Biochemical Pharmacology, Vol. 27, pp. 1515–1518.

0006-2952/78/0601-1515/$02.00/0

COMMENTARY

EXPERIMENTAL CRITERIA FOR EVALUATING PROSTAGLANDIN BIOSYNTHESIS AND INTRINSIC FUNCTION

PHILIP NEEDLEMAN
Department of Pharmacology, Washington University Medical School, St. Louis, MO 63110, U.S.A.

Perusal of most current biomedical journals will quickly indicate the tremendous outpouring of papers that deal with prostaglandins (PG). This rapid expansion of information has largely been based on the discovery of the biosynthesis, chemistry and biological properties of new arachidonic acid metabolites including the endoperoxides [1] and thromboxane [2], and of 6-keto-$PGF_{1\alpha}$ and PGI_2 [3–9]. The flood of new information has either been enlightening or confusing depending upon the reader's background or especially on the adequacy of the experimental protocols to generate unambiguous data. In an effort to cope with this ever-expanding literature and to establish basic guidelines for our investigations, we have adopted certain criteria for evaluating experimental data involving prostaglandins. These criteria will be the subject of this commentary.

Arachidonate metabolic pathway—Vintage 1977

The prostaglandins are synthesized by virtually every tissue in the body [10] and have been detected in the venous effluent from numerous mammalian organs. The release of PG from organs such as kidney and heart can be stimulated by various means including: hormones (e.g. bradykinin and angiotensin 2), nerve stimulation or exogenous neurotransmitters, mechanical damage and decreased oxygen tension [11–18]. Since there is no evidence for storage of PG, release reflects *de novo* biosynthesis [18, 19]. However, few, if any, prostaglandins are detected in arterial blood, largely because of the efficient pulmonary destruction by PG-dehydrogenase [12–18]. Thus, PG should be considered as local hormones which are synthesized at or near their site of action and are largely inactivated (locally) by diffusion and dilution. Prostaglandins formed in any tissue have the capacity to affect local vascular tone. In addition, endogenous biosynthesis of PG by blood vessels can modulate or mediate vascular responses to hormones, neuronal activity or changes in oxygen tension.

Prostaglandins are formed by the enzymatic oxygenation of certain polyunsaturated fatty acids [20]. The most abundant precursors are 5,8,11,14-eicosatetranoic acid (arachidonic acid) and 8,11,14-eicosatrienoic acid (dihomo-γ-linolenic acid), which form PG of the 2, and 1 series respectively [20–22]. However, there is a lack of free fatty acid precursor available, since fatty acids are almost entirely bound to phospholipids and triglycerides. Thus, formation of PG must be preceded by the activation of lipases (e.g. phospholipase A_2) (Fig. 1) which release bound fatty acid [23–26]. Released PG precursor (principally arachidonic acid) is acted upon by an enzyme, cyclo-oxygenase, with the incorporation of oxygen [20] resulting in the formation of the cyclic endoperoxides, PGG_2 and PGH_2 (Fig. 1). Indomethacin and other aspirin-like drugs inhibit this enzyme and, therefore, abolish biosynthesis of all prostaglandins [27]. Arachidonic acid is also converted by a lipoxygenase in platelets to a non-cyclized product, 12-hydroxy-eicosatetraenoic acid (HETE) [1]. Lipoxygenase as well as the cyclo-oxygenase is inhibited by eicosatetraynoic acid (ETYA) [28] (Fig. 1).

The endoperoxides possess considerable biological activity (contract smooth muscle and cause platelet aggregation [1, 29, 30]) and serve as intermediates for the synthesis of other prostaglandins. The endoperoxides are unstable in aqueous media and spontaneously decompose ($T_{\frac{1}{2}}$ of 4–6 min) to a mixture of PGE_2 and PGD_2. The endoperoxides are enzymatically converted to a variety of products (Fig. 1). Each tissue may possess differing synthetic enzymes that require endoperoxide substrate and, therefore, produce different types and amounts of

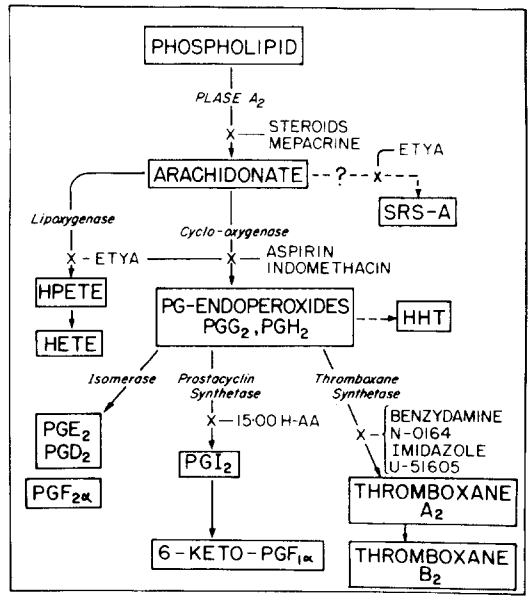

Fig. 1. Sequence of enzymatic steps (in italics) in the biosynthesis of the primary prostaglandins and related products (in boxes) as well as some of the known enzymatic inhibitors (site of action denoted with an ×).

B.P. 27/11—A

PG. Furthermore, the various prostaglandins have different biological actions (either complementary or antagonistic). Thus, the ultimate response of an organ to activation of prostaglandin biosynthesis often represents the algebraic sum of a number of complex interactions. Platelets predominantly synthesize the potent labile vasoconstrictor and aggregatory substance thromboxane A_2, as well as HETE and 12-hydroxy-heptadecatrienoic acid (HHT) [1, 2]. Heart, blood vessels and stomach predominantly synthesize the vasodilator PGI_2 [2, 5–9]. Some tissues possess several enzymes which use the endoperoxides as substrate. For example, the rabbit kidney normally synthesizes from endogenous arachidonate the renal vasodilator PGE_2 as its primary metabolite; however, with ureter obstruction the kidney unmasks the synthesis of the vasoconstrictor thromboxane A_2 [31, 32]. Furthermore, the kidney has recently been found to have the enzymatic capacity to convert exogenous arachidonate into PGI_2 [33]. Thus, depending upon the experimental conditions the isolated perfused rabbit kidney can synthesize PGE_2, thromboxane A_2 or PGI_2. Similarly, the lung has been demonstrated to synthesize these three arachidonate metabolites [18, 34], and very recently it has been demonstrated that slow reacting substance of anaphylaxis (SRS-A) also appears to be an arachidonate metabolite [35].

Prostaglandin synthesis inhibitors

At present there are no highly specific, potent prostaglandin receptor antagonists available. Thus, the pharmacological modification of the prostaglandin system is limited to the use of inhibitors of enzymatic synthesis. The enzymatic conversion of PGH_2 to thromboxane A_2 is inhibited by: imidazole [32, 36, 37], benzydamine [38], the phloretin phosphonate analog, sodium-*p*-benzyl-4-[1-oxo-2-(4-chlorobenzyl)-3-phenyl-propyl]phenyl phosphonate (*N*-0164) [39, 40], the prostaglandin analogs, 9,11-azoprosta-5-13 dienoic acid (U-51605) [41] and 9,11-epoxymethano-prostanoic acid [42]. Hydroperoxy fatty acids block the synthesis *in vitro* of PGI_2 [43], but no inhibitors of the $PGH_2 \rightarrow PGE_2$ isomerase have been discovered. Unfortunately, these inhibitors of the enzymatic conversion of the endoperoxide are most effective in subcellular preparations and have either little or no effect in intact organs or *in vivo*.

Problems encountered in experiments with exogenous prostaglandins or with prostaglandin–cyclo-oxygenase inhibitors

The ubiquitous synthesis of prostaglandins and their wide variety of biological effects stimulated substantial research in evaluating these compounds as potential mediators or modulators of physiological and pathological processes. Initial experimentation largely focused on the utilization of exogenous PG to mimic and produce biological responses. However, mimicking the response of a known stimulus with exogenous prostaglandin does not necessarily indicate that the endogenous process following the stimulus is actually due to PG. The discovery that aspirin-like drugs can inhibit the prostaglandin-synthetase (cyclo-oxygenase) [27] provided an important tool to determine the impact of intrinsic prostaglandin biosynthesis. The utilization of exogenous PG and inhibitors of cyclo-oxygenase must be validated and recognition of certain pitfalls is essential.

Some of the problems encountered with exogenous prostaglandins are: (a) available compounds are utilized (e.g. PGE_2, $PGF_{2\alpha}$, PGA_2, PGE_1, etc.) rather than determining the endogenous substance produced by the system under study; (b) exogenous prostaglandins are readily degraded *in vivo* prior to reaching their site of action; (c) biosynthesis at the site of action (e.g. blood vessels) would produce high local agonist concentrations that would be very difficult to match with exogenous substances; and (d) exogenous substances could systemically activate other receptor sites or humoral systems, and produce actions different from locally synthesized PG.

Among the problems encountered with prostaglandin–cyclo-oxygenase inhibitors are: (a) inadequate dose employed for complete inhibition; thus, treatment is uncertain unless PG levels are measured, (b) differences in the duration of action of the numerous cyclo-oxygenase inhibitors; (c) differential sensitivity of tissues to the various inhibitors, e.g. platelets are much more sensitive to aspirin than are blood vessels; and (d) blockade of the synthesis of all prostaglandin products by eliminating the availability of the endoperoxide as substrate for the numerous synthetases and reductase, and, therefore, elimination of desired metabolites.

Criteria that endogenous prostaglandin mediates a biological process

The strongest evidence for a prostaglandin being a mediator of a physiological or pathological event requires the isolation, identification and quantitation of the PG produced. The prostaglandin assay techniques available include: (a) biological assay, especially by employing selected smooth muscles in a superfusion cascade; (b) radiochemical experiments which utilize isotope labeling of precursors; (c) radioimmunoassay; (d) receptor binding assay; and (e) gas chromatography–mass spectrometry. Each of these approaches has inherent advantages and disadvantages, but the subject of methods is beyond the scope of this commentary. However, when appropriate attention is devoted to controls, standard curves, recovery calculations and, especially, when more than one technique is employed, valid data have been obtained.

We believe the following parameters should be fulfilled to establish that endogenous prostaglandin mediates or modulates a physiological or pathological event: (a) the concentration of the produced mediator (i.e. PG) should be proportional to the dose of the stimulus (e.g. hormone stimulation with bradykinin, or angiotensin, norepinephrine, etc.); (b) there should be temporal and quantitative correlation between changes in concentration of the PG with changes in the functional status of the organ; (c) exogenous administration of the identified mediator should mimic the physiologic response; (d) abolition of the synthesis of the PG should abolish the physiologic action of the stimulus; and

(e) exogenous administration of the precursor of the mediator should produce the physiologic response.

Thromboxane A_2 and PGI_2 characteristics

The identity of the arachidonate product involved relies specifically on the chemical and biological properties of the metabolite under investigation. The identification of thromboxane A_2 should require that the generated substance: (a) contracts isolated blood vessels; (b) is enzymatically synthesized (e.g. by platelet microsomes) from either arachidonic acid (but not dihomo-γ-linolenic acid) or PGH_2 (but not PGH_1) [44]; (c) has an aqueous decay $T_{\frac{1}{2}}$ of 30 sec; (d) aggregates platelets [2]; and (e) spontaneously forms the stable metabolite thromboxane B_2 (identified by thin-layer chromatography in several solvent systems, radioimmunoassay, or gas chromatography–mass spectrometry). Furthermore, its biosynthesis *in vitro* should be blocked by a thromboxane synthetase antagonist such as imidazole or U-51605 [36, 37, 41].

The identity of PGI_2 requires that the generated substance: (a) relaxes isolated bovine or canine coronary artery assay strips [33, 45, 46]; (b) inhibits platelet aggregation [3]; (c) is synthesized from arachidonic acid (but not dihomo-γ-linolenic acid) or PGH_2 (but not PGH_1) [8]; (d) is unstable in aqueous solution especially in acid conditions; and (e) is degraded to the stable 6-keto-$PGF_{1\alpha}$ [3–9]. Furthermore, its biosynthesis should be blocked *in vitro* by hydroperoxy fatty acids [43].

General comment

In the tradition of the problem-solving approach that evolved in characterizing the autonomic nervous system, cyclic nucleotides, and receptors; criteria emerged as the benchmarks by which experiments are evaluated. With parameters for acceptable research, the intrinsic role of prostaglandins in biological processes might be ascertained and a rationale for pharmacological intervention might evolve. In addition, a framework is available from which the discovery and characterization of other prostaglandins and related products will probably emerge.

Acknowledgements—Work done in the author's laboratory was supported by NIH grants SCOR HL-17646, HE-14397 and HL-20787.

REFERENCES

1. M. Hamberg, J. Svensson and B. Samuelsson, *Proc. natn. Acad. Sci. U.S.A.* **71**, 3824 (1974).
2. M. Hamberg, J. Svensson and B. Samuelsson, *Proc. natn. Acad. Sci. U.S.A.* **72**, 2994 (1975).
3. S. Moncada, R. Gryglewski, S. Bunting and J. R. Vane, *Nature, Lond.* **263**, 663 (1976).
4. C. Pace-Asciak and L. S. Wolfe, *Biochemistry* **10**, 3657 (1971).
5. P. S. Kulkarni, R. Roberts and P. Needleman, *Prostaglandins* **12**, 337 (1976).
6. A. Raz, P. C. Isakson, M. S. Minkes and P. Needleman, *J. biol. Chem.* **252**, 1123 (1977).
7. R. J. Gryglewski, S. Bunting, S. Moncada, R. J. Flower and J. R. Vane, *Prostaglandins* **12**, 685 (1976).
8. P. C. Isakson, A. Raz, S. E. Denny, E. Pure and P. Needleman, *Proc. natn. Acad. Sci. U.S.A.* **74**, 101 (1977).
9. P. Needleman, P. S. Kulkarni and A. Raz, *Science, N.Y.* **195**, 409 (1977).
10. E. J. Christ and D. A. Van Dorp, *Advances in the Biosciences* (Ed. G. Raspe), Vol. 9, p. 35. Pergamon Press, New York (1972).
11. J. W. Aiken and J. R. Vane, *J. Pharmac. exp. Ther.* **184**, 678 (1973).
12. S. H. Ferreria and J. R. Vane, *Nature, Lond.* **216**, 868 (1967).
13. J. C. McGiff, N. A. Terragno, J. Colina, D. A. Terragno and A. Nasjletti, *Chemistry and Biology of the Kallekrein System in Health and Disease* (Ed. J. J. Pisano), in press.
14. J. C. McGiff and H. Itskovitz, *Circulation Res.* **33**, 479 (1973).
15. P. Needleman, G. R. Marshall and B. E. Sobel, *Circulation Res.* **37**, 802 (1975).
16. P. Needleman, S. L. Key, S. E. Denny, P. C. Isakson and G. R. Marshall, *Proc. natn. Acad. Sci. U.S.A.* **72**, 2060 (1975).
17. P. Needleman, *Fedn Proc.* **35**, 2376 (1976).
18. P. Piper and J. R. Vane, *Ann. N.Y. Acad. Sci.* **180**, 363 (1971).
19. P. W. Ramwell, J. E. Shaw, W. W. Douglas and A. M. Poisner, *Nature, Lond.* **210**, 273 (1966).
20. B. Samuelsson, *J. Am. chem. Soc.* **87**, 3011 (1965).
21. S. Bergstrom, H. Danielsson and B. Samuelsson, *Biochim. biophys. Acta* **90**, 207 (1964).
22. D. A. Van Dorp, R. K. Beerthuis, D. A. Nugteren and H. Vonkerman, *Nature, Lond.* **203**, 839 (1964).
23. W. Vogt, T. Suzuki and S. Babilli, *Mem. Soc. Endocr.* **14**, 137 (1966).
24. W. Hsueh, P. C. Isakson and P. Needleman, *Prostaglandins* **13**, 1073 (1977).
25. P. C. Isakson, A. Raz, S. E. Denny, A. Wyche and P. Needleman, *Prostaglandins*, **14**, 853 (1977).
26. B. B. Vargaftig and N. Dao Hai, *J. Pharm. Pharmac.* **24**, 159 (1972).
27. J. R. Vane, *Nature, Lond.* **231**, 232 (1971).
28. A. L. Willis, D. C. Kuhn and H. J. Weiss, *Science, N.Y.* **183**, 327 (1974).
29. M. Hamberg, J. Svensson, T. Wakabayaski and B. Samuelsson, *Proc. natn. Acad. Sci. U.S.A.* **71**, 345 (1974).
30. M. Hamberg, P. Hedqvist, K. Strandberg, J. Svensson and B. Samuelsson, *Life Sci.* **16**, 451 (1975).
31. K. Nishikawa, A. R. Morrison and P. Needleman, *J. clin. Invest.* **59**, 1143 (1977).
32. A. R. Morrison, K. Nishikawa and P. Needleman, *Nature, Lond.* **267**, 259 (1977).
33. P. Needleman, S. D. Bronson and A. Wyche, *J. clin. Invest.*, in press.
34. W. Dawson, J. R. Boot, A. F. Cockerill, D. N. B. Mallen and D. J. Osborne, *Nature, Lond.* **262**, 699 (1976).
35. B. A. Jakschik, S. Falkenhein and C. W. Parker, *Proc. natn. Acad. Sci. U.S.A.*, **74**, 4577 (1977).
36. P. Needleman, A. Raz, J. A. Ferrendelli and M. S. Minkes, *Proc. natn. Acad. Sci. U.S.A.* **74**, 1716 (1977).
37. S. Moncada, S. Bunting, K. Mullane, P. Thorogood, J. R. Vane, A. Raz and P. Needleman, *Prostaglandins* **13**, 611 (1977).
38. S. Moncada, P. Needleman, S. Bunting and J. R. Vane, *Prostaglandins* **12**, 323 (1976).
39. P. S. Kulkarni and K. E. Eakins, *Prostaglandins* **12**, 465 (1976).
40. K. E. Eakins and P. S. Kulkarni, *Br. J. Pharmac.* **60**, 135 (1977).
41. R. Gorman, G. Bundy, D. Peterson, F. Sun, O. Miller and F. Fitzpatrick, *Proc. natn. Acad. Sci. U.S.A.*, in press.

42. F. F. Sun, *Biochem. biophys. Res. Commun.* **74**, 1432 (1977).
43. S. Moncada, R. J. Gryglewski, S. Bunting and J. R. Vane, *Prostaglandins* **12**, 715 (1976).
44. P. Needleman, M. S. Minkes and A. Raz, *Science, N.Y.* **193**, 163 (1976).
45. P. Needleman, A. Raz, P. S. Kulkarni, E. Pure, A. Wyche, S. E. Denny and P. C. Isakson, *Biochemical Aspects of Prostaglandins and Thromboxanes*, p. 199. Academic Press, New York (1977).
46. G. J. Dusting, S. Moncada and J. R. Vane, *Prostaglandins* **13**, 3 (1977).