generation for modulation of vascular tone. *Clin. Sci. Mol. Med.*, in press.

McGIFF, J.C., TERRAGNO, D.A., TERRAGNO, N.A., COLINA, J. & NASJLETTI, A. (1976). Prostaglandins as modulators and mediators of kinins. In *Chemistry and Biology of the kallikrein-kinin system in Health and Disease*. Fogarty International Center Proceedings No. 27, ed. J.J. Pisano, K.F. Austen. U.S. Government Printing Office, Washington, pp. 267–273.

MONCADA, S. & VANE, J.R. (1977). Discovery of prostacyclin (PGX): A fresh insight into arachidonic acid metabolism. In *Biochemical Aspects of Prostaglandins and Thromboxanes*. Ed., N. Kharasch, J. Fried. Academic Press, London pp. 155–177.

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

VANE, J.R. & FERREIRA, S.H. (1976). Interactions between bradykinin and prostaglandins. In *Chemistry and Biology of the kallikrein-kinin system in Health and Disease*. Fogarty International Center Proceedings, No. 27, Ed. J.J. Pisano, K.F. Austen. U.S. Government Printing Office, Washington, pp. 255–266.

Activity of prostacyclin, a stable analogue, 6ß-PGI_1 and 6-oxo-$PGF_{1\alpha}$ on canine isolated parietal cells

A.H. SOLL & B.J.R. WHITTLE

Wadsworth V.A. Hospital, U.C.L.A. School of Medicine, Los Angeles, U.S.A.
and
Department of Prostaglandin Research, Wellcome Research Laboratories, Langley Court, Beckenham Kent BR3 3BS

Prostacyclin (PGI_2) and its stable 5–6 dihydro analogue, 6β-PGI_1, inhibit acid secretion from the perfused gastric mucosa of the rat *in vivo* and *in vitro* (Whittle, Boughton-Smith, Moncada & Vane, 1978). The present study concerns the activity of prostacyclin, its chemical breakdown product 6-oxo-$PGF_{1\alpha}$ and 6β-PGI_1 on [^{14}C]-aminopyrine accumulation, an index of acid secretory activity (Berglindh, Helander & Obrink, 1976; Soll, 1978a) in canine isolated parietal cells. Further, since the anti-secretory action of prostaglandin E_2 has previously been linked to reduction in the parietal cell level of cyclic AMP, (cAMP) the potential intracellular mediator of gastric secretion (Soll, 1978a; Major & Scholes, 1978), the effects of prostacyclin on cAMP levels and concomitant parietal-cell function were investigated.

Cells were isolated from the acid-secreting fundic area of the canine mucosa by sequential treatment with crude collagenase and EDTA, and a parietal-cell enriched (60–80% of the total cells) fraction was obtained from an elutriator rotor using a sedimentation velocity technique (Soll, 1978b). Cells were incubated for 20 min in a modified Hanks' medium (pH 7.4 at 37°C) containing histamine, and freshly prepared prostacyclin or the other prostaglandins. [^{14}C]-Aminopyrine ([^{14}C]-AP) accumulation, which depends on the ionization and trapping of this weak base within the parietal-cell during hydrogen-ion elaboration and secretion was measured in aliquots of the cells, and cAMP was determined by radio-immunoassay (Soll, 1978a).

Histamine (10^{-7} – 10^{-4} M) caused a dose-dependent uptake and trapping of [^{14}C]-AP in the cells, as previously shown (Soll, 1978a) and this was inhibited in a dose-dependent manner by concurrent incubation with the prostaglandin (10^{-9} – 10^{-5} M). Using a sub-maximal dose of histamine (10^{-5} M), the ID_{50} (dose causing 50% inhibition) was 0.9×10^{-6} M for prostacyclin, 5×10^{-7} M for 6β-PGI_1, 5×10^{-4} M for 6-oxo-$PGF_{1\alpha}$ and 10^{-8} M for PGE_2. The lower activity of prostacyclin compared to PGE_2 could reflect the rapid breakdown of prostacyclin under the incubation conditions used. In other systems where stability is of limited importance, prostacyclin is some 500–1,000 times more active than 6-oxo-$PGF_{1\alpha}$ and 20–200 times more active than 6β-PGI_1 (Whittle, *et al.*, 1978) whereas in the present study prostacyclin was less potent than this stable analogue.

Histamine (10^{-5} M) increased the cAMP levels in the parietal-cell fraction and this was dose-dependently inhibited by simultaneous incubation with prostacyclin (10^{-8} – 10^{-5} M) in the same concentration range which reduced the parietal-cell accumulation of ^{14}C-AP. As with PGE_2, higher concentrations (10^{-4} M) of prostacyclin revealed a biphasic relationship, with cAMP concentrations becoming elevated. This could reflect stimulation of adenylate cyclase in the few contaminating cells or the parietal cells themselves at high prostaglandin concentrations.

The present observations suggest that the potent gastric antisecretory actions of prostacyclin and its analogue *in vivo* (Whittle, *et al.*, 1978) are the result of direct effects on the parietal cells. As previously suggested for PGE_2 (Soll, 1978a; Major & Scholes, 1978) prostacyclin may exert its gastric antisecretory actions by a direct inhibitory effect on parietal-cell adenylate cyclase. It is not yet known whether prostacyclin, which can be generated by the gastric mucosa (Moncada, Salmon, Vane & Whittle, 1978), can act as an endogenous modulator of parietal cell function *in vivo*.

References

BERGLINDH, T., HELANDER, H.F. & OBRINK, K.J. (1976). Effects of secretagogues on oxygen consumption, aminopyrine accumulation and morphology in isolated gastric glands. *Acta. Physiol. Scand.*, **97**, 401–414.

SOLL, A.H. (1978a). Prostaglandin inhibition of histamine-stimulated aminopyrine uptake and cyclic AMP generation by isolated canine parietal cells. *Gastroenterology*, **74**, 1146.

SOLL, A.H. (1978b). The actions of secretagogues on oxygen uptake by isolated mammalian parietal cells. *J. Clin. Invest.*, **61**, 370–380.

MAJOR, J.S. & SCHOLES, P. (1978). The localization of a histamine H_2-receptor adenylate cyclase system in canine parietal cells and its inhibition by prostaglandins. *Agents & Actions*, **8**, 324–331.

MONCADA, S., SALMON, J.A., VANE, J.R. & WHITTLE, B.J.R. (1978). Formation of prostacyclin and its product 6-oxo-$PGF_{1\alpha}$ by the gastric mucosa of several species. *J. Physiol. Lond.*, **275**, 4–5P.

WHITTLE, B.J.R., BOUGHTON-SMITH, N.K., MONCADA, S. & VANE, J.R. (1978). The relative activity of prostacyclin (PGI_2) and a stable analogue 6β-PGI_1 on the gastrointestinal and cardiovascular system. *J. Pharm. Pharmac.*, **30**, 597–599.

Prostaglandin and thromboxane production by rat macrophages

N.S. CONNOR, E.M. DAVIDSON, M.V. DOIG & A.W. FORD-HUTCHINSON

Biochemical Pharmacology Research Unit, Department of Chemical Pathology, King's College Hospital Medical School, Denmark Hill, London, SE5 8RX

Macrophages derived from peritoneal inflammatory exudates have been shown to release substantial amounts of prostaglandin E (PGE) like activity during short term *in vitro* culture as measured by bioassay and radioimmunoassay (Bray, Gordon & Morley, 1974). In addition murine macrophages have been reported to release thromboxane B_2 (TxB_2), as determined by radioimmunoassay (Weidemann, Peskar, Wrogemann, Rietschel, Staudinger & Fischer, 1978), and 6-keto prostaglandin $F_{1\alpha}$ tentatively identified by thin layer chromatography (Humes, Bonney, Pelus, Dahlgren, Sadowski, Kuehl & Davies, 1977). In the present work we have used a more specific gas chromatography-mass spectrometry technique to examine the release of prostaglandins and thromboxanes from a pure population of rat peritoneal macrophages.

A mixed white cell population was collected by peritoneal lavage. The adherent cells were aged for 72 h prior to use, producing a pure macrophage population. These cells were then cultured for a further 48 h in fresh medium from which the prostaglandins were obtained by acidic extraction into diethyl ether. The prostaglandins and thromboxanes present in the sample were derivatized to form their methyl ester, methoxime, tertiarybutyldimethylsilyl ethers. These were chromatographed using a 5% OV 101 column interfaced to a VG Micromass 16B mass spectrometer.

In supernatants obtained from cultures of rat macrophages, containing 4×10^7 cells, there were consistently found nanogram amounts of PGE_2, $PGF_{2\alpha}$, 6-keto-$PGF_{1\alpha}$ and TXB_2, characteristic mass spectra being obtained for all these compounds. The release of these compounds along with β-glucuronidase was increased when the macrophages were exposed to opsonised zymosan. This work shows that 'resting' rat peritoneal macrophages synthesize prostaglandins and thromboxanes from endogenous arachidonic acid as shown by the presence of PGE_2, $PGF_{2\alpha}$, 6-keto-$PGF_{1\alpha}$ and TXB_2 in the supernatant. The work is being extended to provide quantitative data for the relative amounts of prostaglandins and thromboxanes produced in this system.

We are grateful to the Science Research Council (M.V.D.), Lilly Research, Ltd., and the Wates Foundation (E.M.D.) for financial support, to Dr. Pike of the Upjohn Company for the prostaglandin standards and to Professor M.J.H. Smith and Dr. W. Dawson for help and advice.

References

BRAY, M.A., GORDON, D. & MORLEY, J. (1974). Role of prostaglandins in reactions of cellular immunity. *Br. J. Pharmac.*, **52**, 453P.

HUMES, J.L., BONNEY, R.J., PELUS, L., DAHLGREN, M.E., SADOWSKI, S.J., KUEHL, Jr. F.A. & DAVIES, P. (1977). Macrophages synthesize and release prostaglandins in response to inflammatory stimuli. *Nature, Lond.*, **269**, 149–151.

WEIDEMANN, M.J., PESKAR, B.A., WROGEMANN, K., RIETSCHEL, E.Th., STAUDINGER, H. & FISCHER, H. (1978). Prostaglandin and thromboxane synthesis in a pure macrophage population and the inhibition by E-type prostaglandins or chemiluminescence. *FEBS Lett.*, **89**, 136–140.