J. Pharm. Pharmacol. 1983, 35: 724–728
Received January 5, 1983

Elimination from the circulation of cats of 6-keto-prostaglandin E_1 compared with prostaglandins E_2 and I_2

ULRICH FÖRSTERMANN* AND BRIGITTE NEUFANG

Department of Pharmacology, University of Freiburg, Hermann-Herder-Str. 5, D-7800 Freiburg, Federal Republic of Germany

6-keto-PGE_1, when injected intravenously (i.v.) or into the aortic arch of cats, produced similar dose-dependent decreases in mean arterial blood pressure. The hypotensive effect of PGI_2 was slightly less pronounced compared with that of 6-keto-PGE_1, but was equally potent after i.v. and intra-arterial administration. PGE_2 had a more potent depressor effect than the two other compounds when injected into the aortic arch, but was much less effective after i.v. injection. Plasma concentrations of 6-keto-PGE_1, 6-keto-$PGF_{1\alpha}$ and PGE_2 were measured by radioimmunoassay after bolus injections of 6-keto-PGE_1, PGI_2 and PGE_2. The half-life ($t\frac{1}{2}$) of the initial rapid decrease in immunoreactivity, indicating the rate of elimination, was found to be 1·04–2·19 min for 6-keto-PGE_1, 1·16–2·01 min for PGI_2 and 0·29–1·08 min for PGE_2. We have confirmed in the cat that 6-keto-PGE_1, like PGI_2 but unlike PGE_2, is not substantially inactivated by the lungs and thus could act as a circulating hormone. However, despite its higher chemical stability, the $t\frac{1}{2}$ of 6-keto-PGE_1 in the circulation is similar to that of PGI_2. PGE_2 has a shorter $t\frac{1}{2}$, probably due to its extensive pulmonary inactivation. Thus 6-keto-PGE_1 seems to be removed from circulation as quickly as PGI_2 by organs other than the lung and is unlikely to mediate prolonged effects of PGI_2 in the systemic circulation.

Prostacyclin (PGI_2), the main product of prostaglandin endoperoxide metabolism in blood vessels is a potent vasodilator and inhibits platelet aggregation (Moncada & Vane 1977). PGI_2 is chemically unstable ($t\frac{1}{2}$ at 37 °C and pH 7·6 approx. 3 min) and spontaneously breaks down to 6-keto-$PGF_{1\alpha}$. A new metabolic pathway has recently been described for prostacyclin and 6-keto-$PGF_{1\alpha}$ (Wong et al 1980a, b; Spokas et al 1981) leading to the formation of 6-keto-PGE_1 catalysed by a NAD^+ linked 9-hydroxyprostaglandin dehydrogenase (Wong et al 1980b). This enzyme has been observed in-vitro in rabbit liver (Wong et al 1980b), in human platelets (Wong et al 1980a) and in rabbit renal cortex (Spokas et al 1981; Gans & Wong 1981). The 6-keto-PGE_1-pathway is of special interest in that its product was shown to be equally potent (Wong et al 1979) or slightly less potent (Miller et al 1980) than PGI_2 as an inhibitor of platelet aggregation. Furthermore 6-keto-PGF_1 was found to possess potent vasodilating properties (Quilley et al 1979; Lippton et al 1980; Hyman & Kadowitz 1980; Jackson et al 1981). These findings have led to the hypothesis that 6-keto=PGE_1, formed by some tissues from either PGI_2 or 6-keto-$PGF_{1\alpha}$, could act synergistically with PGI_2 and might mediate some prolonged effects of PGI_2 (Quilley et al 1979; Wong et al 1980b). One presupposition for this would be, that 6-keto-PGE_1, like PGI_2, is not substantially inactivated in the lungs and thus could act as a circulating hormone. This has been claimed by Quilley et al (1979) in the rat and Hyman & Kadowitz (1980) in the cat. However, Tsunoda et al (1982) demonstrated in dogs that 6-keto-PGE_1 underwent more efficient pulmonary extraction than PGI_2. We have therefore compared the rates of elimination of 6-keto-PGE_1, PGI_2 and PGE_2 in cats.

MATERIALS AND METHODS

Cats of either sex (2·7–4·3 kg) were anaesthetized with sodium pentobarbitone (30–40 mg kg^{-1}, i.v.), intubated and respired artificially to give an endtidal CO_2-concentration of 3·5–4·0 vol%. Rectal

* Correspondence.

temperature was maintained between 37 °C and 38 °C using a heating pad. Polyethylene cannulae (PE 60) containing heparinized 0·9% (w/v) saline were inserted into different vessels, for measurement of blood pressure using a pressure transducer (Statham, P23Db) connected to a pen recorder (Watanabe WTR 281), for the administration of prostanoids, and for collecting blood samples. Unless used otherwise, all catheters were continuously infused with saline (2·5 ml h^{-1}) to prevent clotting. After completion of surgery the animals were heparinized (500 i.u. kg^{-1}, i.v.). A time interval of 90 min passed before the beginning of each experiment.

Blood pressure

The hypotensive response to pre- and post-pulmonary infusions of PGI_2, PGE_2, and 6-keto-PGE_1 were used to assess the extraction of the compounds by the lungs. A venous catheter was advanced from the right femoral vein into the right atrium, and an arterial catheter pushed down from the left carotid artery into the aortic arch. Systemic blood pressure was measured from the left femoral artery. Randomized doses of the three prostaglandins (10 ng kg^{-1} –10 μg kg^{-1}) were administered in a constant volume of 0·1 ml kg^{-1} and washed in with 0·5 ml of 0·9% NaCl. The sequence of intra-aortic and i.v. administration was changed in each experiment. After each injection blood pressure was allowed to return to normal and an interval 6 min allowed before the next dose was administered.

Radioimmunoassay

Systemic arterial pressure was measured from the left carotid artery. PGI_2, PGE_2, and 6-keto-PGE_1 (5 μg kg^{-1}) were injected into the right femoral vein. The interval between two drug administrations was 90 min. Blood samples (0·6 ml) were collected from the left femoral artery before and after the injections according to a time schedule. Blood was mixed in chilled tubes with 10%, v/v, of a solution containing Na_2EDTA (77 mM, pH 7·4) and indomethacin (0·1 mM, final concentration in blood) and immediately centrifuged to obtain the plasma which was stored at −20 °C until analysis. PGE_2 and 6-keto-$PGF_{1\alpha}$, the stable hydrolysis product of PGI_2, were determined directly by radioimmunoassays, described by Jobke et al (1973), Peskar & Hertting (1973) and Machleidt et al (1981). A goat anti-rabbit-γ-globulin (Calbiochem, San Diego, CA) was used to separate free and antibody-bound prostaglandins. The least detectable amounts were 170 pg ml^{-1} for PGE_2 and 52 pg ml^{-1} for 6-keto-$PGF_{1\alpha}$. Plasma concentrations of 6-keto-PGE_1 were determined using its relative cross-reactions with the antibody against PGE_2 (19% cross-reaction) and against 6-keto-$PGF_{1\alpha}$ (6% cross-reaction). The detection limits for cross-reacting 6-keto-PGE_1 were 860 pg ml^{-1} when the PGE_2-radioimmunoassay was used and 882 pg ml^{-1} with the 6-keto-$PGF_{1\alpha}$-radioimmunoassay. Plasma samples obtained after injection of 6-keto-PGE_1 were submitted to both assays, which produced comparable results. This mode of determination of 6-keto-PGE_1 was applicable despite the relatively low sensitivity attained, since after injection of 6-keto-PGE_1 plasma levels of 6-keto-PGE_1-like material stayed at measurable levels for at least 10 min, a period long enough to calculate the initial rapid component of elimination.

The immunoreactivities were characterized by thin layer chromatography. Two plasma samples (0·3 ml) obtained 1 min and 2 min after the injection of 6-keto-PGE_1 (5 μg kg^{-1}) were pooled. 1·4 ml of phosphate buffered saline was added and the sample acidified to pH 3·0 with 1 M HCl. The prostaglandins were extracted from the mixture using an XAD-2 resin column (Förstermann et al 1982). The extract was then submitted to thin layer chromatography (plates: Merck Kieselgel 60, solvent system: ethyl acetate–distilled water–isooctane–glacial acetic acid, 110:100:50:20, v/v, upper phase, two developments). The chromatogram was cut into 15 zones from origin to solvent front and the immunoreactivities in each zone were analysed in the radioimmunoassays for 6-keto-$PGF_{1\alpha}$ and for PGE_2 as described elsewhere (Machleidt et al 1981; Förstermann et al 1982).

Materials used

PGI_2 (Upjohn Co., Kalamazoo, MI) was dissolved (1 mg ml^{-1}) in 10 mM Tris buffer, pH 10·0. Stock solutions (1 mg ml^{-1}) of 6-keto-PGE_1 (Upjohn Co., Kalamazoo, MI) and PGE_2 (Sigma, Munich, FRG) were prepared in 2 mM Na_2CO_3. The stock solution of 6-keto-$PGF_{1\alpha}$ (1 mg ml^{-1}) was made in 300 μl of acetone and 700 μl of 2 mM Na_2CO_3. The solutions were kept at −20 °C. All prostaglandins were diluted identically with Tris-buffered saline, pH 8·5, immediately before use.

5,6,8,11,12,14,15-^{3}H-PGE_2 (spec. act. 200 Ci mmol^{-1}, New England Nuclear, Dreieich, FRG) was the tracer in the PGE_2-radioimmunoassay. 5,8,9,11,12,14,15-^{3}H-6-keto-$PGF_{1\alpha}$ (spec. act.

100 Ci mmol^{-1}, New England Nuclear, Dreieich, FRG) was the labelled ligand in the 6-keto-PGF$_{1\alpha}$-assay.

RESULTS

Blood pressure

No vehicle effects on arterial blood pressure were noted. PGI_2, 6-keto-PGE_1, and PGE_2 produced a dose-dependent fall in mean arterial blood pressure when injected into the aortic arch. Intravenous administration of the three compounds also produced dose-dependent relationships between log-dose and response. Responses to PGI_2 and to 6-keto-PGE_1 were not significantly different after either route of administration (Figs 1, 2). In contrast, PGE_2 proved to be about 30 times less potent when injected i.v. (Fig. 1). Intra-aortic PGE_2 was

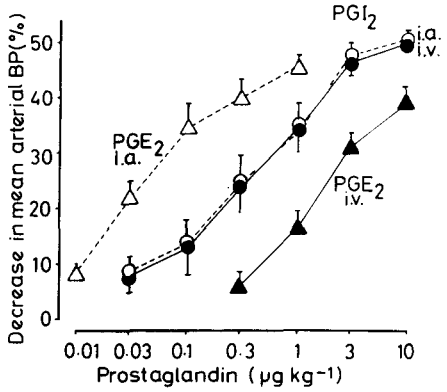

FIG. 1. Decrease in mean arterial blood pressure produced by PGI_2 administered i.v. into the right atrium (●——●, n = 4) or i.a. into the aortic arch (○- - -○, n = 4), and decrease in mean arterial pressure induced by PGE_2 injected i.v. into the right atrium (▲——▲, n = 5) or i.a. into the aortic arch (△- - -△, n = 5). Each point is the mean ± s.e.m. of the number of experiments indicated.

the most potent vasodepressor prostaglandin, causing a significant fall in blood pressure at 10 ng kg^{-1} (Fig. 1). 6-keto-PGE_1 proved to be slightly more potent in lowering systemic blood pressure than PGI_2 (compare Figs 1, 2). Both compounds significantly lowered blood pressure at 30 ng kg^{-1}. 6-keto-PGF$_{1\alpha}$ was without effect on blood pressure up to a dose of 10 µg kg^{-1} given i.v. or into the aortic arch (Fig. 2).

From the dose-response curves obtained with each cat the doses which produced a 20% decrease in blood pressure were derived. These data were then used to quantify the per cent pulmonary extraction of each compound in each animal, calculated as (i.v.-dose–i.a.-dose) × 100/i.v.-dose (Gerkens et al 1978).

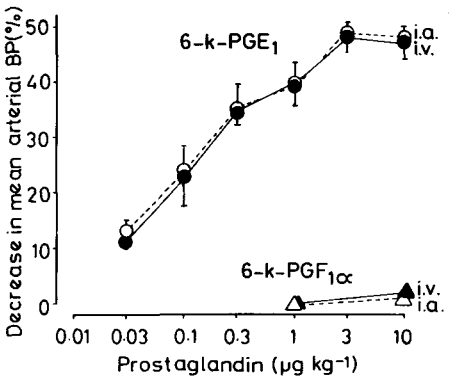

FIG. 2. Effect on mean arterial blood pressure produced by 6-keto-PGE_1 injected i.v. into the right atrium (●——●, n = 5) or i.a. into the aortic arch (○- - -○, n = 5) and effect of two doses of 6-keto-PGF$_{1\alpha}$ administered by both routes (both n = 3). Each point represents the mean ± s.e.m. of the number of experiments indicated.

As shown in Table 1 PGE_2 was extracted to more than 95%, whereas neither PGI_2 nor 6-keto-PGE_1 were significantly inactivated by the lungs.

Table 1. Percentage extraction (mean ± s.e.m.) of 6-keto-PGE_1, PGI_2 and PGE_2 by the pulmonary circulation.

Compound	% Extraction	Number of experiments
6-keto-PGE_1	5·71 ± 6·12	5
PGI_2	0·81 ± 7·95	4
PGE_2	95·72 ± 0·33	5

Radioimmunological measurements

The injection of PGI_2 (5 µg kg^{-1} i.v.) caused a rapid rise in plasma concentrations of 6-keto-PGF$_{1\alpha}$-immunoreactivity, which then decreased, as shown in Fig. 3. The t½ of the initial rapid decline (calculated between 1 min and 4 min), reflecting the rate of elimination, was found to be 1·16–2·01 min (mean 1·46 min). When 6-keto-PGE_1 was injected a similar graph was derived for the 6-keto-PGE_1-like material (Fig. 3). The t½ of the rapid decline was found to be 1·04–2·19 min (mean 1·41 min), which was not substantially different from data obtained for 6-keto-PGF$_{1\alpha}$-immunoreactivity after PGI_2 administration. Peak concentrations measured after 1 min were also similar. As the 6-keto-PGE_1-like material was determined using its cross-reactions in the radioimmunoassays for 6-keto-PGF$_{1\alpha}$ and PGE_2, the immunoreactive material was further characterized by thin layer chromatography. As shown in Fig. 4 the predominant immunoreactive material found by both assays after injection of 6-keto-PGE_1 into

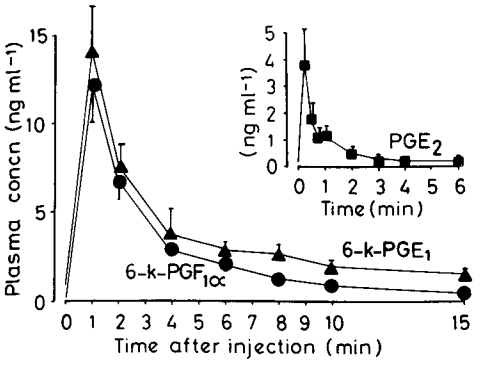

FIG. 3. Plasma concentrations of 6-keto-PGF$_{1\alpha}$-immunoreactive material (●——●, n = 10) after i.v.-injection of 5 μg kg^{-1} PGI$_2$, plasma concentrations of 6-keto-PGE$_1$-like material (▲——▲, n = 8) after i.v.-injection of 5 μg kg^{-1} 6-keto-PGE$_1$ as determined by its cross-reaction in the 6-keto-PGF$_{1\alpha}$-radioimmunoassay, and plasma concentrations of PGE$_2$-immunoreactive material (■——■, n = 5) after i.v.-injection of 5 μg kg^{-1} PGE$_2$ (insert). Each point represents the mean ± s.e.m. of the number of experiments given above.

the cat, co-chromatographed with authentic 6-keto-PGE$_1$.

After injection of PGE$_2$ (5 μg kg^{-1} i.v.) the maximal plasma concentrations of PGE$_2$-immunoreactivity attainable (measured after 0·25 min) were much lower than those of 6-keto-PGF$_{1\alpha}$- or 6-keto-PGE$_1$-like material after their i.v. injection (5 μg kg^{-1}). Additionally, plasma concentrations of PGE$_2$-immunoreactivity after injection of PGE$_2$ declined much more rapidly (Fig. 3). The t½ of the initial rapid component (calculated between 0·25 and 1·00 min after the injection) was found to be 0·29–1·08 min (mean 0·47 min).

Thus as far as pulmonary extraction and t½ in the circulation are concerned, 6-keto-PGE$_1$ has properties which are closely similar to PGI$_2$ but different from PGE$_2$.

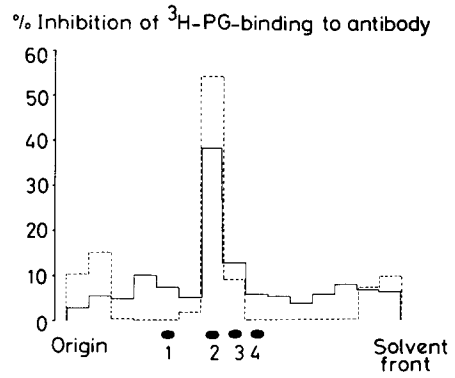

FIG. 4. Thin layer chromatogram on a silica gel plate (developed twice in ethyl acetate–distilled water–isooctane–glacial acetic acid, 110:100:50:20, v/v, organic phase) of a plasma pool obtained 1 and 2 min after the i.v.-injection of 5 μg kg^{-1} 6-keto-PGE$_1$ into a cat. The chromatogram was cut into 15 zones from origin to solvent front. The eluates of the different zones were submitted to a 6-keto-PGF$_{1\alpha}$-radioimmunoassay (continuous line) and to a PGE$_2$-assay (broken line). The predominant immunoreactive peaks in both assays co-chromatographed with authentic 6-keto-PGE$_1$. Standards: spot 1: 6-keto-PGF$_{1\alpha}$, spot 2: 6-keto-PGE$_1$, spot 3: 6,15-diketo-PGF$_{1\alpha}$, spot 4: 6,15-diketo-13,14-dihydro-PGF$_{1\alpha}$.

DISCUSSION

This study shows that the PGI$_2$-metabolite 6-keto-PGE$_1$, in addition to its anti-platelet-aggregatory activity (Wong et al 1979; Miller et al 1980), has in the cat systemic vasodilating capacities, which are slightly greater than those of PGI$_2$ (see Figs 1, 2).

Our data confirm the observation of Hyman & Kadowitz (1980) that 6-keto-PGE$_1$ like PGI$_2$, is not significantly extracted during passage across the cat lungs. Similar results have been reported in the rat (Quilley et al 1979), lamb (Lock et al 1979) and dog (Van Dam et al 1981). All of these results are at variance with the apparent inactivation of 6-keto-PGE$_1$ in the dog pulmonary circulation reported by Tsunoda et al (1982). However we found an extensive extraction of PGE$_2$ in the lungs, which is in accordance with earlier well established findings (Ferreira & Vane 1967; Piper et al 1970). As the lung is the most important organ of clearance, the strong extraction of PGE$_2$ by this organ can explain its smaller maximal plasma concentrations reached, and its shorter t½ in the circulation.

Since 6-keto-PGE$_1$ escapes substantial pulmonary metabolism in the cat, it has a similar potential as PGI$_2$ to act as a circulating hormone. However, the in-vivo actions of prostaglandins at sites distant from their site of synthesis are limited by two factors: their chemical stability and their rate of systemic enzymatic inactivation. Of these two, the latter seems to be the more important, for under physiological conditions PGE$_2$ and PGF$_{2\alpha}$ show a remarkable chemical stability (Karim et al 1968), whereas PGI$_2$ has only a chemical t½ of about 3 min (Dusting et al 1978). In-vivo, however, the 'classical' prostaglandins are extracted from the circulation by the lungs within seconds (Granström & Samuelsson 1978; present results). Even PGI$_2$, which is not substantially inactivated by the lungs and thus may be expected to have a longer t½, disappears from the circulation with a t½ of much less than 3 min (Salmon et al 1979; Machleidt et al 1981; present results). Although 6-keto-PGE$_1$ is not inactivated in the cat lung it is obviously

rapidly metabolized by other tissues e.g. liver and kidney. Thus its longer chemical t½ seems to be of minor importance in the systemic circulation and it is thus unlikely that 6-keto-PGE_1 is responsible for the unexpectedly long duration of effect of PGI_2 (Higgs et al 1977; Armstrong et al 1978).

As we could not determine basal levels of endogenous 6-keto-PGE_1 with our radioimmunological technique, the data presented give no information about the conversion of endogenous PGI_2 to 6-keto-PGE_1 in the cat. It has recently been reported that endogenous plasma levels of 6-keto-PGE_1 are very low in humans (<30 pg ml^{-1}) and that exogenous PGI_2 is not converted to an appreciable extent to this metabolite in man (Jackson et al 1982). However, this does not completely exclude 6-keto-PGE_1 from perhaps being responsible for some biological actions of *endogenous* PGI_2 due to *localized* biotransformation. Additionally, prolonged in-vitro effects of PGI_2 such as stimulation of renin release (Whorton et al 1977) and inhibition of platelet aggregation (MacIntyre et al 1979) might be explained by the formation of 6-keto-PGE_1.

REFERENCES

Armstrong, J. M., Lattimer, N., Moncada, S., Vane, J. R. (1978) Br. J. Pharmacol. 62: 125–130

Dusting, G. J., Moncada, S., Vane, J. R. (1978) Ibid. 64: 315–320

Ferreira, S. H., Vane, J. R. (1967) Nature (London) 216: 868–873

Förstermann, U., Neufang, B., Hertting, G. (1982) Biochim. Biophys. Acta 712: 684–691

Gans, K. R., Wong, P. Y-K. (1981) Fed. Proc. Fed. Am. Soc. Exp. Biol. 40: 681

Gerkens, J. R., Friesinger, G. C., Branch, R. A., Shand, D. G., Gerber, J. G. (1978) Life Sci. 22: 1837–1842

Granström, E., Samuelsson, B. (1978) in: Frölich, J. C. (ed.) Advances in Prostaglandin and Thromboxane Research, vol. 5, Raven Press, New York, pp 1–13

Higgs, G. A., Moncada, S., Vane, J. R. (1977) Br. J. Pharmacol. 61: 137P

Hyman, A. L., Kadowitz, P. J. (1980) J. Pharmacol. Exp. Ther. 213: 468–472

Jackson, E. K., Herzer, W. A., Zimmerman, J. B., Branch, R. A., Oates, J. A., Gerkens, J. F. (1981) Ibid. 216: 24–27

Jackson, E. K., Goodman, R. P., Fitzgerald, G. A., Oates, J. A., Branch, R. A. (1982) Ibid 221: 183–187

Jobke, A., Peskar, B. A., Peskar, B. M. (1973) FEBS Lett. 37: 192–196

Karim, S. M. M., Devlin, J., Hillier, K. (1968) Eur. J. Pharmacol. 4: 416–420

Lippton, H. L., Chapnick, B. M., Hyman, A. L., Kadowitz, P. J. (1980) Prostaglandins 19: 299–310

Lock, J. E., Olley, P. M., Coceani, F., Hamilton, F., Doubilet, G. (1979) Ibid. 18: 303–309

MacIntyre, D. O., Steer, M., Galgoci, B., Khorana, J., Levine, L., Salzman, E. (1979) Abstracts of the Fourth International Prostaglandin Conference, p 73

Machleidt, C., Förstermann, U., Anhut, H., Hertting, G. (1981) Eur. J. Pharmacol. 74: 19–26

Miller, O. V., Aiken, J. W., Shebuski, R. J., Gorman, R. R. (1980) Prostaglandins 20: 391–399

Moncada, S., Vane, J. R. (1977) in: Kharash, N., Fried, J. (eds) Biochemical Aspects of Prostaglandins and Thromboxanes, Academic Press, New York, pp 155–177

Peskar, B. Hertting, G. (1973) Naunyn-Schmiedeberg's Arch. Pharmacol. 279: 227–234

Piper, P. J., Vane, J. R., Wyllie, J. H. (1970) Nature (London) 225: 600–604

Quilley, C. P., Wong, P. Y-K., McGiff, J. C. (1979) Eur. J. Pharmacol. 57: 273–276

Salmon, J. A., Mullane, K. M., Dusting, G. J., Moncada, S., Vane, J. R. (1979) J. Pharm. Pharmacol. 31: 529–532

Spokas, E. G., Wong, P. Y-K., McGiff, J. C. (1981) Fed. Proc. Am. Soc. Exp. Biol. 40: 548

Tsunoda, S., Jackson, E. K., Branch, R. A., Gerkens, J. F. (1982) Eur. J. Pharmacol. 77: 147–151

Van Dam, J., Fitzpatrick, T. M., Friedman, L. S., Ramwell, P. W., Rose, J. C., Kot, P. A. (1981) Proc. Soc. Exp. Biol. Med. 166: 76–79

Whorton, A. R., Misono, K., Hollifield, J., Frölich, J. C., Inagami, T., Oates, J. A. (1977) Prostaglandins 14: 1095–1104

Wong, P. Y-K., McGiff, J. C., Sun, F. F., Lee, W. H. (1979) Eur. J. Pharmacol. 60: 245–248

Wong, P. Y-K., Lee, W. H., Chao, P. H-W., Reiss, R. F., McGiff, J. C. (1980a) J. Biol. Chem. 255: 9021–9024

Wong, P. Y-K., Malik, K. U., Desiderio, D. M., McGiff, J. C., Sun, F. F. (1980b) Biochem. Biophys. Res. Commun. 93: 486–494