Effect of Dexamethasone on In Vivo Prostanoid Production in the Rabbit

Anikó Náray-Fejes-Tóth, Géza Fejes-Tóth, Christine Fischer, and Jürgen C. Frölich
Fischer-Bosch Institute of Clinical Pharmacology, D-7000 Stuttgart, Federal Republic of Germany

Abstract. To investigate the effects of antiinflammatory steroids on in vivo prostaglandin production, urinary excretion rates of six different cyclo-oxygenase products were determined before, during, and after the administration of dexamethasone (1 mg/kg per d). Urine was collected in metabolism cages and was analyzed for prostaglandins E_2 and $F_{2\alpha}$ (PGE_2 and $PGF_{2\alpha}$) by radioimmunoassay after open-column chromatography; 6-keto-prostaglandin $F_{1\alpha}$ (6-keto-$PGF_{1\alpha}$) and thromboxane B_2 (TxB_2) were determined by radioimmunoassay after organic solvent extraction and reversed-phase high performance liquid chromatography; 7α-hydroxy-5,11-diketo-tetranorprostane-1,16-dioic acid (PGE-M) and 5α,7α-dihydroxy-11-keto-tetranorprostane-1,16-dioic-acid (PGF-M), the major urinary metabolites of prostaglandins E and F, were determined by gas chromatography-mass spectrometry and by radioimmunoassay, respectively. Dexamethasone failed to cause a statistically significant change in the excretion rate of PGE_2 (control, 250.4±40.8; dexamethasone, 297.6±78.7 ng/kg per d). In contrast, $PGF_{2\alpha}$ excretion decreased during administration of dexamethasone (from 1,036±228 to 449±158 ng/kg per d; $P < 0.05$). The urinary excretion rates of 6-keto-$PGF_{1\alpha}$, TxB_2, PGE-M, and PGF-M were not significantly altered by dexamethasone. (Control and dexamethasone values were, respectively, 63.6±7.9 and 103.5±17.9 ng/kg per d for 6-keto-$PGF_{1\alpha}$; 13.0±3.0 and 14.8±2.1 ng/kg per d for TxB_2; 1,251±217 and 1,905±573 ng/kg per d for PGE-M; and 4,131±611 and 4,793±600 ng/kg per d for PGF-M.) Urine flow was significantly higher during dexamethasone administration (control, 159±24; dexamethasone, 305±29 ml/24 h; $P < 0.01$). However, no correlation could be detected between changes in urine flow and changes in the excretion rate of any of the prostanoids investigated.

It is concluded that the administration of pharmacological doses of glucocorticoids does not affect the basal rate of total body prostanoid synthesis.

Introduction

Since the discovery that nonsteroidal antiinflammatory drugs inhibit prostaglandin (PG)[1] synthesis, a central role for PG in inflammation has been postulated (1, 2). Thus, it was obvious to suppose that glucocorticoids also exert their potent antiinflammatory action, at least in part, through the inhibition of PG synthesis. Initial studies, which used cell-free systems, failed to demonstrate any effect of antiinflammatory steroids on PG production (1, 3, 4). Recently, however, evidence was obtained that glucocorticoids decrease PG production in different in vitro systems via inhibition of phospholipase activity (5–13). Although this action of glucocorticoids seems to be fairly reproducible in in vitro systems, especially with high doses of steroids, almost no data are available on the effect of glucocorticoids on PG synthesis in vivo. In order to fill this gap, in the present study we attempted to assess the effect of dexamethasone on total body and renal prostanoid production by measuring the excretion rates of major metabolites of prostaglandins E and F (PGE and PGF), prostacyclin, and thromboxane A_2 on one hand, and by determining urinary prostaglandins E_2 and $F_{2\alpha}$ (PGE_2 and $PGF_{2\alpha}$) excretions on the other.

Methods

Experimental protocol. To determine urinary prostanoid excretion, 11 female rabbits who weighed 2.5–3.5 kg were housed in individual metabolism cages and fed a standard laboratory chow (Altromin GmbH; Lage, FRG). After 2 wk of acclimatization, two 24-h control urine collections were made. The rabbits were then given dexamethasone phosphate (Fortecortin; Merck, Darmstadt, FRG) at a dosage of 1 mg/

Received for publication 17 May 1983 and in revised form 12 March 1984.

J. Clin. Invest.

0021-9738/84/07/0120/04 $1.00
Volume 74, July 1984, 120–123

1. *Abbreviations used in this paper:* PG, prostaglandin; PGE, PGE_2, PGF, $PGF_{1\alpha}$, $PGF_{2\alpha}$, prostaglandins E, E_2, F, $F_{1\alpha}$, $F_{2\alpha}$; PGE-M, 7α-hydroxy-5,11-diketo-tetranorprostane-1,16-dioic acid; PGF-M, 5α,7α-dihydroxy-11-keto-tetranorprostane-1,16-dioic acid; RIA, radioimmunoassay; TxB_2, thromboxane B_2.

kg per d for 1 wk. Dexamethasone phosphate was dissolved in 50 ml of tap water and offered as drinking fluid. The animals drank this solution within several hours, after which they were given tap water ad lib. On the last day of the dexamethasone administration a venous blood sample was obtained for the determination of plasma dexamethasone concentration by radioimmunoassay (RIA) (14). Urine was collected on the 6th and 7th days of dexamethasone treatment. The animals were then allowed to recover from the effect of the drug for 2 wk, and then urine was again collected for 2 d. All urine collections were made while the collection vessels were immersed in an ice bath.

Determination of urinary prostanoids. Levels of 7α-hydroxy-5,11-diketo-tetranorprostane-1,16-dioic acid (PGE-M) were measured by gas chromatography-mass spectrometry as described earlier (15). Urinary concentration of 5α,7α-dihydroxy-11-keto-tetranorprostane-1,16-dioic acid (PGF-M) was measured by a direct RIA, according to the method of Okhi et al. (16), with an RIA kit which was generously provided by Ono Pharmaceutical Co., Ltd. (Osaka, Japan).

Urinary PGE_2 and $PGF_{2\alpha}$ concentrations were measured by RIA, as described in detail elsewhere (17), by the use of antibodies from the Pasteur Institute (Paris). The authenticity of both assays was established by linearity of sample dilutions and quantitative recovery of exogenous prostaglandins added to the samples before extraction. The PGE_2 RIA was further validated by the finding that treatment of the samples with 0.1 M KOH or $NaBH_4$ resulted in an almost complete loss of immunoreactivity; the reliability of the $PGF_{2\alpha}$ RIA was confirmed by the finding of a good correlation ($r = 0.922$, $n = 42$, $P < 0.001$) between values measured by RIA and gas chromatography-mass spectrometry.

Urinary thromboxane B_2 (TxB_2) and 6-keto-prostaglandin $F_{1\alpha}$ (6-keto-$PGF_{1\alpha}$) concentrations were determined by RIA after high performance liquid chromatography purification of the sample. In brief, after the addition of tracer amounts of [^{3}H]TxB_2 and [^{3}H]6-keto-$PGF_{1\alpha}$, urine (5 ml) was extracted at pH 8.1 with 20 ml of ethyl acetate; the water phase was then acidified to pH 3.4 by the addition of formic acid and was reextracted twice with 20 ml of ethyl acetate. The organic phases from the second and third extractions were washed with 2 ml of water, evaporated in vacuo, and redissolved in 1 ml of 0.1 M triethylamine formate, pH 3.5/acetonitrile/benzene (84.8:15:0.2, vol/vol). High performance liquid chromatography was performed on Zorbax ODS columns (4.6 × 250 mm; 7 μm particles; DuPont Instruments, Wilmington, DE) with a pump and an injector (models 6000A and U6K, respectively; Waters Associates; Milford, MA). Isocratic elution was performed with 0.1 M triethylamine formate, pH 3.5/acetonitrile/benzene (75.8:24:0.2, vol/vol) at a flow rate of 1.5 ml/min. Fractions corresponding to the elution position of [^{3}H]TxB_2 and [^{3}H]6-keto-$PGF_{1\alpha}$ were collected and analyzed by RIA (18, 19). Values were corrected for individual recoveries. Urinary sodium and potassium concentrations were determined by flame photometry and creatinine was measured by Jaffe's reaction.

Statistical analysis. Values obtained in the two control, two experimental, and two postexperimental control days, respectively, were averaged. Statistical evaluation of the data was performed by Friedman's test for two-way analysis of variance by ranks.

Results

Changes in the excretion rate of different prostanoids are summarized in Table I. Although the excretion of $PGF_{2\alpha}$ was significantly decreased, the excretion rate of all other prostanoids investigated was, if anything, increased during dexamethasone treatment.

Because of the opposing tendencies in urinary PGE_2 and $PGF_{2\alpha}$ excretion, the $PGE_2/PGF_{2\alpha}$ ratio increased markedly during dexamethasone administration (from 0.34±0.08 to 1.50±0.43; $P < 0.01$), and returned toward the control value (0.30±0.06; $P < 0.01$) in the postexperimental control period. The ratio of PGE-M to PGF-M was, however, not significantly altered by dexamethasone.

Urine flow increased from a control value of 61.15±9.23 to 112.96±10.74 ml/kg per d ($P < 0.01$) during dexamethasone, and it decreased to 62.13±7.72 ml/kg per d ($P < 0.01$) after the drug was discontinued. We were unable to demonstrate any correlation between urine flow and excretion rate of the different prostanoids. Urinary sodium, potassium, and creatinine excretion were not significantly altered by dexamethasone.

Plasma concentration of dexamethasone averaged 42.65 ±20.5 ng/ml.

Discussion

The capability of nonsteroidal antiinflammatory drugs to decrease prostaglandin production both in vitro and in vivo is well established (1–3). The effect of antiinflammatory steroids on prostaglandin synthesis is, however, controversial. Although

Table I. Effect of Dexamethasone on Urinary Prostanoid and Creatinine Excretion

	PGE-M	PGF-M	TxB_2	6-Keto-$PGF_{1\alpha}$	PGE_2	$PGF_{2\alpha}$	Creatinine
Control	1251±217	4131±611.4	12.98±2.97	63.65±7.85	250.4±40.8	1036±228	35.02±2.27
Dexamethasone	1905±573	4793±600.9	14.77±2.09	103.46±17.86	297.6±78.7	449±158*	28.80±3.1
Recovery	1602±370	5087±491.0	11.68±4.31	88.51±14.81	220.0±43.8	1009±298	36.05±1.66
n	9	11	11	11	11	11	11

Rabbits were treated with dexamethasone (1 mg/kg per d) for 1 wk. Two 24-h urine collections were made before the treatment began, on the last two days of the treatment, and after a 2-wk recovery period. Values given are nanograms per kilogram per day for prostanoids, and milligrams per kilogram per day for creatinine. Values obtained on the two control, two experimental, and two postexperimental control days, respectively, were averaged. *n*, number of animals. * $P < 0.05$; *P* refers to changes from the mean of the control and recovery periods.

several lines of recent evidence obtained from in vitro studies suggest that glucocorticoids may also decrease prostaglandin production via the inhibition of phospholipase A_2 activity (5–12), this finding is not universal, as some investigators were unable to show an inhibitory effect (4, 20, 21), and data on the effects of glucocorticoids on in vivo prostaglandin production are rather fragmentary. Although it was reported earlier that the major urinary metabolite of $PGF_{2\alpha}$ was not decreased after prednisolone treatment in four healthy volunteers (22), a systemic investigation of the effect of in vivo glucocorticoid treatment on total body PG production is still lacking.

In the present experiments, the administration of dexamethasone to intact rabbits failed to cause any decrease in the excretion rate of major urinary metabolites of different prostanoids; in fact, it caused a slight increase in the excretion rate of 6-keto-$PGF_{1\alpha}$ and TxB_2, which are major urinary metabolites of prostacyclin (23) and thromboxane A_2 (24), respectively. Total body and renal PGE production evaluated by the excretion rate of PGE-M and PGE_2, respectively, also trended upward without reaching statistical significance. In contrast, dexamethasone caused a significant fall in urinary $PGF_{2\alpha}$ excretion which was accompanied by an unaltered total body PGF production. Because a considerable proportion of total body PGF production originates in the kidney, unaltered PGF-M excretion coupled with a decreased renal production might indicate an enhanced PGF synthesis by extra-renal sites. The reason for the diminished $PGF_{2\alpha}$ excretion is not readily apparent. It might be explained by decreased synthesis via reduction of prostaglandin H_2 endoperoxide, or by decreased conversion from PGE_2 by PG-9-keto-reductase.

The failure of dexamethasone to decrease total body prostanoid production is difficult to reconcile with those in vitro data that indicate that glucocorticoids significantly reduced prostaglandin release (5, 6, 9). This discrepancy is unlikely to be related, however, to insufficient doses of dexamethasone, as plasma concentration in the present experiments averaged around 100 nM, which is sufficient to decrease prostaglandin production in vitro (5, 6, 9) and which results in a >90% occupancy of glucocorticoid receptors (25). One possible explanation for the apparent discrepancy between our in vivo data and the in vitro results could be that dexamethasone in vivo, beside inhibiting phospholipase A_2 activity, also initiates secondary reactions that counterbalance its action to reduce prostaglandin production. For instance, the lipolysis that characteristically accompanies glucocorticoid administration (26) may also lead to the liberation of unsaturated fatty acids, which in turn can serve as substrates for prostaglandin synthesis.

Another explanation might be that dexamethasone decreases prostaglandin production only in some cell types (i.e., monocytes-macrophages); because the contribution of these cells to total body prostanoid synthesis might be small, this effect could have been easily overlooked. One should also keep in mind that in most in vitro studies on steroid effects, prostaglandin synthesis was vastly stimulated either by inflammatory stimuli (5, 8, 11, 12) or as a consequence of tissue injury (10, 13); in our case the basal rate of prostaglandin production was measured. It is possible that the pathways leading to the production of free arachidonic acid are different in the two situations and that only the stimulated pathway is sensitive to glucocorticoids. If this were, indeed, the case, or if glucocorticoids inhibited prostaglandin production only in cell types that are potentially involved in inflammation, then this would render glucocorticoids superior to nonsteroidal antiinflammatory agents by reducing the side effects that result from the inhibition of prostaglandin synthesis in noninflamed tissues. Unfortunately, however, from the present results it is impossible to make inferences in favor of or against the role of prostaglandin synthesis inhibition in the mechanism of action of antiinflammatory steroids, as we studied prostaglandin production only in the absence of inflammatory stimuli. Nevertheless, the data collected in this paper seem to indicate that dexamethasone-sensitive phospholipases (10, 11) do not contribute significantly to the basal rate of prostaglandin synthesis in vivo. Thus, the effect of glucocorticoids on prostaglandin production is clearly distinct from the effects of nonsteroidal antiinflammatory drugs, which cause marked inhibition of basal prostaglandin synthesis both in vitro as well as in vivo (27).

Acknowledgments

The authors are indebted to Dr. M. Sawada for measuring PGF-M concentrations; to Dr. D. Schenzle, University of Tübingen, for performing the statistical analysis; and to the Ono Pharmaceutical Co. for providing the PGF-M RIA kit. Professor Vecsei, Heidelberg, kindly provided the dexamethasone antibody. The excellent technical assistance of Sonja Stucke, Birgit Siems, Bart Rigter, and Frank Schönberger is gratefully acknowledged.

This study was supported by the Robert Bosch Foundation, Stuttgart.

References

1. Vane, J. R. 1971. Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. *Nat. New Biol.* 231:232–235.

2. Vane, J. R. 1979. Prostaglandins as mediators of inflammation. *Adv. Prostaglandin Thromboxane Res.* 2:791–801.

3. Flower, R., R. Gryglewski, K. Herbaczynska-Cedro, and J. R. Vane. 1972. Effects of anti-inflammatory drugs on prostaglandin biosynthesis. *Nat. New Biol.* 238:104–106.

4. Lewis, G. P., and P. J. Piper. 1975. Inhibition of release of prostaglandins as an explanation of some of the actions of anti-inflammatory corticosteroids. *Nature (Lond.).* 254:308–311.

5. Kantrowitz, F., D. R. Robinson, M. B. McGuire, and L. Levine. 1975. Corticosteroids inhibit prostaglandin production by rheumatoid synovia. *Nature (Lond.).* 258:737–739.

6. Tashjian, A. H., Jr., E. F. Voelkel, J. McDonough, and L. Levine. 1975. Hydrocortisone inhibits prostaglandin production by mouse fibrosarcoma cells. *Nature (Lond.).* 258:739–741.

7. Hong, S. C., and L. Levine. 1976. Inhibition of arachidonic acid release from cells as the biochemical action of anti-inflammatory corticosteroids. *Proc. Natl. Acad. Sci. USA.* 73:1730–1734.

8. Bray, M. A., and Gordon, D. 1978. Prostaglandin production by

macrophages and the effect of anti-inflammatory drugs. *Br. J. Pharmacol.* 63:635–642.

9. Russo-Marie, F., M. Paing, and D. Duval. 1979. Involvement of glucocorticoid receptors in steroid-induced inhibition of prostaglandin secretion. *J. Biol. Chem.* 254:8498–8504.

10. Flower, R. J. and G. J. Blackwell. 1979. Anti-inflammatory steroids induce biosynthesis of a phospholipase A_2 inhibitor which prevents prostaglandin generation. *Nature (Lond.).* 278:456–459.

11. Hirata, F., E. Schiffmann, K. Venkatasubramanian, D. Salomon, and J. Axelrod. 1980. A phospholipase A_2 inhibitory protein in rabbit neutrophils induced by glucocorticoids. *Proc. Natl. Acad. Sci. USA.* 77:2533–2536.

12. Blackwell, G. J., R. Carnuccio, M. Di Rosa, R. J. Flower, L. Parente, and P. Persico. 1980. Macrocortin: a polypeptide causing the anti-phospholipase effect of glucocorticoids. *Nature (Lond.).* 287:147–149.

13. Danon, A., and G. O. Assouline. 1978. Inhibition of prostaglandin biosynthesis by corticosteroids requires RNA and protein synthesis. *Nature (Lond.).* 273:552–554.

14. Haak, D., D. Vecsei, K. Lichtwald, H. R. Klee, K. H. Gless, and M. Weber. 1980. Some experiences on radioimmunoassays of synthetic glucocorticoids. *Allergology.* 3:259–267.

15. Green, K., B. Hamberg, B. Samuelson, M. Smigel, and J. C. Frölich. 1978. Measurement of prostaglandins, thromboxanes, prostacyclin and their metabolites by gas liquid chromatography-mass spectrometry. *Adv. Prostaglandin Thromboxane Res.* 5:39–94.

16. Ohki, S., T. Hanyu, K. Imaki, N. Nakazawa, and F. Hirata. 1974. Radioimmunoassays of prostaglandin $F_{2\alpha}$ and prostaglandin $F_{2\alpha}$-main urinary metabolite with prostaglandin-^{125}I-Tyrosine methylester amide. *Prostaglandins.* 6:137–148.

17. Fejes-Tóth, G., J. C. Frölich, and A. Náray-Fejes-Tóth. 1983. Effect of aprotinin on the renal response to vasopressin in diabetes insipidus rats. *J. Physiol. (Lond.).* 339:585–590.

18. Mohammed, S. F., W. H. Anderson, J. B. Smith, H. Y. K. Chuang, and R. G. Mason. 1981. Effects of heparin on platelet aggregation and release and thromboxane A2 production. *Am. J. Pathol.* 104:132–41.

19. Czervionke, R. L., J. B. Smith, G. L. Fry, J. C. Haak, and D. L. Haycraft. 1979. Inhibition of prostacyclin by treatment of endothelium with aspirin. Correlation with platelet adherence. *J. Clin. Invest.* 63:1089–1092.

20. Zusman, R. M., and H. R. Keiser. 1977. Prostaglandin biosynthesis by rabbit renomedullary interstitial cells in tissue culture. Stimulation by angiotensin II, bradykinin, and arginine vasopressin. *J. Clin. Invest.* 60:215–223.

21. Hammarström, S. 1982. Biosynthesis and biological actions of prostaglandins and thromboxanes. *Arch. Biochem. Biophys.* 214:431–445.

22. Brash, A. R. 1980. Metabolite measurement as an index of prostaglandin synthesis in vivo. *In* Prostaglandins, Prostacyclin, and Thromboxanes Measurement. J. M. Boeynaems and A. G. Herman, editors. Martinus Nijhoff Publishers, The Hague. 123–141.

23. Rosenkranz, B., C. Fischer, K. E. Weimer, and J. C. Frölich. 1980. Metabolism of Prostacyclin and 6-keto-prostaglandin $F_{1\alpha}$ in man. *J. Biol. Chem.* 255:10194–10198.

24. Roberts, L. J., B. J. Sweetman, and J. A. Oates. 1981. Metabolism of thromboxane B_2 in man. *J. Biol. Chem.* 256:8384–8393.

25. Munck, A. 1976. General aspects of steroid hormone-receptor interactions. *In* Receptors and Mechanism of Action of Steroid Hormones. J. R. Pasqualini, editor. Marcel Dekker, Inc., New York. 1:1–40.

26. Dreiling, D. A., E. L. Bierman, A. F. Debons, P. Elsbach, and I. L. Schwartz. 1962. Effect of ACTH, hydrocortisone and glucagon on plasma nonesterified fatty acid concentration (NEFA) in normal subjects and in patients with liver disease. *Metab. Clin. Exp.* 11:572–578.

27. Robinson, H. J., and J. R. Vane. 1974. Prostaglandin Synthetase Inhibitors. Raven Press, New York. 9–19, 99–106.