Title

The cyclo-oxygenase pathway in the avascular heart of the frog, Rana esculenta L.

Journal

Vascular Pharmacology, 15(4)

ISSN

1537-1891

Authors

Ghiara, P

Parente, L

Piomelli, D

Publication Date

1984

DOI

10.1016/0306-3623(84)90006-5

Gen. Pharmac. Vol. 15, No. 4, pp. 309–313, 1984
Printed in Great Britain.

0306-3623/84 $3.00 + 0.00

THE CYCLO-OXYGENASE PATHWAY IN THE AVASCULAR HEART OF THE FROG, *RANA ESCULENTA* L.

P. GHIARA*, L. PARENTE and D. PIOMELLI†
Department of Experimental Pharmacology, University of Naples, Via L. Rodinò 22, 80138 Naples, Italy

(*Received* 24 *November* 1983)

Abstract—1. A modification of Vane's cascade is reported, allowing the superfusion bioassay of prostaglandin-like substances (PLS) in the outflow of isolated and perfused heart of the frog *Rana esculenta L.*

2. Using both this technique and radioimmunoassay determination, the cyclo-oxygenase pathway in perfused frog heart has been investigated.

3. Arachidonate (AA) (2–20 μg) injected into the perfusing fluid, was transformed by the heart into PLS, as shown by the response of the bioassay tissues (rat stomach strip, chick rectum, rat colon). A compound capable of relaxing rabbit mesenteric artery and a rabbit aorta contracting substance were also generated. The release was inhibited by indomethacin (1.0×10^{-5} M).

4. Radioimmunoassay determination of PGE_2, TXB_2 and 6-keto-$PGF_{1\alpha}$ in frog heart effluent, before and after AA injection (20 μg), gave the following yields (ng/ml of effluent). Basal: $PGE_2 = 0.45 \pm 0.15$; $TXB_2 = 0.46 \pm 0.13$; 6-keto-$PGF_{1\alpha} = 2.21 \pm 0.3$. Following AA: $PGE_2 = 1.55 \pm 0.35$; 6-keto-$PGF_{1\alpha} = 3.4 \pm 0.4$; $TXB_2 = 1.00 \pm 0.06$.

5. Our results suggest that prostacyclin is a major product of the cyclo-oxygenase pathway in frog perfused heart. The biological significance of this finding is discussed in relation to both the absence of a coronary circulation in amphibians and to the spongy nature of frog myocardium.

INTRODUCTION

In amphibians, the development of a massive cutaneous and buccopharyngeal respiration dramatically alters the features of cardiac nourishment. The portion of blood which passes through skin and oral mucosa is there actively oxygenated and, entering in this form into the right auricle, mixes with the venous blood coming from the other regions of the body: the striking reduction of coronary vasculature, common to all amphibians (Grant and Regnier, 1926), is presumed to be a consequence of this change in respiratory patterns (Foxon, 1955). In fact, this lack of coronary supply is compensated by the transformation of the architecture of the ventricular wall into a complex interlacing of muscular fibers, separated by lacunar spaces extended to the epicardium, which allow the direct oxygenation of cardiac cells. In mammals, the products of the cyclo-oxygenase pathway (prostaglandins, thromboxane and prostacyclin, mainly synthesized starting from arachidonate, AA) take part in the control of vascular reactivity and blood pressure, modulating the vasoconstrictor action of pressor hormones and moderating adrenergic nervous activity (McGiff *et al.*, 1981).

Furthermore, platelet aggregation and vascular tone are adjusted by a balance between the vasodilating and anti-aggregatory actions of prostacyclin (PGI_2) and the vasoconstricting and pro-aggregatory actions of thromboxane A_2 (TXA_2), produced by platelets (Moncada and Vane, 1979).

In the isolated and perfused heart of the guinea-pig, not only PGI_2 is a potent dilator of the coronary vasculature, but it is also the major AA metabolite (Schrör *et al.*, 1978). A comparable predominance of PGI_2 biosynthesis in perfused rabbit and rat heart has been demonstrated (De Deckere *et al.*, 1977; Needleman *et al.*, 1978).

AA metabolism in amphibian heart has not so far been elucidated.

The objectives of the present study, performed on the Anuran *Rana esculenta* L., were:

(1) to devise a bioassay technique suitable for the investigation of the frog perfused heart;

(2) to define, both by bioassay and radioimmunoassay, the features of the cyclo-oxygenase pathway in this experimental model;

(3) finally, to compare these features with those of an Urodele heart, namely that of the newt, *Triturus cristatus* Laur.

MATERIALS AND METHODS

The cascade superfusion bioassay, developed by Vane (1964), has been widely applied to mammalian physiopharmacology of prostaglandins (Moncada *et al.*, 1978). In particular, the release of bioactive AA metabolites from isolated perfused organs has been studied by bathing the assay tissues with the outflow of the organs.

In applying this technique to isolated organs of poikilothermic vertebrates, some problems have to be solved, since mammalian and amphibian salines markedly differ both in ionic composition (especially Ca^{2+} concentration) and temperature. Therefore, the outflow of the perfused poikilothermic organ cannot bathe, as it is, the mammalian

*Present address: Laboratory of Immunopharmacology, Sclavo Research Center, Via Fiorentina 1, Siena, Italy.
†Author to whom correspondence should be addressed. Present address: Department of Pharmacology, College of Physicians and Surgeons of Columbia University, 360 W, 168th Street, NYC, NY 10032, U.S.A.

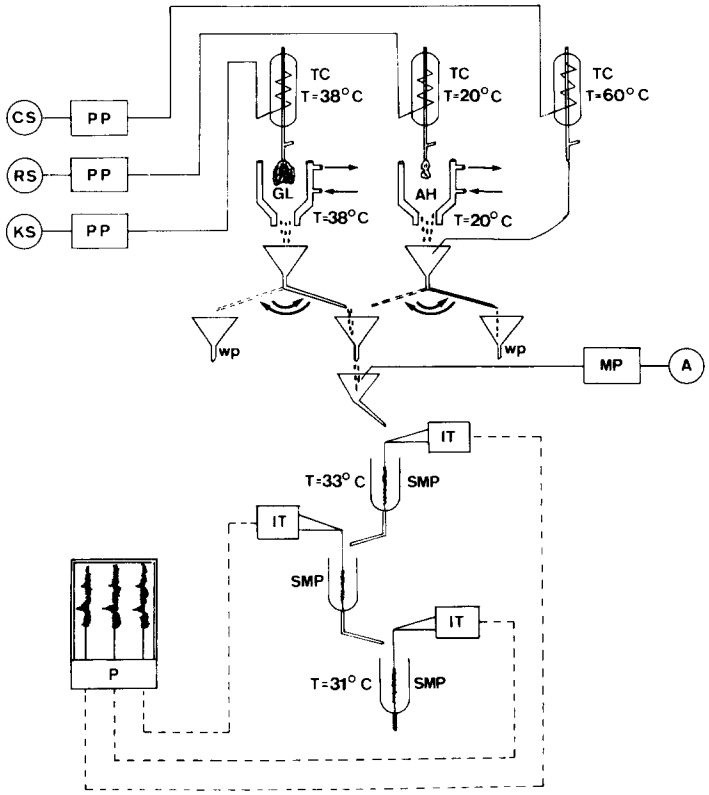

Fig. 1. Diagram of the experimental apparatus. Solid lines represent perfusion channels and connections; dashed lines represent electronic connections. A: antagonist mixture; AH: amphibian heart; CS: correcting solution (flow: 1.5 ml/min); GL: guinea-pig lungs; IT: isotonic transducer; KS: Krebs solution (flow: 3.0 ml/min); MP: micropump; P: Beckman polygraph; PP: peristaltic pump; R: polygraph recording; RS: amphibian Ringer solution (flow: 1.5 ml/min); SMP: smooth muscle preparations; TC: thermostating coils; WP: waste pipe.

assay tissues. In the modification of Vane's cascade proposed here, the effluent from amphibian heart is continuously mixed with a correcting solution capable of adapting it to the ionic and thermic requirements of mammalian smooth muscle preparations. A diagram of the experimental apparatus is shown in Fig. 1. Frog heart (AH) was perfused with amphibian saline via the left systemic artery at a constant flow of 1.5 ml/min (Table 1), maintained at a constant temperature (20°C) by means of a thermostating coil. The heart outflow was constantly mixed with an equal flow of correcting solution (Table 1) warmed at 60°C.

The resulting saline superfused the following smooth muscle preparations: rat stomach strip (RSS), rabbit aorta spiral strip (RbA), rabbit mesenteric artery (RbM), rat colon (RC) and chick rectum (CR).

The temperature of the assay tissues varied from 33 down to 31°C. Changes in muscle tension were recorded by means of isotonic transducers (IT) connected to a polygraph (P). A mixture of antagonists with the following composition was continously added by a micropump to the superfusing saline: methysergide maleate (2.7×10^{-7} g/ml), propranolol HCl (2.6×10^{-6} g/ml), atropine sulfate (10^{-7} g/ml) and indomethacin (2×10^{-6} g/ml) (Moncada *et al.*, 1978).

Guinea-pig lungs were also perfused at a constant flow of 5.0 ml/min, with Krebs bicarbonate (Table 1), maintained at 37°C. The outflow could be directed either to a waste-pipe (WP), or to the assay preparations. In the latter case the outflow of AH was diverted to the WP. Thus, assay tissues could be superfused either with the mammalian or the amphibian heart effluent.

Animals

Frogs and newts of both sexes (weighing 60–80 and 10–20 g respectively) were housed in sinks containing circulating tapwater (20°C). Male Wistar rats (250–300 g), New Zealand rabbits (1.5–2.0 kg), guinea-pigs (300–400 g) and chicks (50–100 g) were also used.

Cannulation techniques

Frogs and newts were pithed and the heart exposed. A

Table 1. Composition of the salines: Concentrations expressed in g/l of solution

Fluid	NaCl	KCl	$CaCl_2$	$MgCl_2$	$MgSO_4 \cdot 7H_2O$	$NaHCO_3$	NaH_2PO_4	KH_2PO_4	Glucose
Krebs Bicarbonate $T = 37°C$	6.9	0.35	0.28		0.29	2.1		0.16	2
Amphibian Ringer $T = 20°C$	6.5	0.14	0.12			0.2			1
Correcting solution $T = 60°C$	9.72	0.72	0.44		0.54	0.39	0.33		4.4

polyethylene cannula was inserted into the left systemic artery and tied in place at the level of the truncus arteriosus. The heart was then excised and perfused according to Langendorff's technique (see above).

Guinea-pig isolated lungs were perfused through the pulmonary artery with Krebs bicarbonate (37°C) at 5 ml/min as described by Piper and Vane (1969).

Radioimmunoassay

After cannulation, 20 min of stabilization were given to the perfused beating heart. The effluent was collected at fixed intervals for 1 min both in basal conditions and after AA administration (20 μg) and left to rest for 2 hr at room temperature.

Radioimmunoassay (RIA) of PGE_2, 6-keto-$PGF_{1\alpha}$ and TXB_2 was performed according to Salmon and Flower (1979).

Substances

The following substances were used: arachidonic acid, histamine HCl (Sigma), indomethacin (Merck, Darmstadt), TXB_2 (Upjohn, Kalamazoo), PGE_2, PGI_2, PGF_2, 6-keto-$PGF_{1\alpha}$ (Wellcome Laboratories), ^{3}H-PGE_2, ^{3}H-TXB_2 and ^{3}H-6-keto-$PGF_{1\alpha}$ (The Radiochemical Center, Amersham). For PGE_2-, 6-keto-$PGF_{1\alpha}$- and TXB_2-antisera, see Salmon and Flower (1979).

RESULTS

Reliability of the bioassay technique

The functionality of isolated assay preparations in our experimental conditions was tested by injecting into the perfusing saline standard doses (up to 20 ng) of synthetic prostaglandins E_2, $F_{2\alpha}$ and I_2.

Responsivity of rabbit aorta to endoperoxides and TXA_2 was tested by injecting AA (5 μg) through a perfused guinea-pig lung and allowing the effluent to pass over the tissue. As shown in Fig. 2, where a typical tracing of these experiments is reported, responsivity and sensitivity of the assay preparations were not significantly affected by the change in superfusing saline.

Smooth muscle viability remained essentially unmodified in a range of at least 4 hr continuous work.

After each change of saline, tissue stabilization was usually reached in less than 1 hr.

Release of prostaglandin-like material and RCS from frog heart

Injection of AA (2–10 μg) into the saline perfusing the frog heart was followed by the release of prostaglandin-like substances (PLS), as shown by the contraction of rat stomach strip, rat colon and chick rectum (Fig. 3). Furthermore, a slight relaxation of rabbit mesenteric artery and contraction of rabbit aorta were observed (Fig. 2). A similar, dose-dependent release of PLS occurred after histamine injection (2.5–25 μg) (Fig. 4).

The production of PLS, rabbit mesenteric relaxing substance (RRS) and rabbit aorta contracting substance (RCS) was completely prevented by in-

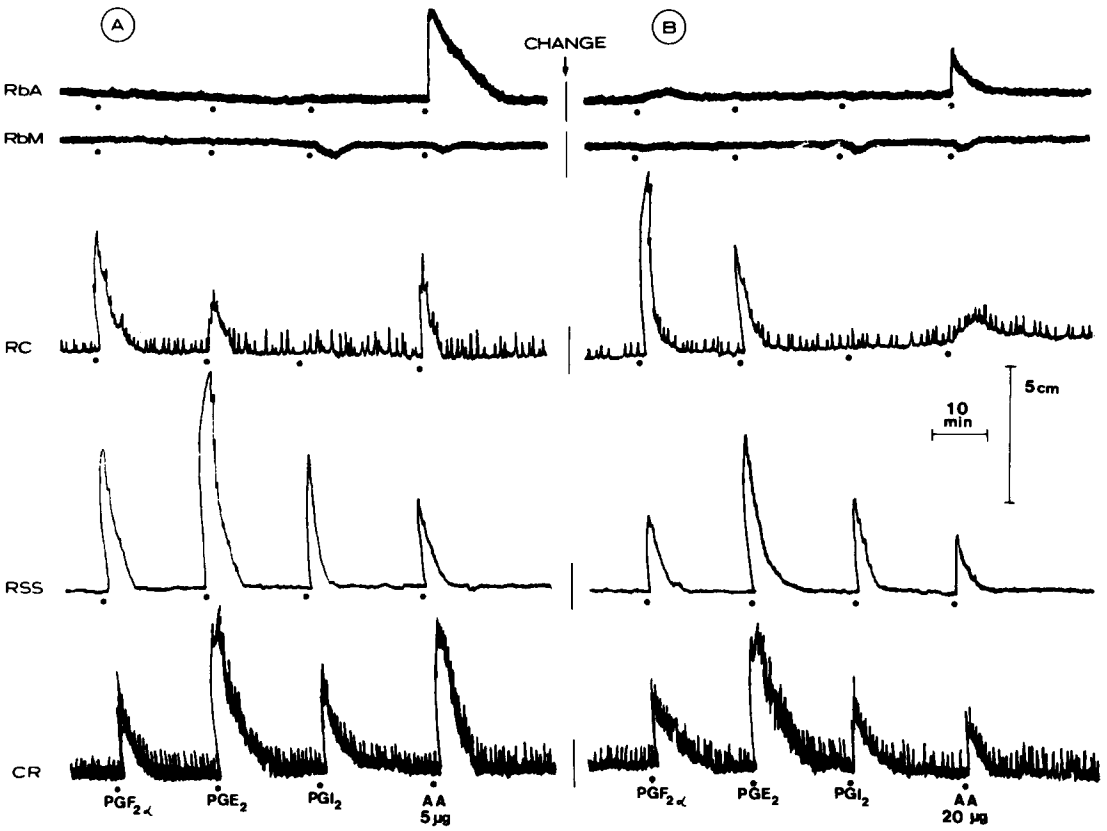

Fig. 2. Responsivity and sensitivity of the assay preparations superfused either with (A) Krebs bicarbonate or (B) with Ringer + correcting solution. RbA: rabbit aorta; RbM; rabbit mesenteric artery; RC: rat colon; RSS: rat stomach strip; CR: chick rectum. Prostaglandins were injected into the superfusing saline: arachidonate (AA) was administered via (A) guinea-pig lungs or (B) frog heart.

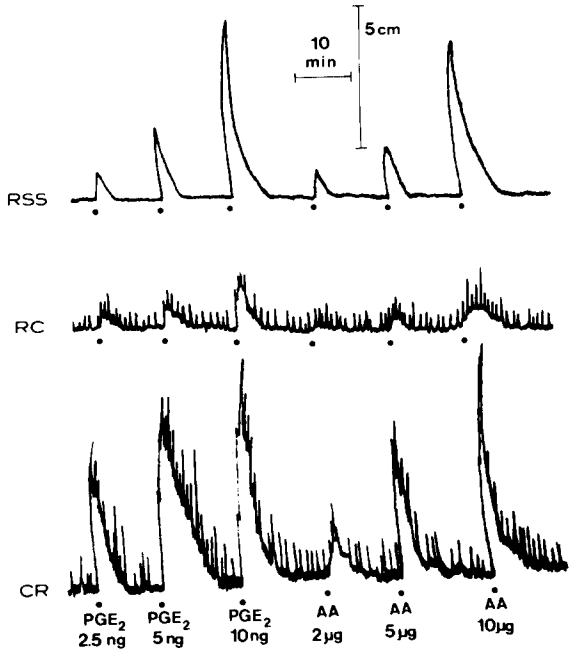

Fig. 3. Release of prostanoids from frog perfused heart, after arachidonate (AA) injection (2–10 μg), as detected by bioassay. The production was prevented by indomethacin (1.0×10^{-5} M) infusion (not shown in Fig.). RSS = rat stomach strip; RC = rat colon; CR = chick rectum. Prostaglandin E_2 was injected into the superfusing saline; arachidonate was administered via frog heart.

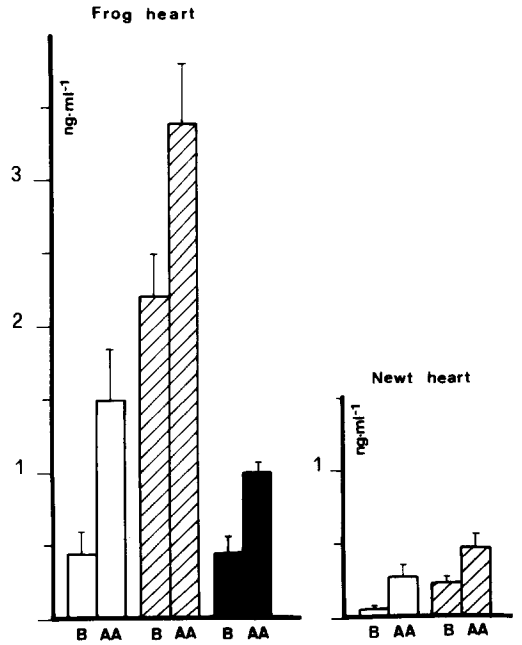

Fig. 5. Radioimmunoassay of frog and newt heart effluent. Values, expressed in ng/ml of heart outflow, are the mean ± SEM (frogs, $n = 12$; newts, $n = 9$). B: basal production; AA: after arachidonate administration (20 μg). Open columns = PGE_2; hatched columns = 6-keto-$PGF_{1\alpha}$; solid columns = TXB_2.

domethacin (1.0×10^{-5} M) treatment (not shown in figures).

Radioimmunoassay of frog and newt heart effluent

In a third series of experiments, perfused frog heart outflow was examined by RIA to determine PGI_2, PGE_2 and TXB_2 production, both in basal conditions and after injection of AA (20 μg).

PGI_2 and TXA_2 were detected from their stable metabolites, 6-keto-$PGF_{1\alpha}$ and TXB_2, respectively.

The average yields, expressed in ng/ml of heart effluent ± SEM (see Fig. 5) in basal conditions were: $PGE_2 = 0.45 \pm 0.15$; 6-keto-$PGF_{1\alpha} = 2.21 \pm 1.01$; $TXB_2 = 0.46 \pm 0.14$. After AA stimulation: $PGE_2 = 1.55$; 6-keto-$PGF_{1\alpha} = 3.4 \pm 1.8$; $TXB_2 = 1.00 \pm 0.06$. Similarly, the presence of PGE_2 and 6-keto-$PGF_{1\alpha}$ was detected in newt heart effluent. The average yields (ng/ml effluent ± SEM) were as follows (Fig. 5): in basal conditions: $PGE_2 = 0.06 \pm 0.01$; 6-keto-$PGF_{1\alpha} = 0.24 \pm 0.05$. After AA stimulation: $PGE_2 = 0.27 \pm 0.8$; 6-keto-$PGF_{1\alpha} = 0.48 \pm 0.09$.

DISCUSSION

Administration of exogenous AA to the perfused frog heart was followed by the immediate formation of PLS, RCS and a rabbit mesenteric relaxing substance, as shown by bioassay experiments.

The release of bioactive compounds was reversibly blocked by indomethacin infusion, thus indicating that isolated frog heart can metabolize AA to PLS.

Similar results were obtained by stimulating the heart with histamine, a well known phospholipase A_2 activator. These findings extend to amphibians the previous knowledge on the mammalian heart (Needleman, 1976) and are in agreement with the ability, demonstrated by the blood vessels of several inframammalian species, to use both exogenous and

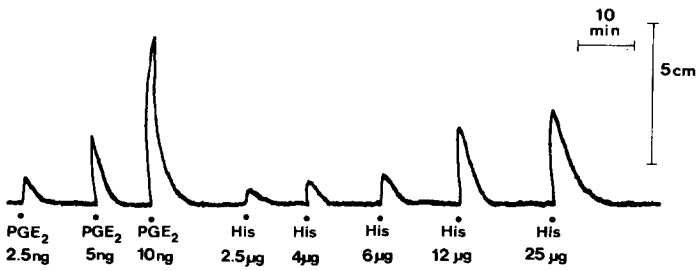

Fig. 4. Dose-related release of prostaglandin-like material, after histamine (His) injection (2,5–25 μg) through perfused frog heart. Bioassay preparation: rat stomach strip. Prostaglandin E_2 was injected into the superfusing saline; histamine was administered via frog heart.

endogenous AA as a cyclo-oxygenase substrate (Leffler *et al.*, 1980; Herman *et al.*, 1982; Piomelli *et al.*, 1983).

The occurrence of rabbit mesenteric relaxing substance and of RCS production after AA injection is also in agreement with the earliest findings reported by Schrör *et al.* (1978), who found a RCS activity in perfused guinea-pig heart effluent. In mammalian heart this activity is presumed to be due to non-metabolized endoperoxides (Schrör *et al.*, 1978; Sivakoff *et al.*, 1979) rather than to a mixture of TXA_2 and endoperoxides, as occurs in lungs, spleen and platelets (Moncada and Vane, 1979).

Furthermore, relaxation of rabbit coeliac and mesenteric arteries and of bovine coronary artery, observed by several authors (Moncada *et al.*, 1978) is a result of PGI_2 liberation from mammalian heart.

Our RIA results confirmed and extended those obtained by bioassay: in fact, a basal prostaglandin production from endogenous AA stores, undetectable with biological methods, was also observed.

Interestingly, 6-keto-$PGF_{1\alpha}$ (the stable metabolite of PGI_2) was the predominant AA product detected, both in the absence and presence of exogenous AA. PGI_2 is at the same time the most abundant product of the cyclo-oxygenase pathway in the isolated heart of rabbit, rat and guinea-pig (Schrör *et al.*, 1978; De Deckere *et al.*, 1979). Within the heart the coronary blood vessels have been identified as the major source of the total cardiac prostaglandins, while the myocardium is presumed to contribute only to a slight degree (Sivakoff *et al.*, 1979). Hence, the generation of PGI_2 may contribute to the protection of the coronary vessel wall against deposition of platelet aggregates.

Our results, similar to those obtained in mammals, suggest that PGI_2 is a major component of the prostaglandin system in frog perfused heart, thus indicating that the biological importance of this autacoid is retained also in a type of heart which is virtually devoid of coronary vasculature.

This conclusion is strengthened by the similar, partial results obtained in the newt, which shares the avascular organization of the frog heart, while being markedly different as regards many other cardiovascular features (Foxon, 1955).

As a matter of fact, in the intertrabecular spongework of the amphibian myocardium, where blood can be trapped for the duration of a systole, the presence of an effective anti-aggregating agent may possibly be required: PGI_2 might be a candidate for this function.

REFERENCES

De Deckere E. A. M., Nugteren D. H., Ten Hoor F. (1977) Prostacyclin is the major prostaglandin released from the isolated perfused rabbit and rat heart. *Nature* **268,** 160–163.

Foxon G. E. H. (1955) Problems in the double circulation of vertebrates. *Biol. Rev.* **30,** 196–228.

Grant R. T. and Regnier M. (1926) The comparative anatomy of the cardiac coronary vessels. *Heart* **13,** 205.

Herman C. A., McCloskey M. M. and Doolittle K. (1982) Prostaglandins of the one, two and three series affect blood pressure in american bullfrog, *Rana catesbeiana. Gen. comp. Endocr.* **48,** 491–498.

Leffler C. W., Hanson R. C. and Schneider E. G. (1980) Effects of prostaglandins and prostaglandin precursors on the arterial pressure of the bullfrog, *Rana catesbeiana. Comp. Biochem. Physiol.* **66C,** 199–202.

McGiff J. C., Spokas E. G. and Wong P. Y. K. (1981) Prostaglandin mechanisms in blood pressure regulation and hypertension. In *The Prostaglandin System* (Edited by Berti F. and Velo E. P.) pp. 235-246. Plenum Press, New York.

Moncada S. and Vane J. R. (1979) Pharmacology and endogenous roles of prostaglandin endoperoxides, thromboxane A_2 and prostacyclin. *Pharmacol. Rev.* **30,** 293-331.

Moncada S., Ferreira S. H. and Vane J. R. (1978) Bioassay of prostaglandins and biologically active substances derived from arachidonic acid. In *Advances in Prostaglandin and Thromboxane Research* (Edited by Fröhlich J. C.) Vol. 5, pp. 211–237. Raven Press, New York.

Needleman P. (1976) The synthesis and function of prostaglandins in the heart. *Fedn Proc. Fedn Am. Socs exp. Biol.* **35,** 2376–2381.

Needleman P., Bronson S. D., Wyche A., Sivakoff M. and Nicolaou K. C. (1978) Cardiac and renal prostaglandin I_2 biosynthesis and biological effects in isolated perfused rabbit tissues. *J. clin. Invest.* **61,** 839–849.

Piomelli D., Pinto A. and Tota B. (1984) The prostaglandin system in gills vascular function of teleost and elasmobranch fish. *J. comp. Physiol.* Submitted for publication.

Piper P. J. and Vane J. R. (1969) Release of additional factors in anaphylaxis and its antagonism by anti-inflammatory drugs. *Nature* **233,** 29–35.

Salmon J. A. and Flower R. (1979) Prostaglandins and related compounds. In *Hormones in Blood* (Edited Gray C. and James V. H. T.) Vol. 2, 3rd edn, pp. 237–319. Academic Press, New York.

Schrör K., Moncada S., Ubatuba F. B. and Vane J. R. (1978) Transformation of arachidonic acid and prostaglandin endoperoxides by the guinea pig heart. Formation of RCS and prostacyclin. *Eur. J. Pharmac.* **47,** 103–114.

Sivakoff M., Pure E., Hsueh W. and Needleman P. (1979) Prostaglandins in the heart. *Fedn Proc. Fedn Am. Socs exp. Biol.* **38,** 78–82.

Vane J. R. (1964) The use of isolated organs for detection of active substances in circulating blood. *Br. J. Pharmac.* **23,** 360–373.