Biochemical Pharmacology, Vol. 26, pp. 1463–1468. Pergamon Press, 1977. Printed in Great Britain.

EFFECT OF GLUCOCORTICOID ON PROSTAGLANDIN PRODUCTION DURING SIMULATED SHOCK CONDITIONS IN THE PERFUSED CAT LIVER*

RICHARD P. CARLSON†, JOHN T. FLYNN‡ and ALLAN M. LEFER

Department of Physiology, Jefferson Medical College, Thomas Jefferson University, Philadelphia, PA 19107, U.S.A.

(*Received* 13 *August* 1976; *accepted* 15 *October* 1976)

Abstract—The production of prostaglandins E_2 and $F_{2\alpha}$ by isolated perfused cat livers was quantitated during separate simulated shock conditions (i.e. ischemia, acidosis and hypoxia). Control livers showed little change in perfusate prostaglandin concentrations over a 150-min perfusion period. Neither ischemia nor acidosis produced significant increases in prostaglandin concentrations in the perfusate after 150 min of perfusion. Hypoxia resulted in a 4.5-fold increase in PGE_2 and a 3.5-fold increase in $PGF_{2\alpha}$ concentrations during this same period. Lactic acid dehydrogenase and cathepsin D activities were also elevated in the perfusate during hypoxia. Addition of methylprednisolone (10^{-3} M) to the perfusate significantly retarded the release of PGE_2, $PGF_{2\alpha}$ and cathepsin D into the perfusate during severe hypoxia, without significantly altering the increase in lactic acid dehydrogenase activity. The action of methylprednisolone on prostaglandin and lysosomal enzyme release in hypoxic livers may be the result of a membrane-stabilizing action of the glucocorticoid. One proposed mechanism for the inhibition by glucocorticoids of the increased prostaglandin production during hypoxia is that endogenous arachidonic acid is stabilized within the cell membrane phospholipids. Thus, less substrate is available for utilization by the prostaglandin synthetase system.

Several investigators have demonstrated increased circulating concentrations of prostaglandins during a variety of forms of circulatory shock. These include hemorrhagic [1–3], splanchnic artery occlusion [4], and endotoxic [5–7] shock. Although it has been suggested that the increased circulating prostaglandin concentrations may have resulted from an increase in the rate of synthesis and release of these fatty acids from various organs such as the kidney [3, 6, 8–10], the lung [11, 12] or the splanchnic region [4], the precise stimuli for prostaglandin synthesis and release during the shock states have not been determined. Studies in intact animals do not permit a precise localization of the source of prostaglandin production, since certain blood cells are a rich source of prostaglandins. In contrast, isolated perfused organs can be more carefully controlled by removal of the organ from neural and endogenous humoral factors. Moreover, interpretation of data is facilitated since individual parameters such as flow, PO_2 and pH of the perfusate can be separately varied or controlled. Thus, the isolated perfused cat liver can be independently subjected to acidosis, ischemia or hypoxia. Under these conditions, hypoxia is the stimulus most detrimental to liver integrity based upon increased perfusate activities of cytoplasmic and lysosomal enzymes, and depression of hepatic reticuloendothelial system (RES) function as indices of cellular damage [13].

The present study was designed to determine the concentrations of prostaglandins (PG) E_2 and $F_{2\alpha}$ in the perfusate of isolated cat livers subjected to hypoxia, ischemia or acidosis in order to determine the effect of these stimuli on prostaglandin synthesis and release. Since glucocorticoids have been shown to prevent lysosomal enzyme release and to protect against the depression of reticuloendothelial function during severe hypoxia [14], it was of interest to determine the effect of methylprednisolone on prostaglandin production under control and hypoxic conditions. Additional experiments were conducted with liver homogenates in order to determine whether disrupted liver cells respond in a similar manner as intact cells to glucocorticoid.

MATERIALS AND METHODS

Animal preparation. Cats of either sex weighing between 2.5 and 3.5 kg and fed *ad lib.* were anesthetized with sodium pentobarbital (30 mg/kg, i.v.). After intravenous administration of heparin (1000 units), a laparotomy was performed. The liver was isolated and excised within 2–3 min after cannula placement. The liver was placed in oxygenated Krebs–Henseleit (KH) solution held at 4° and transferred to the perfusion apparatus as previously described [13].

Perfusion technique. Livers weighing between 70 and 100 g were placed in a water-jacketed (37°) plexiglass chamber. The livers were perfused through an inflow cannula placed in the portal vein. After a rapid washout of trapped red blood cells, an outflow catheter placed in the vena cava was connected to a 1000-ml perfusate reservoir for a 10-min equilibration period. The Krebs–Henseleit buffer contained the following ingredients expressed as m-moles/liter: KCl, 4.8; KH_2PO_4, 1.2; $MgSO_4$, 1.2; $CaCl_2$, 2.5; $NaHCO_3$,

* Supported in part by Research Grant HL-17688 from the National Institutes of Health.

† Predoctoral Fellow.

‡ NIH Postdoctoral Fellow (HL-05017).

12.5; NaCl, 118.4; glucose, 10; and N-2, hydroxyethyl piperazine-*N'*2 ethanesulfonic acid (HEPES), 25. The buffer also contained 5% dextran (40,000 mol. wt, Rheomacrodex, Pharmacia). The osmolarity of the perfusate was 325 mOsM. The perfusate pH was maintained between 7.2 and 7.4 with 25 mM HEPES. Perfusate samples were collected from the outflow catheter at 0-, 60-, 90-, 120- and 150 min sampling times for the determinations of hepatic flow, pH as well as lactic acid dehydrogenase (LDH), and cathepsin D activities. Oxygen consumption was calculated from the difference in PO_2 values of samples collected simultaneously at the inflow and outflow catheters at the 0-, 90- and 150-min sampling times multiplied by the flow rate according to a previously described procedure [13]. Inflow pressure to the liver was initially set at 25 cm H_2O by adjustment of the perfusate inflow rate. A Haake constant temperature circulatory bath was used to maintain perfusate temperature at $37 \pm 0.2°$ within the perfusion system. A gas mixture of 95% O_2 + 5% CO_2 was bubbled through the perfusate to achieve a PO_2 of 300–450 mm Hg at the inflow cannula. Particulate matter was removed by an in-line 100 mesh nylon filter. Hypoxia was induced by gassing the perfusate with a mixture of 95% N_2 + 5% CO_2. This procedure resulted in a perfusate PO_2 of 25–35 mm Hg. Acidosis was induced by titrating the HEPES-buffered perfusate to pH 6.9 prior to perfusion. Ischemia was initiated by manually clamping the inflow tubing to reduce flow to 40 per cent of control. Methylprednisolone (MP) sodium succinate was added to some perfusates at a concentration of 400 μg/ml (10^{-3} M) just prior to the start of the experiment. In other experiments, the glucocorticoid vehicle, sodium succinate in benzyl alcohol, was added to the perfusate.

In a separate set of perfusion experiments, arachidonic acid (Sigma, 99 per cent pure)(1–5 μg/ml) was added at zero time to the perfusate. During the equilibration period, 0.1 M Na_2CO_3 was added to the perfusate as a vehicle control. In these experiments, the perfusate was not recirculated. In an additional experimental group, the livers were treated with methylprednisolone (10^{-3} M) prior to and after administration of arachidonic acid. Samples for prostaglandin and enzyme determinations were collected at 0, 2, 5 and 10 min.

Analytical procedures. An International Laboratories model 313 blood gas analyzer was used to determine perfusate PO_2 in mm Hg. Samples were read immediately upon collection. Lactic acid dehydrogenase activity was determined in perfusate samples spectrophotometrically at 340 nm using pyruvate as substrate. Activities are expressed as changes in units of absorbance of NADH/min at 25°. Samples of perfusate were also assayed for cathepsin D activity according to the method of Anson [15] using bovine hemoglobin as substrate. Activities are expressed as m-equiv. of tyrosine × 10^{-4} produced/hr at 37°/ml of perfusate.

Liver large granule fraction preparation. Cat livers were rapidly excised and placed in 6 vol. of 0.02 M Tris buffer (pH 7.3) containing 0.25 M sucrose at 4°. The livers were homogenized, and large granule fractions were obtained according to the method of Glenn and Lefer [16]. In one set of experiments, large granule fractions were preincubated for 5 min at 37° with MP (10^{-3} M) or its vehicle and followed with an incubation for 2 min at 37° and a pH of 7.3 with either arachidonic acid (2 μg/ml) or its vehicle. The suspended fractions were subsequently assayed for prostaglandin E_2 and $F_{2\alpha}$ by radioimmunoassay.

Prostaglandin extraction and chromatography. Liver perfusate samples (10 ml) were collected in plastic centrifuge tubes containing 100 μg indomethacin to inhibit prostaglandin synthesis *in vitro*. These samples were cooled, centrifuged at 10,000 *g* for 10 min at 4°, and frozen at −20°. Homogenate samples (3 ml) were also collected in plastic centrifuge tubes and frozen at −20°. Prostaglandins were extracted by a modification of the method of Unger *et al.* [17]. Ten-ml perfusate or 3-ml homogenate samples diluted with 0.9% NaCl to a volume of 10 ml, were mixed with 10 ml of 0.9% NaCl, 20 ml of 95% ethanol, and a known amount of [^{3}H]PGA_1 which was added to assess the per cent of recovery. The mixtures were washed with petroleum ether, and the pH was adjusted to 4.5 with 5% formic acid. The prostaglandins were partitioned twice into chloroform which was subsequently flash evaporated at 40°. The residues were dissolved in 70% ethanol, mixed with Fuller's Earth, and centrifuged at 2000 *g* for 10 min. The prostaglandins were repartitioned to chloroform at pH 4.5, dried under nitrogen, and applied to silicic acid chromatography columns for the separation of A, E and F series prostaglandins according to the method of Jaffe *et al.* [18] using a modification of solvent ratios as previously described [19]. The entire PGA fraction was collected, evaporated to dryness, and counted in a liquid scintillation spectrometer for the calculation of per cent of recovery throughout the extraction and chromatography procedures.

The PGE and PGF fractions were evaporated to dryness under 100% nitrogen and assayed by specific radioimmunoassay.

PG radioimmunoassay. The dried PGE and PGF residues were dissolved in 1.0 ml of 95% ethanol. Aliquots were transferred to assay tubes and evaporated to dryness under nitrogen. Both the $PGF_{2\alpha}$ and PGE_2 assays were carried out in 0.06 M phosphate buffer (pH 7.3) containing 0.1% gelatin using a specific antibody directed against $PGF_{2\alpha}$. PGE fractions were treated with sodium borohydride which reduced the E-type prostaglandins to F-type prostaglandins. After a 1 hr incubation period at room temperature, bound and free hapten were separated by adding dextran-coated charcoal followed by centrifugation at 1500 *g* for 10 min. Supernatants were decanted into vials, dissolved in a suitable counting medium, and counted in a liquid scintillation spectrometer. After calculations of dis./min, the average binding of [^{3}H]prostaglandin was determined for duplicate samples comprising a standard curve and the unknown samples for each assay. Sample values were corrected for aliquot volume and recovery, and the PGE_2 values are corrected for their 50 per cent conversion to $PGF_{2\alpha}$ and $PGF_{2\beta}$, the latter of which did not react with the anti-$PGF_{2\alpha}$ antibody. Prostaglandin concentrations are reported as ng prostaglandin/ml of perfusate or homogenate. Specific characteristics of the PGE_2 and $PGF_{2\alpha}$ radioimmunoassays have been described previously [20].

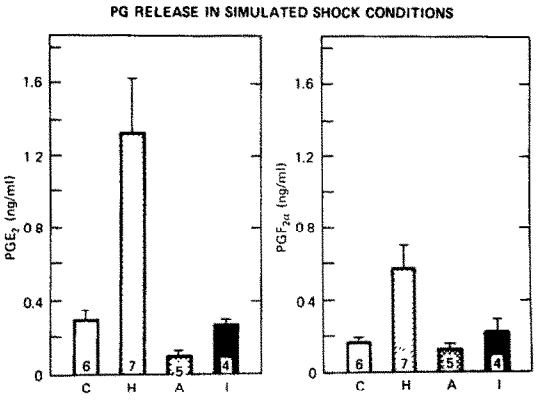

Fig. 1. Prostaglandin concentrations in perfusates of experimental groups after 150 min of perfusion. The groups are: control (C), hypoxia (H), acidosis (A) and ischemia (I). The numbers in the bars represent the number of livers in each group. Standard errors of the means are represented by the brackets above the bars.

RESULTS

The isolated, perfused cat liver is a viable and stable model for the investigation of the effects of various hemodynamic and metabolic alterations which simulate the shock state. The perfusion pressure of 25 cm H_2O resulted in a flow of 95–105 ml/min which was maintained for the duration of the experiment. In ischemic livers, flow was set at 40 per cent of the control period value and held at that value for the entire 150-min observation period. No statistical differences in flow were seen between the control, hypoxic or acidotic livers at any time period. The rate of flow of the ischemic livers returned to within 10 per cent of the control period flow when the flow clamp was released at 150 min.

Total hepatic oxygen consumption averaged between 9.5 and 12.0 ml/min/g $\times 10^{-3}$. These values were consistent, and are in very good agreement with those previously described [13]. Although control and acidotic livers did not experience significant alterations in O_2 consumption, this function in ischemic livers decreased to 40–50 per cent of control, and hypoxic livers exhibited reductions in O_2 consumptions of 90–95 per cent during the 150-min experimental period.

The HEPES buffer in the perfusate maintained the pH between 7.15 and 7.35 in all groups except the acidotic group, and the pH in this non-buffered system was maintained at 6.9 for the entire 150-min experimental period.

The circulating lactic acid dehydrogenase activities, a representative cytoplasmic enzyme, and cathepsin D, a lysosomal enzyme, did not significantly increase above initial values in the control, ischemic or acidotic groups of livers during the 150-min experimental period. In contrast, hypoxia increased LDH activity 15 times (i.e. about 4500 vs 300 units/ml) by 150 min when compared to the control group. Cathepsin D activity increased 3.9 times (from 1.8 to 7.0 units/ml) over the same time period in hypoxic preparations.

Figure 1 illustrates the concentrations of prostaglandins E_2 and $F_{2\alpha}$ in the perfusate at the end of the 150-min perfusion period in livers subjected independently to the simulated shock conditions of hypoxia, ischemia and acidosis. Livers subjected to acidosis or ischemia did not demonstrate any significant increase in PGE_2 or $PGF_{2\alpha}$ concentrations at 150 min when compared to control livers. In contrast, hypoxia resulted in a 4.5-fold increase in the PGE_2 concentration, and a 3.5-fold increase in the $PGF_{2\alpha}$ concentration at 150 min when compared to controls. In general, the PGE_2 concentrations were approximately twice those of $PGF_{2\alpha}$ during most experimental conditions in the perfused cat liver.

The time courses of the release of PGE_2 and $PGF_{2\alpha}$ under control and hypoxic conditions are shown in Fig. 2. Although only slight changes are seen in the PGE_2 or $PGF_{2\alpha}$ concentrations of the control groups as a function of time, hypoxia results in a progressive increase in the prostaglandin concentrations of the perfusate. The 90-min concentrations of PGE_2 and $PGF_{2\alpha}$ were elevated but not significantly. The concentrations of both prostaglandins were significantly elevated above control values at 150 min.

Since hypoxia is a strong stimulus for endogenous prostaglandin synthesis and release by the perfused cat liver, the hypoxic liver is a suitable preparation in which to assess the action of methylprednisolone (10^{-3} M). As shown in Fig. 3, addition of methylprednisolone to the perfusate virtually abolished the production of PGE_2 and also significantly attentuated the production of $PGF_{2\alpha}$ by the liver in response to hypoxia. Methylprednisolone treatment did not significantly affect PGE_2 and $PGF_{2\alpha}$ perfusate concentrations of livers under control conditions. The vehicle for methylprednisolone (i.e. sodium succinate in benzyl alcohol) had no effect on any of the variables measured in this study.

In an attempt to delineate the mechanism of action of the steroid in preventing prostaglandin accumulation in the perfusate in response to hypoxia, exogenous arachidonic acid was added to the perfusate of control livers in the presence or absence of the glucocorticoid. This experimental design facilitated study of the action of the glucocorticoid at the level

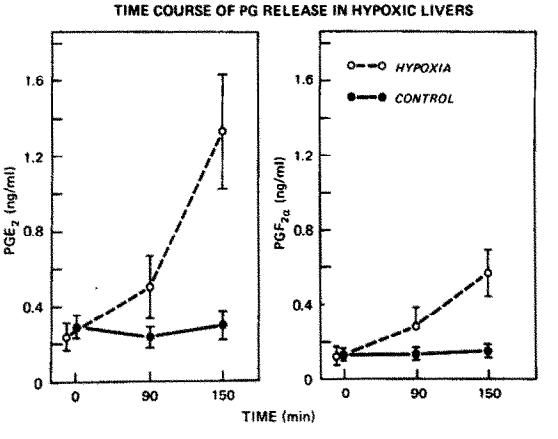

Fig. 2. Concentrations of prostaglandins released into the perfusate at specific times from 0 to 150 min during control and hypoxic experiments. The number of experiments in each group are: control (6) and hypoxia (7). Brackets represent standard errors of the mean.

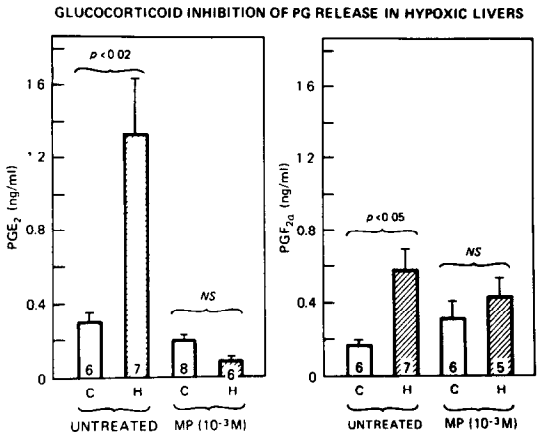

Fig. 3. Effect of methylprednisolone (MP) in control (C) and hypoxic (H) experiments on prostaglandin titers after 150 min of perfusion. The numbers in the bars represent the number of livers. Standard errors of the means are represented by the brackets above the bars.

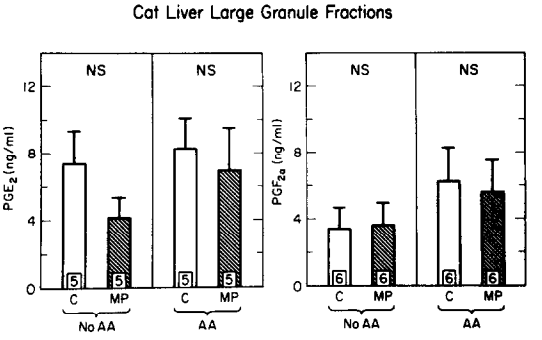

Fig. 5. Prostaglandin concentrations in suspensions from a large granule fraction of liver after incubation at 37° with control (C) and methylprednisolone (MP)-treated groups. Arachidonic acid (AA) at 2 μg/ml was given to stimulate increased synthesis of prostaglandins. The numbers in the bars represent the number of livers in each group. Standard errors of the means are represented by the brackets above the bars.

of prostaglandin substrate availability. Figure 4 summarizes the effect of arachidonic acid administration on the capacity of perfused livers to produce and release prostaglandins E_2 and $F_{2\alpha}$. In this non-recirculated perfusion system, arachidonic acid (1–5 μg/ml) increased the PGE_2 and $PGF_{2\alpha}$ concentrations within 10 min from four to six times above the initial values. As in other experiments, prostaglandin E_2 was formed and released to a greater degree than was prostaglandin $F_{2\alpha}$. In contrast to the finding that methylprednisolone strongly inhibited the accumulation of prostaglandins in the perfusate of hypoxic livers, methylprednisolone had no significant effect on the synthesis and release of prostaglandins E_2 and $F_{2\alpha}$ resulting from the addition of exogenous substrate for the prostaglandin synthetase system.

The synthetic capacity of the prostaglandin synthetase system was directly assessed in a large granule fraction (LGF) of cat liver homogenates (Fig. 5). Incubation of 2 min in either the presence or absence of arachidonic acid (2 μg/ml) resulted in similar increases in PGE_2 and $PGF_{2\alpha}$ concentrations. Thus, the homogenization procedure appears to provide adequate substrate for the prostaglandin synthetase system. Pretreatment of the LGF with methylprednisolone at 10^{-3} M had no significant effect on the production of either PGE_2 or $PGF_{2\alpha}$ in any of the groups. Under these same conditions, the non-steroidal anti-inflammatory drug, sodium meclofenamate, at a concentration of 1 μg/ml, inhibited the synthesis of both PGE_2 and $PGF_{2\alpha}$ in the fractions (i.e. 85–95 per cent below control values). Thus, glucocorticoids do not directly inhibit the prostaglandin synthetase system in cat liver.

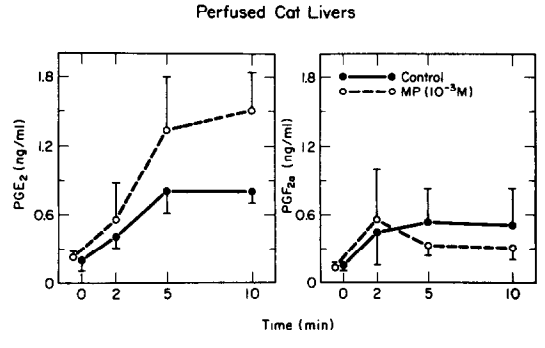

Fig. 4. Time course of PGE_2 and $PGF_{2\alpha}$ release in control and methylprednisolone (MP)-treated groups after arachidonic acid (1–5 μg/ml) was added to the perfusate at zero time. The number of experiments for the groups are: control (5) and MP (7). Brackets represent standard errors of means.

DISCUSSION

Prostaglandins are present in many tissues and are synthetized and released during a variety of forms of circulatory shock. Several investigators [1–3] have described a 2 to 3-fold increase in arterial blood prostaglandins after hemorrhagic shock, and others [4–7] have shown similar increases during endotoxic and splanchnic ischemia shock. In addition, the kidney and splanchnic organs have been shown to release prostaglandins during stress states [3, 4, 6, 8, 9, 21, 22]. While the splanchnic area has been shown to be a good source of prostaglandins during shock [4], the ability of the liver to release prostaglandins during shock or shock-like conditions had not been previously studied.

Hepatic ischemia produced no increase in circulating prostaglandin concentrations in our experiments. Similarly, acidosis did not influence cellular integrity or increase prostaglandin concentrations in the perfusate during 150 min of perfusion. In fact, prostaglandin concentrations were slightly lower in acidotic preparations. Our experiments indicate that hypoxia, as it occurs in severe shock, significantly increases $PGF_{2\alpha}$ and PGE_2 concentrations 3–5-fold in liver perfusates. These prostaglandin concentrations reflect the net effect of increased prostaglandin synthesis and release, modified by degradation. The elevation of prostaglandins in the perfusate in response to hypoxia occurred at a time when circulating LDH and cathepsin D activities were also increasing. These latter substances appear during severe hypoxia because cell membrane integrity and RES function are impaired

in this state [13, 14]. Although we cannot definitively establish that the release of lysosomal enzymes in our preparation either directly or indirectly enhanced prostaglandin production, other investigators have presented data supporting this hypothesis [23].

During hypoxia, hepatic cells and subcellular organelles undergo a loss of integrity. In this connection, glucocorticoids have been reported to stabilize hepatic membranes [24]. Our experiments confirm a preservation of cellular integrity during glucocorticoid administration in that methylprednisolone (10^{-3} M) significantly inhibited the appearance of cathepsin D in the hepatic perfusate during 150 min of severe hypoxia. Also, lower prostaglandin concentrations were observed in the perfusates of hypoxic livers to which methylprednisolone was given.

Recently, several investigators reported that glucocorticoids inhibit prostaglandin release into the extracellular space. There are three stages in cellular prostaglandin production which may be influenced by glucocorticoids. These are: (a) the level of substrate availability, (b) the enzymatic conversion of arachidonic acid into prostaglandins, and (c) the transport of prostaglandins from the intracellular into the extracellular fluid. Floman *et al.* [25] showed both reduced synthesis and release of PG in inflamed rabbit synovia and uvea after glucocorticoid administration. In some of these experiments, arachidonic acid administration was able to restore the prostaglandin concentrations in the extracellular medium after inhibition by glucocorticoids. The exogenous arachidonic acid apparently provided the prostaglandin synthetase system with sufficient substrate to produce prostaglandins. In contrast, experiments by Lewis and Piper [26] using rabbit adipose tissue suggest that transcellular prostaglandin transport is the step in which glucocorticoids affect prostaglandin release, since intracellular levels of PGE_2 are high after hydrocortisone treatment whereas PG concentrations were found to be relatively low in the perfusing medium. The data presented from our laboratory strongly support the hypothesis that endogenous arachidonic acid is less available to the intracellular prostaglandin synthetase system after administration of pharmacologic doses of glucocorticoid.

We have demonstrated that glucocorticoids significantly inhibit prostaglandin production by perfused livers in response to hypoxia. The conditions of these experiments promoted the utilization of endogenously released arachidonic acid as substrate for the prostaglandin synthetase system. These data do not exclude a glucocorticoid action on the enzymatic conversion of arachidonic acid into prostaglandins or on the transport of prostaglandins out of cells. However, data gathered from large granule fractions of homogenized livers demonstrate that methylprednisolone, even at high concentrations, has no effect on prostaglandin synthesis in broken cell preparations. Under the conditions of this experiment, substrate availability was not the rate-limiting step in prostaglandin synthesis. Moreover, this lack of inhibition of prostaglandin synthetase by glucocorticoids is in close agreement with the results of others [27–30], and is in contrast to the effect of non-steroidal anti-inflammatory agents on the prostaglandin synthetase system [31, 32].

Bito and Baroody [33] have presented data which suggest that the removal of prostaglandins from the extracellular fluid of the brain is mediated by a saturable, facilitated-transport process across the blood–brain barrier. If this transport process is common to all cells, glucocorticoids could act to prevent prostaglandin release by inhibiting the transport of prostaglandins across the cell surface. In this regard, glucocorticoids have been shown to inhibit the transport of prostaglandins through adipose cell membranes [26, 34]. Our results do not support this concept. Isolated cat livers perfused with a medium containing exogenous arachidonic acid produced significant quantities of prostaglandins, which were not inhibited by the administration of glucocorticoid. These findings are similar to those of Gryglewski *et al.* [35], Floman *et al.* [25] and Hong and Levine [36], all of whom demonstrated a reversal of the glucocorticoid inhibition of prostaglandin production by exogenous administration of arachidonic acid. Furthermore, Kantrowitz *et al.* [37] demonstrated a reduction of the PGE_2 content of synovial tissue by dexamethasone parallel to the decreased PGE_2 concentration in the medium.

Regarding the sequence of events of hepatic prostaglandin release, hypoxia appears to result in a loss of cellular and lysosomal membrane integrity. As a result, phospholipase A, which is known to cleave arachidonic acid from membrane phospholipids, may be released or activated. An alternate explanation might be that phospholipids lose their binding capacity for arachidonic acid during hypoxia [38]. In either case, prostaglandin substrates are liberated from storage sites in membrane phospholipids, leading to prostaglandin formation and release into the ECF.

Glucocorticoids stabilize cellular and subcellular membranes, probably making arachidonic acid less available to the prostaglandin synthetase system. Although the exact mechanism of glucocorticoid inhibition of prostaglandin substrate availability is not known, there are three major possibilities: (1) direct stabilization of cellular membranes, (2) inhibition of phospholipase A activity or (3) prevention of phospholipase A from reaching its substrate. Phospholipase A activity in homogenates from shock pancreas is not affected by glucocorticoid treatment [39]. It thus appears that glucocorticoids reduce the availability of substrate to the prostaglandin synthetase system by directly stabilizing cellular membranes or by attenuating the release of activated phospholipase A from its intracellular storage sites.

Acknowledgements—We gratefully acknowledge the expert technical assistance of Frances Kuber, Mary Ann Gaffney, Genevieve Deutsch and George Osman throughout the course of the study.

REFERENCES

1. J. T. Flynn, H. E. Appert and J. M. Howard, *Circulat. Shock* **2**, 155 (1975).
2. B. A. Jakschik, G. R. Marshall, J. L. Kourik and P. Needleman, *J. clin. Inves.* **54**, 842 (1974).
3. P. A. Johnston and E. E. Selkurt, *Am. J. Physiol.* **230**, 831 (1976).

4. J. T. Flynn, G. A. Bridenbaugh and A. M. Lefer, *Am. J. Physiol.* **230,** 684 (1976).
5. F. L. Anderson, W. Jubiz, T. J. Tsagaris and H. Kuida, *Am. J. Physiol.* **228,** 410 (1975).
6. J. G. Collier, A. G. Herman and J. R. Vane *J. Physiol., Lond.* **230,** 14P (1973).
7. E. Kessler, R. C. Hughes, E. N. Bennett and S. M. Nadela, *J. Lab. clin. Med.* **81,** 85 (1973).
8. K. Herbaczynska-Cedro and J. R. Vane, *Nature, Lond.* **247,** 492 (1974).
9. B. M. Jaffe, C. W. Parker, G. R. Marshall and P. Needleman, *Biochem. biophys. Res. Commun.* **49,** 799 (1972).
10. J. C. McGiff, K. Crowshaw, N. A. Terragno, A. J. Lonigro, J. C. Strand, M. A. Williamson, J. B. Lee and K. K. F. Ng, *Circulation Res.* **27,** 765 (1970).
11. F. L. Anderson, T. J. Tsagaris, W. Jubiz and H. Kuida, *Am J. Physiol.* **228,** 1479 (1975).
12. P. J. Piper and J. R. Vane, *Ann. N.Y. Acad. Sci.* **180,** 363 (1971).
13. R. P. Carlson and A. M. Lefer, *Am. J. Physiol.* **213,** 1408 (1976).
14. R. P. Carlson and A. M. Lefer, *Inflammation* **1,** 347 (1976).
15. M. L. Anson, *J. gen. Physiol.* **20,** 565 (1936).
16. T. M. Glenn and A. M. Lefer, *Circulation Res.* **27,** 783 (1970).
17. W. G. Unger, I. F. Stamford and A. Bennett, *Nature, Lond.* **233,** 336 (1971).
18. B. M. Jaffe, H. R. Behrman and C. W. Parker, *J. clin. Invest.* **52,** 398 (1973).
19. G. A. Bridenbaugh, J. T. Flynn and A. M. Lefer, *Am. J. Physiol.* **231,** 112 (1976).
20. J. T. Flynn and A. M. Lefer, *Circulation Res.* **40,** 422 (1977).
21. G. Markelonis and J. Garbus, *Fedn Eur. Biochem. Soc. Lett.* **51,** 7 (1975).
22. G. Markelonis and J. Garbus, *Prostaglandins* **10,** 1087 (1975).
23. A. J. Anderson, W. E. Brocklehurst and A. C. Willis, *Pharmac. Res. Commun.* **3,** 13 (1971).
24. G. Weissman, in *Lysosomes in Biology and Pathology* (Eds. J. T. Dingle and H. B. Fell), p. 276. American Elsevier, New York (1969).
25. Y. Floman, N. Floman and Z. Uriel, *Prostaglandins* **11,** 591 (1976).
26. G. P. Lewis and P. J. Piper, *Nature, Lond.* **254,** 308 (1975).
27. R. Flower, R. Gryglewski, K. Herbaczynska-Cedra and J. R. Vane, *Nature New Biol.* **238,** 104 (1972).
28. M. W. Greaves, W. P. Kingston and K. Pretty, *J. Pharmac.* **53,** 470P (1975).
29. M. W. Greaves and W. McDonald-Gibson, *Br. J. Pharmac.* **46,** 172 (1972).
30. A. H. Tashjian, Jr., E. F. Voelkel, J. McDonough and L. Levine, *Nature, Lond.* **258,** 793 (1975).
31. S. H. Ferreira, S. Moncada and J. R. Vane, *Nature New Biol.* **231,** 237 (1971).
32. J. R. Vane, *Nature, Lond.* **231,** 232 (1971).
33. L. Z. Bito and R. Baroody, *Prostaglandins* **7,** 131 (1974).
34. J. Chang, G. P. Lewis and P. J. Piper, *Br. J. Pharmac.* **56,** 342P (1971).
35. R. J. Gryglewski, B. Panczenko, R. Korbut, L. Grodzinska and A. Ocetkiewicz, *Prostaglandins* **10,** 343 (1975).
36. S. L. Hong and L. Levine, *Proc. natn. Acad. Sci. U.S.A.* **73,** 1730 (1976).
37. F. Kantrowitz, D. R. Robinson, M. B. McGuire and L. Levene, *Nature, Lond.* **258,** 737 (1975).
38. G. Gaja, M. E. Ferrero, P. Piccoletti and A. Bernelli-Zazzera, *Expl molec. Path.* **19,** 248 (1973).
39. A. M. Lefer and Y. Barenholz, *Am. J. Physiol.* **223,** 1103 (1972).