Prostaglandins and Medicine 1: 117-120, 1978.

OXYGEN REQUIREMENT FOR PROSTAGLANDIN BIOSYNTHESIS

W. E. M. Lands, J. Sauter, and G. W. Stone
Department of Biological Chemistry, The University of Michigan, Ann Arbor, Michigan 48109 (reprint requests to WEML)

ABSTRACT

The formation of prostaglandins by vesicular gland cyclooxygenase can be regulated by O_2 concentrations below 30 μM (20 mm Hg) with an apparent K_m value of about 5 μM. This result suggests that most mammalian tissues might be expected normally to have sufficient O_2 for some synthesis, and that only occasionally, would the level of oxygen available within some tissues become marginally inadequate to sustain a high rate of prostaglandin biosynthesis.

INTRODUCTION

Two different substrates, oxygen and fatty acid, are needed for the oxidative cyclization of essential fatty acids to form prostaglandins and thromboxane (1). The accessibility of these substrates to the active cyclooxygenase enzyme can influence the rate at which prostaglandin biosynthesis may occur. For example, the fatty acid in tissues is normally bound in ester linkage, and cleavage to the non-esterified form (2) is required before prostaglandin biosynthesis can occur. Several recent reports have supported the concept that release of the fatty acid substrate from precursors by carboxyesterase action is a rate-limiting event controlling prostaglandin formation (3,4). Stimulation of this hydrolytic release may be a major mechanism for tissue responses to bradykinin and angiotensin (5,6) in which increased non-esterified arachidonate allows an increased biosynthesis of prostaglandins.

Non-esterified fatty acid may also increase in hypoxic conditions (7). This increase may result from stimulation of hydrolysis or because of insufficient cellular supplies of ATP to maintain normal fatty acid: CoA ligase activity. In the latter case, a slowed rate of acyl-CoA formation (and subsequent acyl transfer to glycerolipid) would allow the steady state level of non-esterified arachidonate to rise without stimulation of the normal rate of hydrolytic release. An increased level of non-esterified arachidonate (6) appears to facilitate increased rates of prostaglandin formation that are noted in hypoxia and ischemia (8,9). However, this situation provides a paradoxical feature in that decreasing the level of one substrate (oxygen) can increase the rate at which the overall biosynthesis from the two substrates occurs. Clearly, completely removing the oxygen available would reduce the amount of prostaglandin formed.

The following study was conducted to examine the limit to which different levels of oxygen availability can regulate the biosynthesis of prostaglandins by the cyclooxygenase.

MATERIALS AND METHODS

An acetone-dried particulate fraction was prepared from sheep vesicular glands as described by Wallach and Daniels (10) and activated in 0.66 mM phenol (11) for 30 min at room temperature. Arachidonate was purchased from Nuchek Prep and stored as a 15 mM solution in benzene. Aliquots were evaporated to dryness and then dissolved in 0.1 M Tris-chloride buffer (pH 8.5). A partially purified preparation was obtained by solubilization of the crude vesicular gland particles (20,000 xg pellet at pH 5) with Tween -40 and, following ammonium sulfate precipitation, chromatography on DEAE-cellulose.

Oxygenase activity was determined with a Yellow Springs Instrument Co. oxygen monitor fitted with 4 electrodes and a constant temperature bath set at 30 ± 0.1°C. An interval timer and electronic differentiator were attached to provide continual monitoring of both the oxygen content and the rate of oxygen consumption (dO_2/dt), which were directly displayed on a 2-pen recorder. The oxygen content was varied by slightly elevating the electrode and flushing the vapor space in the reaction chambers with N_2 until a desired level of dissolved oxygen was obtained. With the electrode replaced to the surface of the liquid, the oxygen content was constant with negligible drift.

RESULTS AND DISCUSSION

The cyclooxygenase reaction was limited by fatty acid concentration with a half-maximal rate provided by 5-20 µM arachidonate. The effect of oxygen concentration was examined at three different levels of fatty acid substrate (Fig. 1). All of the reactions attained an optimal velocity within 12 sec and then rapidly decreased so that the electronic differentiator was valuable in accurately measuring the rapid rates of reaction and assigning the peak velocity. The velocity was independent of the oxygen concentration above 30 µM oxygen. The oxygen and fatty acid in the reaction mixtures were rapidly consumed at oxygenation rates of 30 - 60 µM O_2/min.

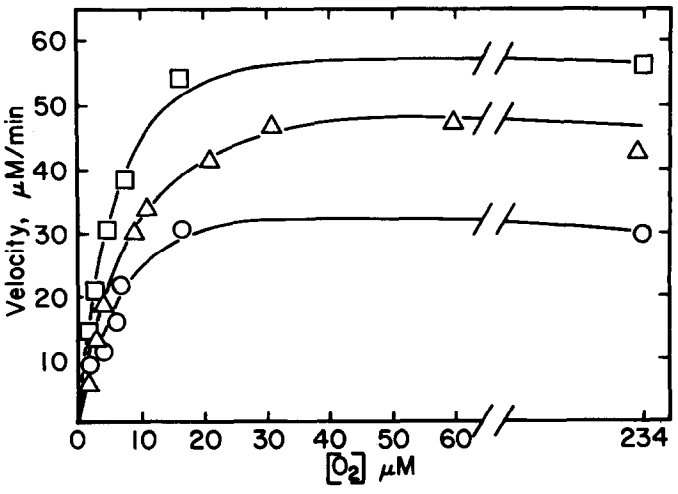

Figure 1. Influence of oxygen concentration upon the rate of sheep cyclooxygenase.

The DEAE-purified enzyme preparation was incubated with added hematin (0.67µM), 0.67mM phenol, and 10 (-O-), 25 (-Δ-), and 50 (- -)µM arachidonate in the presence of varied oxygen levels as indicated.

REFERENCES

1. Samuelsson, B. Ady. Prostaglandin and Thromboxane Res. 1, 1-6, 1976.

2. Lands, WEM, and Samuelsson, B. Biochim. Biophys. Acta 164, 426-428, 1968.

3. Vargaftig, BB, and Dao Hai, N. J. Pharm. Pharmacol. 24, 159, 1972.

4. Flower, RJ, and Blackwell, GJ. Biochem. Pharmacology 25, 285-291, 1976.

5. McGiff, JC, Itskovita, HD, Terragno, AT, Wong, PYK. Fed. Proc. 35, 1976.

6. Hsueh, W, Isakson, PC, and Needleman, P. Prostaglandins 13, 10-73-1091, 1977.

7. Markelonis, G, and Garbus, J. FEBS Letters 51, 7-10, 1975.

8. Block, AJ, Poole, S, and Vane JR. Circ Resh 36, 34-42, 1975).

9. Needleman, P, Key, SL, Isakson, PC, and Kulkarin, PS. Prostaglandins 9, 132-134, 1975.

10. Wallach, DP, and Daniels, EG. Biochim. Biophys, Acta 231, 445-457, 1971.

11. Smith, WL, and Lands, WEM. J. Biol. Chem. 246, 6700-6702, 1971.

12. Whalen, WJ, Nair, P, Burek, D, and Thuning GA. Am. J. Physiol. 227, 1221-1225, 1974.

13. Whalen, WJ, and Nair, P. Am. J. Physiol. 218, 973-980, 1970.

14. Jobsis, FF. in Handbook of Physiology. Respiration, Sect. 3, Vol. 1, Chapt. 2, p. 63-124, Am. Physiol. Soc., Washington, DC. 1964.

15. Hemler, M, Smith, WL, and Lands, WEM. J. Biol. Chem. 251, 5575-5579, 1976.

16. Markelonis, G, and Garbus, J. Prostaglandins 10, 1087-1106, 1975.

17. Moncado, S, Grygleioski, R, Bunting, S, and Vane, JR. Nature 263, 663-665, 1976.

The oxygen concentration giving half maximal velocity for a given level of fatty acid was about 5 μM. This result suggests that normal oxygen levels in tissue may seldom be a rate-limiting feature of prostaglandin synthesis. For example, the oxygen content of arterial blood was reported to be 89 mm Hg (~ 130 μM) while that of adjacent skeletal muscle averaged 17 mm Hg (~ 25 μM) (12). We can expect decreased oxygen contents at increased distances from the vascular endothelium (13). If a gradient similar to that measured in resting muscle existed in other tissues, oxygen concentrations approaching the half-maximal value of 5 μM would occur in regions that were 50 μ distant from the capillary wall. Thus, cells some distance from the capillaries may have sub-optimal oxygen contents while those at the luminal surface would tend to be always optimal for prostaglandin synthesis.

Total tissue oxygen is distributed among the various carriers in accord with their relative affinities. For instance, at pO_2 values of 10 mm Hg (ca. 15 μM), there is an 80% saturation of myoglobin when hemoglobin is only 10% saturated. The oxygen supply apparently does not limit cytochrome oxidase and respiration until the pO_2 becomes less than 4 to 6 mm Hg (6 to 9 μM) (14). Our results indicate that for prostaglandin formation, the rate-limiting step involving oxygen appears to have an affinity similar to that for cytochrome oxidase. Therefore, competition for oxygen among the various heme proteins and the heme prosthetic group of the cyclooxygenase (15) may reduce the synthesis of prostaglandins under conditions when respiration is also reduced. Any uncoupling of the mitochondrial oxidative phosphorylation by the increased non-esterified fatty acids during hypoxia could be expected to increase the tendency of the cytochrome respiratory chain to deplete further the already inadequate oxygen supply.

Free fatty acid may increase in liver mitochondria under hypoxic conditions (7) suggesting that increased substrate acids for prostaglandin synthesis might also become available in hypoxic or anoxic situations in many tissues (9,16) to force faster biosynthesis. If prostaglandins were formed in hypoxia at amounts greater than normal basal levels, one would conclude that the fatty acid supply rather than the oxygen supply was the rate-limiting aspect. If, however, less prostaglandins appear in an experimental hypoxic state, then oxygen content may have been regulatory. Such a differential response was noted for hypoxic and anoxic perfused rabbit hearts by Needleman et al. (9). Our current results suggest a decreased synthesis of prostaglandins and thromboxanes due to severe local anoxia is physiologically feasible, and may occur in cells under severe oxygen limitation. The recognized localization of prostacyclin synthase in vascular endothelium (17) with thromboxane synthase possibly located at deeper layers might allow persistent cyclooxygenase action at the vascular wall (with its relatively high O_2 content) to produce higher PGI_2/TXA_2 ratios than might occur at higher O_2 levels. Further studies will be needed to indicate more clearly those systems where such shifts do occur in physiologic response to lowered oxygen content.

ACKNOWLEDGEMENTS

Support for materials and equipment was provided by a research grant (NSF-BMS-7513157). Sheep vesicular glands were kindly provided by the Upjohn Company. We thank Dr. T. Zenser for his stimulating interest and discussions during the initial phase of this project.