHODS

Isolated, perfused and ventilated rat lung preparation. Male Sprague-Dawley rats (300 g) were studied using the isolated lung model [7]. The lungs were perfused at 10 mL/min with recirculating physiological salt solution containing 4 g/100 mL Ficoll and 0.0157 mM meclofenamate [7]. Perfusate pH was maintained between 7.35 and 7.45 and temperature at 38 ± 0.1°. The lungs were ventilated with 21% O_2, 5% CO_2, 74% N_2 and, during hypoxia, with 5% CO_2 and 95% N_2. Pulmonary arterial perfusion pressure (PAP), airway pressure and lung weight were monitored. After 30 min stabilization, angiotensin II (AII) was added to the perfusate reservoir to prime the vasculature for testing pressor agents [7]; in Protocol 1 (see below), 23 ± 4 μM AII was used to increase PAP by 4 mm Hg at maximal response, whereas in Protocol 2, 13 μM AII was used and increased PAP by 2.1 ± 0.4 mm Hg. Five minutes after the AII, N^{ω}-nitro-L-arginine (NNA) was added to the perfusate (final concentration 470 μM) [8, 9] to inhibit nitric oxide synthase. Four minutes later, the first test cycle was started.

Protocol 1: Effect of DPI on vasoconstriction by AII and hypoxia. In each test cycle, a 100 ng bolus of AII was injected into the pulmonary artery during normoxia. Two minutes later, the lungs were exposed to hypoxia for 5 min, then to normoxia for 3 min before the next cycle was started. Three test cycles of AII and hypoxia were administered. The third cycle was used as the pre-drug baseline. DPI dissolved in 100% dimethyl sulfoxide (DMSO) was then added to give a final perfusate concentration of 0.5, 1, 2 or 4 μM DPI. Control studies were performed with DMSO alone. Three more cycles of AII bolus followed by 5 min hypoxia were given (5, 15 and 25 min after DPI). The peak increases in PAP produced by AII and by hypoxia were measured.

Protocol 2: Effects of DPI on vasoconstriction by AII and U46619 during continuous normoxia, and by hypoxia. In each of three cycles, a 100 ng bolus of AII was injected into the pulmonary artery, and 4 min later, a 50 ng bolus of the thromboxane analogue, U46619 (9,11-dideoxy-11α,9α-epoxymethanoprostaglandin F2α); 6 min was allowed for recovery. The third cycle was used as the pre-drug baseline. DPI dissolved in 100% DMSO was then added to give a perfusate concentration of 4 μM DPI; alternatively, DMSO alone was added. Three further cycles of AII and U46619 (4, 14 and 24 min after DPI) were given. Thereafter, a single 5 min exposure to hypoxia was administered.

* To whom all correspondence should be addressed.