Stimulatory effect of prostaglandin E₂ on 1 α ,25-dihydroxyvitamin D₃ synthesis in rats

Masamichi YAMADA,* Toshio MATSUMOTO,* $\ddagger$ Naoyuki TAKAHASHI, $\dagger$ Tatsuo SUDA $\dagger$ and Etsuro OGATA*

*Fourth Department of Internal Medicine, University of Tokyo School of Medicine, 3-28-6, Nejirodai, Bunkyo-ku, Tokyo 112, Japan, and $\dagger$ Department of Biochemistry, Showa University School of Dentistry, Shinagawa-ku, Tokyo, Japan

(Received 12 July 1983/Accepted 2 September 1983)

The effect of prostaglandin E₂ on accumulation in plasma of 1 α ,25-dihydroxy[³H]vitamin D₃ from 25-hydroxy[³H]vitamin D₃ was studied *in vivo* using vitamin D-deficient thyroparathyroidectomized rats. Intra-arterial infusion of 10–50 μ g of prostaglandin E₂/h caused a significant stimulation of 1 α ,25-dihydroxy[³H]vitamin D₃ production. No significant changes in plasma Ca²⁺ and P_i concentrations or urinary cyclic AMP excretion were observed after prostaglandin E₂ infusion. Theophylline did not enhance the effect of a submaximal dose of prostaglandin E₂ on 1 α ,25-dihydroxy[³H]vitamin D₃ production. These data indicate that prostaglandin E₂ stimulates plasma accumulation of 1 α ,25-dihydroxy[³H]vitamin D₃ independent of the adenylate cyclase/cyclic AMP system, and suggest that prostaglandin E₂ has a modulatory role in the regulation of 25-hydroxyvitamin D₃ 1 α -hydroxylase in the kidney.

It is well-established that 25(OH)D₃ is converted into its active metabolite, 1,25(OH)₂D₃, in the kidney by a mitochondrial enzyme, 1 α -hydroxylase. Parathyrin (Garabedian *et al.*, 1972; Rasmussen *et al.*, 1972; Fraser & Kodicek, 1973), P_i (Tanaka & Deluca, 1973; Hughes *et al.*, 1975; Baxter & Deluca, 1976), calcitonin (Galante *et al.*, 1972; Horiuchi *et al.*, 1979; Kawashima *et al.*, 1981a) and 1,25(OH)₂D₃ itself (Tanaka & Deluca, 1974; Larkins *et al.*, 1974; Omdahl, 1978) have been shown to affect the activity of the enzyme. However, there are some additional factors that can influence 1 α -hydroxylase (Fraser, 1980), and the exact mechanism of activation of the enzyme is still not well understood.

Prostaglandins have been shown to act as local regulators of cell functions and intracellular modulators for various hormonal actions (Samuelsson *et al.*, 1978; Harris *et al.*, 1979), and renal tissue is known to be rich in prostaglandins. According to a recent report by Sraer *et al.* (1982), prostaglandins can be synthesized in the rat renal cortex and PGE₂ is the one that is most abundantly synthesized in the

glomeruli and cortical tubules. Prostaglandins have been thought to be involved in the regulation of many physiological functions of the kidney (Dunn & Hood, 1977). Thus, there is a possibility that prostaglandins are involved in the regulation of the 1 α -hydroxylase activity or modulate the actions of Ca²⁺-regulating hormones on 1 α -hydroxylase.

In previous reports (Horiuchi *et al.*, 1977, 1979) we have demonstrated that vitamin D-deficient rats showed a marked stimulation of the accumulation of [³H]1,25(OH)₂D₃ but thyroparathyroidectomy reduced the rate of conversion of [³H]25(OH)D₃ into [³H]1,25(OH)₂D₃ to approximately one-third of that in the vitamin D-deficient control animals, and that infusion *in vivo* of parathyrin or calcitonin into vitamin D-deficient T-PTX rats caused a stimulation of [³H]1,25(OH)₂D₃ formation. From these observations, it was shown that the perfusion system *in vivo* using vitamin D-deficient T-PTX rats offers a useful system to study the effects *in vivo* of various hormones or agents on the 25(OH)D₃ metabolism.

In the present study, we investigated the effect of the administration of PGE₂ to vitamin D-deficient T-PTX rats on the conversion *in vivo* of [³H]25(OH)D₃ into [³H]1,25(OH)₂D₃. PGE₂ infusion induced a significant stimulation of [³H]1,25(OH)₂D₃ production. The mechanism of activation of 1 α -hydroxylase by PGE₂ is discussed in relation to the role of cyclic AMP.

Abbreviations used: 25(OH)D₃, 25-hydroxyvitamin D₃ (25-hydroxycholecalciferol; calcidiol); 1,25(OH)₂D₃, 1 α ,25-dihydroxyvitamin D₃ (1 α ,25-dihydroxycholecalciferol; calcitriol); PGE₂, prostaglandin E₂; T-PTX, thyroparathyroidectomized.

$\ddagger$ To whom reprint requests should be sent.

Materials and methods

Male Wistar weanling rats weighing about 50 g were maintained on a synthetic vitamin D-deficient diet containing 0.45% calcium and 0.3% P_i (Suda *et al.*, 1970). After 6 weeks on the vitamin D-deficient diet when the rats have been shown to become rachitic and the serum levels of $25(\text{OH})\text{D}_3$ were undetectable, thyroparathyroidectomy was performed and the left femoral artery and vein were cannulated with heparinized polyethylene tubes. The animals were then fixed in a Ballman cage and subjected to a perfusion study as described previously (Horiuchi *et al.*, 1979; Matsumoto *et al.*, 1979). A perfusion medium composed of 10 mM- CaCl_2 , 5 mM- MgCl_2 , 20 mM- NaCl , 2.5 mM- KCl and 4% glucose was infused at a rate of 3 ml/h via a cannulated femoral vein. About 24 h after the operation, PGE_2 (99.8% purity; Ono Pharmaceutical Co., Osaka, Japan) in the perfusion medium or the medium alone was infused into the femoral artery at a rate of 1 ml/h. The end of the arterial cannula was placed just proximal to the renal arteries so that the infused PGE_2 could reach the kidneys directly. When intra-arterial infusion was started, the rate of venous infusion was reduced to 2 ml/h in order to keep the total volume of perfusate constant. For the experiment in which the effect of theophylline was examined, theophylline was added to the perfusion medium from the beginning through the entire course of experiments. At 1 h after the beginning of intra-arterial infusion, 0.5 μCi of $[26,27\text{-}^3\text{H}]\text{25}(\text{OH})\text{D}_3$ (sp. radioactivity 9 Ci/mmol; Amersham International, Amersham, Bucks., U.K.) in 50 μl of ethanol that had been purified by LH-20 column chromatography was injected into the femoral vein. At 6 h thereafter, the blood was drawn into a heparinized syringe. Total lipids were extracted from the plasma by the method of Bligh & Dyer (1959). Extracts were applied to Sephadex LH-20 column chromatography (1.5 cm $\times$ 30 cm) equilibrated with chloroform/n-hexane (13:7, v/v) (Holick & Deluca, 1971).

A portion of each fraction was evaporated to dryness under N_2 and the radioactivity was determined by liquid-scintillation counting. The radioactive $1,25(\text{OH})_2\text{D}_3$ from Sephadex LH-20 column chromatography was identified by high-performance liquid chromatography (Waters model 204). The radioactive peak was collected and co-chromatographed with authentic $1,25(\text{OH})_2\text{D}_3$ using Zorbax-SIL column with the solvent system of propan-2-ol/n-hexane (9:91, v/v).

Plasma concentrations of Ca^{2+} and P_i were determined as described previously (Horiuchi *et al.*, 1979). Cyclic AMP in urine was measured by Yamasa radioimmunoassay kit (Yamasa Co., Chiba, Japan). Each experiment was composed of

three or four rats and each rat was used for a single set of experiments. Statistical analyses were performed using the Student's *t* test.

Results

Over 90% of the radioactivity of the peak presumed to be $1,25(\text{OH})_2\text{D}_3$ from Sephadex LH-20 column chromatography migrated in the same position as authentic $1,25(\text{OH})_2\text{D}_3$ on high-performance liquid chromatography. Fig. 1 shows the effect of PGE_2 infusion on the accumulation in plasma of $[^3\text{H}]1,25(\text{OH})_2\text{D}_3$ in vitamin D-deficient T-PTX rats. Intra-arterial infusion of 10–50 μg of PGE_2/h caused a significant stimulation of the conversion of $[^3\text{H}]\text{25}(\text{OH})\text{D}_3$ into $[^3\text{H}]1,25(\text{OH})_2\text{D}_3$. Plasma levels of Ca^{2+} and P_i did not change significantly after the PGE_2 infusion at any concentration of PGE_2 tested (Fig. 2).

Fig. 1 Effect of graded doses of PGE_2 infusion on plasma $[^3\text{H}]1,25(\text{OH})_2\text{D}_3$ accumulation in vitamin D-deficient T-PTX rats

Rats were infused with graded amounts of PGE_2 intra-arterially starting 24 h after thyroparathyroidectomy. At 1 h after the beginning of the intra-arterial infusion, 0.5 μCi (45 pmol) of $[^3\text{H}]\text{25}(\text{OH})\text{D}_3$ was injected and accumulation *in vivo* of $[^3\text{H}]1,25(\text{OH})_2\text{D}_3$ was measured by collecting plasma 6 h thereafter. Data are means $\pm$ S.E.M. of three or four experiments. * $P < 0.05$; ** $P < 0.01$; for significance of difference from the control value.

Fig. 2. Effect of graded doses of PGE₂ infusion on plasma Ca²⁺ and P_i levels in vitamin D-deficient T-PTX rats

Experiments were performed as described in the legend to Fig. 1. The data are means $\pm$ S.E.M. of three or four experiments.

In order to examine if the stimulatory effect of PGE₂ on the conversion of [³H]25(OH)D₃ into [³H]1,25(OH)₂D₃ is mediated through stimulation of cyclic AMP production, urinary excretion of cyclic AMP during the infusion of PGE₂ was measured. Because surgical cannulation of the urinary bladder of vitamin D-deficient rats caused an unfavourable change to animals, vitamin D-repleted animals were used to examine urinary cyclic AMP excretion.

Infusion of 20 μg of PGE₂/h caused no significant change in urinary excretion of cyclic AMP (before infusion, 5.63 $\pm$ 0.43 nmol/h; after infusion, 5.26 $\pm$ 0.54 nmol/h; $n = 4$, not significant). Although there was no change in urinary cyclic AMP excretion after PGE₂ infusion, there might be a slight increase in intracellular cyclic AMP that caused a stimulation of the accumulation of [³H]1,25(OH)₂D₃. To further define whether the action of PGE₂ on [³H]1,25(OH)₂D₃ production is mediated through increased concentration of intracellular cyclic AMP, the effect of theophylline, a cyclic AMP phosphodiesterase inhibitor, on the action of a submaximal dose of PGE₂ was examined. As shown in Table 1, accumulation of [³H]1,25(OH)₂D₃ after the infusion of 2 μg of PGE₂/h along with 0.5 μmol of theophylline/h was not increased compared with that in animals infused with 0.5 μmol of theophylline/h alone, indicating that theophylline did not potentiate the action of a submaximal dose of PGE₂.

Table 1. Interaction of theophylline with a submaximal dose of PGE₂ on plasma [³H]1,25(OH)₂D₃ accumulation in vitamin D-deficient T-PTX rats

Data are means $\pm$ S.E.M. of three or four experiments.

Chemicals infused into T-PTX rats	Plasma accumulation of [³ H]1,25(OH) ₂ D ₃ (pmol/100 ml)
None	12.21 $\pm$ 0.61
PGE ₂ (2 μg/h)	13.34 $\pm$ 0.49
Theophylline (0.5 μmol/h)	16.58 $\pm$ 0.29*
PGE ₂ (2 μg/h) + theophylline (0.5 μmol/h)	15.38 $\pm$ 0.86†
PGE ₂ (20 μg/h)	19.49 $\pm$ 1.09*

* Significantly different from the control group (none) ($P < 0.01$).

† Significantly different from the control group ($P < 0.05$), but not significantly different from the theophylline group.

Discussion

The present study demonstrates that intra-arterial PGE₂ administration stimulates the plasma accumulation of [³H]1,25(OH)₂D₃ in vitamin D-deficient T-PTX rats. The stimulatory effect of PGE₂ on [³H]1,25(OH)₂D₃ production shown in the present study is not mediated by changes in known regulating factors of 1,25(OH)₂D₃ synthesis since the animals were thyroparathyroidectomized and serum Ca²⁺ and P_i levels remained stable over the entire course of the experimental protocol. Similar doses of PGE₂ have been employed in studies of various effects of PGE₂ in rats (Franklin & Tashjian, 1975; Vanderwiell & Talmage, 1979) and have been shown to cause changes in osteoclast function without affecting the plasma Ca²⁺ level (Vanderwiell & Talmage, 1979). The present observations confirm and extend the previous reports in which the stimulatory effect of PGE₂ on the 1α-hydroxylase activity was shown in chicks *in vitro* using primary kidney cell culture or isolated renal tubules (Trechsel *et al.*, 1980; Wark *et al.*, 1981).

Prostaglandins, especially of the E series, have been suggested to exert at least some of their actions in tissues via the adenylate cyclase/cyclic AMP system (Samuelsson *et al.*, 1978; Harris *et al.*, 1979). The present results, however, indicate that the amount of PGE₂ used in this study does not cause an increase in urinary cyclic AMP excretion and that simultaneous infusion of theophylline does not potentiate the effect of a submaximal dose of PGE₂. These data do not support the idea that the effect of PGE₂ on 1,25(OH)₂D₃ synthesis is mediated through an increase in cyclic AMP production. Our observations are consistent with those by Torikai & Kurokawa (1981) showing that PGE₂ did not

stimulate cyclic AMP production in proximal tubules in rats where 1α -hydroxylase is localized (Brunette *et al.*, 1978; Akiba *et al.*, 1980; Kawashima *et al.*, 1981b).

Previously, we have demonstrated that the stimulation of the accumulation of $1,25(\text{OH})_2\text{D}_3$ by parathyrin is dependent on cyclic AMP production (Horiuchi *et al.*, 1977), whereas that by calcitonin is not (Horiuchi *et al.*, 1979). In addition, Kurokawa and his colleagues have presented convincing evidence showing that parathyrin and calcitonin stimulate 1α -hydroxylase in different segments of the renal nephron: proximal convoluted tubules and proximal straight tubules respectively (Kawashima *et al.*, 1981a,b). From these observations, it is important to define the locus of the effect of PGE_2 on 1α -hydroxylase and the mechanism of activation of the enzyme by PGE_2 , as well as the physiological or pathophysiological significance of this effect of PGE_2 .

In conclusion, the present study demonstrates that PGE_2 stimulates the conversion of $[^3\text{H}]25(\text{OH})\text{D}_3$ into $[^3\text{H}]1,25(\text{OH})_2\text{D}_3$ in vitamin D-deficient T-PTX rats *in vivo*, and that the stimulatory effect of PGE_2 on the accumulation of $[^3\text{H}]1,25(\text{OH})_2\text{D}_3$ is not mediated by the adenylate cyclase/cyclic AMP system. The mechanism of the effect of PGE_2 on $25(\text{OH})\text{D}_3$ metabolism and the physiological significance of this effect remain to be elucidated.

This work was supported in part by Grants-in-Aid for Cancer Research (56-6) from the Ministry of Health and Welfare, and a Grant from the Ministry of Education, Science and Culture of Japan.

References

- Akiba, T., Endo, H., Koseki, C., Sakai, F., Horiuchi, N. & Suda, T. (1980) *Biochem. Biophys. Res. Commun.* **94**, 313–318.
- Baxter, L. A. & Deluca, H. F. (1976) *J. Biol. Chem.* **251**, 3158–3161.
- Bligh, E. G. & Dyer, W. J. (1959) *Can. J. Biochem. Biophys.* **37**, 911–917.
- Brunette, M. G., Chan, M., Ferriere, C. & Roberts, K. D. (1978) *Nature (London)* **276**, 287–289.
- Dunn, M. J. & Hood, V. L. (1977) *Am. J. Physiol.* **233**, F169–F184.
- Franklin, R. B. & Tashjian, A. H. (1975) *Endocrinology* **97**, 240–243.
- Fraser, D. R. (1980) *Physiol. Rev.* **60**, 551–613.
- Fraser, D. R. & Kodicek, E. (1973) *Nature (London)* **241**, 163–166.
- Galante, L., Colston, K. W., MacAuley, S. J. & MacIntyre, I. (1972) *Nature (London)* **238**, 271–273.
- Garabedian, M., Holick, M. F., Deluca, K. F. & Boyle, I. T. (1972) *Proc. Natl. Acad. Sci. U.S.A.* **69**, 1673–1676.
- Harris, R. H., Ramwell, P. W. & Gilmer, P. J. (1979) *Annu. Rev. Physiol.* **41**, 653–668.
- Holick, M. F. & Deluca, H. F. (1971) *J. Lipid Res.* **12**, 460–465.
- Horiuchi, N., Suda, T., Takahashi, H., Shimazawa, E. & Ogata, E. (1977) *Endocrinology* **101**, 969–974.
- Horiuchi, N., Takahashi, H., Matsumoto, T., Takahashi, N., Shimazawa, E., Suda, T. & Ogata, E. (1979) *Biochem. J.* **184**, 269–275.
- Hughes, M. R., Brumbaugh, P. F., Haussler, M. R., Wergedahl, J. E. & Baylink, D. J. (1975) *Science* **190**, 578–580.
- Kawashima, H., Torikai, S. & Kurokawa, K. (1981a) *Nature (London)* **291**, 327–329.
- Kawashima, H., Torikai, S. & Kurokawa, K. (1981b) *Proc. Natl. Acad. Sci. U.S.A.* **78**, 1199–1203.
- Larkins, R. G., MacAuley, S. J. & MacIntyre, I. (1974) *Nature (London)* **252**, 412–414.
- Matsumoto, T., Horiuchi, N., Suda, T., Takahashi, H., Shimazawa, E. & Ogata, E. (1979) *Metabolism* **28**, 925–927.
- Omdahl, J. L. (1978) *J. Biol. Chem.* **253**, 8474–8478.
- Rasmussen, H., Wong, M., Bikle, D. & Goodman, D. B. P. (1972) *J. Clin. Invest.* **51**, 2502–2504.
- Samuelsson, B., Goldyne, M., Granström, E., Hamberg, M., Hammerström, S. & Malmsten, C. (1978) *Annu. Rev. Biochem.* **47**, 997–1029.
- Sraer, J., Siess, W., Doleris, L. M., Oudinet, J. P., Dray, F. & Ardaillou, R. (1982) *Biochim. Biophys. Acta* **710**, 45–52.
- Suda, T., Deluca, H. F. & Tanaka, Y. (1970) *J. Nutr.* **100**, 1049–1052.
- Tanaka, Y. & Deluca, H. F. (1973) *Arch. Biochem. Biophys.* **154**, 566–574.
- Tanaka, Y. & Deluca, H. F. (1974) *Science* **183**, 1198–1200.
- Torikai, S. & Kurokawa, K. (1981) *Prostaglandins* **21**, 427–438.
- Trechsel, U., Taylor, C. M., Bonjour, J. P. & Fleisch, H. (1980) *Biochem. Biophys. Res. Commun.* **93**, 1210–1216.
- Vanderwiel, C. J. & Talmage, R. V. (1979) *Endocrinology* **105**, 588–594.
- Wark, J. D., Larkins, R. G., Eisman, J. A. & Wilson, K. R. (1981) *Clin. Sci.* **61**, 53–59.