Br. J. Pharmac. (1985), **86**, 491–496

Effect of nonsteroidal anti-inflammatory drugs on glycogenolysis in isolated hepatocytes

Eric P. Brass[1] & Maureen J. Garrity

Departments of Medicine and Pharmacology, University of Colorado Health Sciences Center, Denver, Colorado, U.S.A.

1 E-series prostaglandins have previously been demonstrated to inhibit hormone-stimulated glycogenolysis when added to isolated hepatocytes of the rat. In the present study, the effect of nonsteroidal anti-inflammatory drugs, which inhibit cyclo-oxygenase activity, on glycogenolysis was examined in the hepatocyte model. Ibuprofen (80 μM), indomethacin (50 μM) and meclofenamate (60 μM) all increased rates of glycogenolysis when added under basal conditions. In contrast, piroxicam (50 μM) had no effect on glycogenolysis in the hepatocyte system. Concentrations of ibuprofen below 80 μM did not significantly increase rates of glycogenolysis.

2 Ibuprofen (80 μM) had no effect on glycogenolysis in the presence of 10^{-5}M adrenaline or 5×10^{-7}M glucagon, but did increase glycogenolytic rates in the presence of 5×10^{-8}M glucagon.

3 Ibuprofen-stimulated glycogenolysis was inhibited by addition of prostaglandin E_2 (PGE_2). Under conditions where glucagon-stimulated glycogenolysis was inhibited by exogenous PGE_2, addition of ibuprofen (80 μM) increased the rate of glycogenolysis.

4 Ibuprofen had no effect on basal or glucagon-stimulated hepatocyte adenylate cyclase activity.

5 In conclusion, these results demonstrate that nonsteroidal anti-inflammatory drugs which are carboxylic acids can increase the rate of glycogenolysis in isolated hepatocytes. The high concentrations of drug required to stimulate glycogenolysis, the lack of effect of piroxicam, and the demonstration of stimulation by ibuprofen in the presence of exogenous PGE_2 all suggest that the stimulation of glycogenolysis by ibuprofen, indomethacin and meclofenamate is independent of cyclo-oxygenase inhibition. These observations are consistent with reports that carboxylic acid nonsteroidal anti-inflammatory drugs can interfere with hepatic intracellular calcium handling.

Introduction

Prostaglandins are known to regulate cellular processes in a number of tissues. The observations that addition of E-series prostaglandins (PGE) inhibits hormone-stimulated glycogenolysis in isolated hepatocytes (Brass *et al.*, 1984; Brass & Garrity, 1985) and that liver plasma membranes contain a PGE-specific receptor (Robertson *et al.*, 1980; Rice *et al.*, 1981), suggest that PGE may have a role in the regulation of hepatic metabolism. Liver is also known to synthesize (Morita & Murota, 1978; Hewertson *et al.*, 1984) and degrade (Garrity *et al.*, 1984) PGE, both of which are required if a regulatory function for PGE in liver is to be hypothesized.

The biosynthesis of prostaglandins is thought to be limited by the availability of the precursor fatty acid, arachidonic acid, and its subsequent metabolism by fatty acid cyclo-oxygenase (Hassid, 1982). Nonsteroidal anti-inflammatory drugs inhibit fatty acid cyclo-oxygenase, and thus inhibit endogenous prostaglandin synthesis (Vane, 1971). This property of the nonsteroidal anti-inflammatory drugs has provided a useful tool to suggest actions of cyclo-oxygenase products related to regulation of biological processes. *In vivo* studies have demonstrated that nonsteroidal anti-inflammatory agents can alter hepatic glucose metabolism (Ganguli *et al.*, 1979; Miller *et al.*, 1983). To determine if endogenous cyclo-oxygenase products modulate the rate of glycogenolysis in isolated hepatocytes, we studied the effect of a number of nonsteroidal anti-inflammatory drugs on the regulation of glycogenolysis in the hepatocyte model. The results suggest that nonsteroidal anti-inflammatory drugs which contain a carboxylic acid group stimulate glycogenolysis by a mechanism independent of cyclo-oxygenase inhibition.

[1] Author for correspondence

Methods

Hepatocyte isolation and incubations

Hepatocytes were isolated from male, fed, Sprague Dawley rats (270 ± 8 g, mean ± s.e.mean, $n = 37$) by a modification of the method of Berry & Friend (1969) as previously described (Brass *et al.*, 1984). Hepatocyte preparations used in this study were 92 ± 1% viable on the basis of trypan blue exclusion ($n = 38$) and in all cases were greater than 85% viable. Hepatocytes averaged 11.6 ± 0.5 mg wet weight per 10^6 cells ($n = 27$). Cell yields averaged $54 \pm 4 \times 10^6$ cells per 100 g body weight ($n = 37$).

Incubations were conducted at 37°C under an atmosphere of 95% O_2/5% CO_2 in a shaking incubator bath. Hepatocytes ($2.5-5.0 \times 10^6$ cells ml^{-1}) were added to the incubation buffer (composition, mM: NaCl 128.5, KCl 5.2, $MgSO_4$ 0.9, $CaCl_2$ 1.2, Na_2HPO_4 3.0, glucose 5.0 and Tris (hydroxymethyl) aminoethane 10, pH 7.4) and preincubated for 30 min. Following the preincubation, drugs, hormones or prostaglandins were added at time zero. At times indicated, aliquots were removed from the incubation, placed in chilled tubes and immediately spun in a high speed micro-centrifuge. Supernatants were stored at −20°C for further analysis.

Glycogenolysis

Glycogenolysis was determined as the rate of glucose production in the incubations in the absence of gluconeogenic substrates (Garrison & Haynes, 1973). Glucose concentration in the incubation was measured after 0, 10, 20 and 30 min by a glucose oxidase method (Hjelm & DeVerdier, 1963). The rate of glycogenolysis was determined by the slope of the linear least squares regression line through the glucose-time points. The increase in glucose concentration was linear with time over the incubation period (Brass *et al.*, 1984).

Adenylate cyclase

Adenylate cyclase activity was measured in a crude membrane fraction prepared from isolated hepatocytes. Cells ($2-3 \times 10^6$) were lysed by freeze-thawing, homogenized and centrifuged at 23,000 *g* for 20 min at 4°C. The pellet was resuspended in 20 mM Tris (hydroxymethyl) amninoethane, pH 7.5, to a final concentration equivalent to 10^6 cells ml^{-1}. Adenylate cyclase activity was then assayed by the method of Salomon *et al.* (1974) as previously described (Robertson *et al.*, 1980). Results are expressed as picomoles adenosine 3′:5′-cyclic monophosphate (cyclic AMP) generated per mg membrane protein per 10 min.

Reagents

All chemicals and solvents used were of reagent grade. Collagenase (type II) was obtained from Worthington Diagnostic Systems, Inc., Freehold, New Jersey. Glucagon was a gift of Eli Lilly and Company and was stored at −20°C in 10 mM NaOH. (−)-Adrenaline was obtained from Sigma Chemicals, St Louis and was dissolved in 10 mM HCl on the day of use. PGE_2 was obtained from Upjohn Pharmaceuticals, Kalamazoo, Michigan. [α-^{32}P]-ATP was obtained from New England Nuclear Company, Boston, Massachusetts. Piroxicam and indomethacin were obtained from Sigma Chemicals, ibuprofen was obtained from Upjohn Pharmaceuticals, naproxen from Syntex Laboratories and meclofenamate from Warner-Lambert Pharmaceuticals. The non-steroidal anti-inflammatory drugs were dissolved on the day of use in 100 mM Na_2CO_3 and then the solution adjusted to pH 7.5–8.5 with 1.2 N HCl. In some experiments, a stock solution of ibuprofen in 95% ethanol was used. Studies using comparable concentrations of Na_2CO_3/HCl or ethanol diluents alone showed no effect on any of the processes measured.

Data analysis

In each study, sets of hepatocyte incubations were conducted containing all conditions of interest. For example, basal, basal plus drug, basal plus drug plus PGE_2, or basal, hormone, hormone plus drug. All comparisons were made within incubation sets. *n* refers to the number of incubation sets, each run with

Table 1 Effect of nonsteroidal anti-inflammatory drugs on basal glycogenolysis in isolated hepatocytes

	Ibuprofen (80 μM, $n = 16$)	*Indomethacin* (50 μM, $n = 9$)	*Meclofenamate* (60 μM, $n = 7$)	*Naproxen* (70 μM, $n = 8$)	*Piroxicam* (50 μM, $n = 7$)
Basal	1.54 ± 0.20	0.93 ± 0.14	1.70 ± 0.26	1.24 ± 0.22	1.19 ± 0.12
Drug	2.56 ± 0.28*	1.27 ± 0.16*	2.30 ± 0.39*	1.42 ± 0.22†	1.21 ± 0.20

Hepatocyte incubations were conducted as detailed in the text. At time zero, drugs were added at the concentrations indicated. Values are rates of glycogenolysis in μg glucose per 10^6 cells min^{-1}, and are mean ± s.e.mean. * $P < 0.05$; † $0.2 > P > 0.1$ basal *vs.* drug.

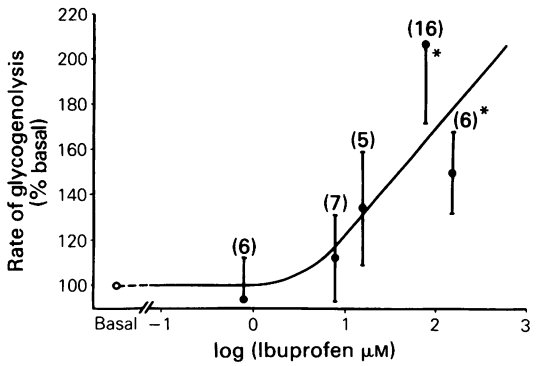

Figure 1 Effect of ibuprofen on basal glycogenolysis. Hepatocyte incubations were conducted as detailed in the text. Data are normalized to basal glycogenolysis equals 100% for each set of incubations (mean 100% value = 1.51 ± 0.15 μg glucose per 10^6 cells min^{-1}, $n = 23$). Values are mean with s.e.mean shown by vertical lines. * $P < 0.05$ ibuprofen *vs.* basal. Number in parentheses = n.

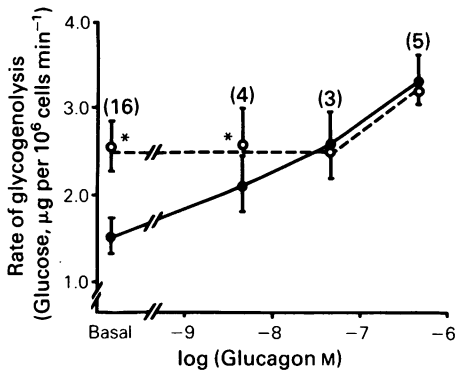

Figure 2 Effect of ibuprofen on glucagon-stimulated glycogenolysis. Hepatocyte incubations were conducted as detailed in the text. (●) Without ibuprofen; (○) presence of 80 μM ibuprofen. Values are mean with s.e.mean shown by vertical lines. * $P < 0.05$ ibuprofen *vs.* absence of ibuprofen. Number in parentheses = n.

separate hepatocyte preparations. Data were analysed for statistical significance using Student's t test (single-tailed); $P < 0.05$ was considered significant.

Results

The effect of several structurally distinct nonsteroidal anti-inflammatory drugs on basal glycogenolysis in isolated rat hepatocytes is shown in Table 1. Ibuprofen, indomethacin and meclofenamate all increased the rate of glycogenolysis; naproxen caused a smaller increase in glycogenolysis and piroxicam had no effect. The increase in rate of glycogenolysis was dose-dependent with respect to ibuprofen, and was only statistically significant at ibuprofen concentrations of 80 μM or above (Figure 1).

In contrast to the effect seen on basal glycogenolysis, ibuprofen had no effect on the rates of glycogenolysis in the presence of maximal stimulatory concentrations of adrenaline or glucagon (Table 2). The glucagon dose-response curve for stimulation of glycogenolysis in the absence or presence of 80 μM ibuprofen demonstrates that ibuprofen increased glycogenolysis only at glucagon concentrations below 5×10^{-8} M (Figure 2).

Addition of PGE to hepatocyte incubations is known to inhibit hormone-stimulated glycogenolysis (Brass *et al.*, 1984; Brass & Garrity, 1985). Because the nonsteroidal anti-inflammatory drugs would be expected to inhibit PGE generation by the hepatocytes it was of interest to study the interaction of the nonsteroidal anti-inflammatory drugs and exogenous PGE. PGE_2 was added at 10 min intervals during the incubation as previously described (Brass *et al.*, 1984) to compensate for rapid catabolism (Garrity *et al.*, 1984). PGE_2 reversed the increase in rate of glycogenolysis caused by addition of ibuprofen under basal conditions (Table 3). This demonstrates that ibuprofen did not interfere with the effects of prostaglandins once they were generated. However, when exogenous PGE_2 was used to inhibit glucagon-stimulated glycogenolysis, addition of 80 μM ibuprofen increased the rate of glycogenolysis. If the effect of ibuprofen on glycogenolysis was solely through the inhibition of cyclo-oxygenase, it would be expected to have no effect in the presence of large concentrations of exogenous PGE_2.

Table 2 Effect of ibuprofen on hormone-stimulated glycogenolysis in isolated hepatocytes

Conditions	*Rate of glycogenolysis* (μg glucose per 10^6 cells min^{-1})
Set 1 ($n = 7$)	
Basal	1.93 ± 0.19
Adrenaline (10^{-5} M)	3.30 ± 0.38
Adrenaline (10^{-5} M) + ibuprofen (80 μM)	3.03 ± 0.34
Set 2 ($n = 5$)	
Basal	1.54 ± 0.29
Glucagon (5×10^{-7} M)	3.31 ± 0.32
Glucagon (5×10^{-7} M) + ibuprofen (80 μM)	3.31 ± 0.27

Hepatocyte incubations were conducted as detailed in the text. At time zero, hormone with or without ibuprofen was added. Values are mean ± s.e.mean.

Table 3 Interaction of ibuprofen and prostaglandin E_2 (PGE_2) on glycogenolysis in isolated hepatocytes

Conditions	*Rate of glycogenolysis* (μg glucose per 10^6 cells min^{-1})
Set 1 ($n = 6$)	
Basal	1.07 ± 0.30
Ibuprofen (80 μM)	1.76 ± 0.32†
Ibuprofen (80 μM) + PGE_2	1.48 ± 0.20*
Set 2 ($n = 4$)	
Basal	1.38 ± 0.23
Glucagon (5×10^{-7} M)	3.09 ± 0.15
Glucagon (5×10^{-7} M) + PGE_2	2.37 ± 0.23*
Glucagon (5×10^{-7} M) + PGE_2 + ibuprofen (80 μM)	3.11 ± 0.24†

Hepatocyte incubations were conducted as detailed in the text. At time zero, hormone with/or without ibuprofen were added at the concentrations indicated. PGE_2 was added at a concentration of 1.7 μM at times zero, 10 and 20 min of incubation to compensate for PGE catabolism. Values are mean ± s.e.mean.
*$P<0.05$ PGE_2 *vs.* absence of PGE_2, †$P<0.05$ ibuprofen *vs.* absence of ibuprofen.

The high concentrations of nonsteroidal anti-inflammatory drugs required to stimulate glycogenolysis, the lack of effect of piroxicam (which inhibits liver prostaglandin formation, Burch *et al.*, 1983) and the stimulatory effect of ibuprofen in the presence of exogenous PGE_2, suggested these drugs were acting independently of cyclo-oxygenase inhibition. If the action of ibuprofen, indomethacin and meclofenamate in stimulating glycogenolysis is independent of cyclo-oxygenase inhibition, their action might be through alterations in either cyclic AMP generation or regulation of intracellular calcium concentrations which are key factors in the control of glycogenolysis (Exton, 1982). Recent reports from other laboratories provide evidence that drugs such as ibuprofen can interfere with intracellular calcium handling at the concentrations reported here to increase glycogenolysis (Northover, 1973; Northover, 1977; Burch *et al.*, 1983). To determine whether ibuprofen could directly affect cyclic AMP production, the effect of the drug on adenylate cyclase activity was studied on isolated hepatocyte membranes. No effect of 80 μM ibuprofen on adenylate cyclase was observed under basal or glucagon-stimulated conditions (Table 4).

Table 4 Effect of ibuprofen on hepatocyte adenylate cyclase

Conditions	*Adenylate cyclase* (Cyclic AMP pmol mg^{-1} protein per 10 min)
Basal	112 ± 6 ($n = 5$)
Ibuprofen (80 μM)	120 ± 3 ($n = 3$)
Glucagon (10^{-7} M)	1250 ± 200 ($n = 3$)
Glucagon (10^{-7} M) + ibuprofen (80 μM)	1370 ± 230 ($n = 3$)

Plasma membranes were prepared from hepatocytes and assayed for adenylate cyclase as described in the text. Values are mean ± s.e.mean.

Discussion

A physiological role for prostaglandins has been identified in many tissues. We have recently demonstrated that exogenous E-series prostaglandins can inhibit hormone-stimulated glycogenolysis in isolated hepatocytes (Brass *et al.*, 1984; Brass & Garrity, 1985). However, no direct evidence exists that endogenously generated prostaglandins act to modulate liver metabolism. *In vivo* studies have shown that interfering with hepatic prostaglandin metabolism results in altered hepatic glucose metabolism (Ganguli *et al.*, 1979; Miller *et al.*, 1983), but it is difficult to exclude completely indirect effects, such as changes in hormone levels, as being responsible for these *in vivo* observations. In an attempt to address the question of the effect of endogenously generated hepatic prostaglandins on hepatic glucose metabolism, we studied the actions of several cyclo-oxygenase inhibitors on glycogenolysis in rat isolated hepatocytes. Ibuprofen, indomethacin and meclofenamate all stimulated basal rates of glycogenolysis but this effect appears to be independent of cyclo-oxygenase inhibition.

Several observations support the concept that the increase in rates of glycogenolysis observed with ibuprofen, indomethacin and meclofenamate is not dependent on inhibition of prostaglandin synthesis. The concentrations of drug required to stimulate glycogenolysis (Table 1, Figure 1) are 10 to 100 fold higher than those usually required to inhibit cyclo-oxygenase activity (Metz, 1981). Specifically, in rat liver the concentration required for 50% inhibition of prostaglandin synthesis has been reported to be less than 1 μM for meclofenamate (Burch *et al.*, 1983) and 4–10 μM for indomethacin (Burch *et al.*, 1983; Hewertson *et al.*, 1984). These concentrations are consistent with those required for cyclo-oxygenase inhibition in other tissues (Metz, 1981). Additionally, if inhibition of cyclo-oxygenase resulted in an increase in glycogenolysis, this effect should have been seen with piroxicam which has been shown to inhibit arachidonic acid metabolism in rat liver (Burch *et al.*, 1983) but did not

stimulate glycogenolysis in the present study (Table 1). Finally, if ibuprofen were stimulating glycogenolysis exclusively by removing inhibitory endogenous prostaglandins, then no effect of ibuprofen would be expected in the presence of high concentrations of exogenous prostaglandins, which should mask any effect of the endogenous compounds. As Table 3 demonstrates, ibuprofen stimulated the rate of glycogenolysis when added to incubations containing glucagon and PGE_2.

The studies described here do not demonstrate a mechanism by which ibuprofen, indomethacin and meclofenamate might stimulate glycogenolysis independent of cyclo-oxygenase inhibition. Recently, Burch *et al.* (1983) have extended earlier observations by Northover (1973, 1977) and demonstrated that nonsteroidal anti-inflammatory drugs which are carboxylic acids interfered with intracellular sequestration of calcium and membrane binding of calcium in liver tissue. These effects would tend to increase cytoplasmic calcium concentrations, an effect known to stimulate glycogenolysis (Exton, 1982). Ibuprofen, indomethacin and meclofenamate, all of which stimulated glycogenolysis (Table 1), all contain carboxylic acid groups, and hence would be expected to demonstrate this effect on calcium metabolism. Burch *et al.* (1983) demonstrated that the effects of these drugs on calcium metabolism were independent of cyclo-oxygenase inhibition and were only observed at higher drug concentrations, as was observed here for stimulation of glycogenolysis. Piroxicam, which is not a carboxylic acid, did not stimulate glycogenolysis (Table 1) nor did it interfere with hepatic calcium metabolism (Burch *et al.*, 1983). Naproxen, while a carboxylic acid, had a smaller effect on glycogenolysis, and was found by Burch *et al.* (1983) to be less potent than meclofenamate or indomethacin in affecting calcium sequestration.

Other observations reported here are also consistent with the nonsteroidal anti-inflammatory drugs stimulating glycogenolysis through a calcium-dependent mechanism. No effect of ibuprofen was demonstrated on hepatic adenylate cyclase (Table 4), which, in addition to cytoplasmic calcium, plays a major role in regulating glycogenolysis. PGE_2 inhibited ibuprofen-stimulated glycogenolysis (Table 3). Because PGE_2 has no effect on cyclic AMP-dependent stimulation of glycogenolysis distal to cyclic AMP generation, but does appear to inhibit calcium-dependent stimulation of glycogenolysis at a site after the increase in cytoplasmic calcium (Brass & Garrity, 1985), the inhibitory effect of PGE_2 seen here would be consistent with ibuprofen acting to increase cytoplasmic calcium concentrations.

Figure 2 suggests that the stimulation of glycogenolysis by ibuprofen and submaximal concentrations of glucagon were not additive. For example, the rate of glycogenolysis in the presence of 80 μM ibuprofen and 5×10^{-8} M glucagon was less than the sum of the rates with 80 μM ibuprofen and 5×10^{-8} M glucagon alone. While no clear explanation for this non-additive phenomenon is evident, it is possible that this may also relate to a calcium-mobilizing effect of ibuprofen. Under some conditions, calcium can be shown to inhibit hormone-stimulated adenylate cyclase activity and cyclic AMP accumulation (Grinde & Ichihara, 1983; Dorflinger *et al.*, 1984). Thus, the suggested ibuprofen-induced increase in calcium, in addition to stimulating glycogenolysis directly may inhibit glucagon-stimulated cyclic AMP accumulation, and therefore glucagon-stimulated glycogenolysis. The response of the system to both ibuprofen and glucagon would then be less than the sum of the individual agents because of the blunted glucagon component of the response.

The concentrations of drugs observed to stimulate glycogenolysis in the present study are high but might be reached during *in vivo* therapeutic use of these agents (Metz, 1981). However, direct comparison of the *in vitro* concentrations used and those seen *in vivo* is difficult because the drugs are highly protein bound (Metz, 1981) and the more important free drug concentration is not known. Salicylate intoxication is well known to be associated with enhanced hepatic glycogenolysis (Thurston *et al.*, 1970). Several mechanisms have been suggested to explain salicylate-induced glycogenolysis (Thurston *et al.*, 1970), but it is interesting to note that salicylate is a carboxylic acid and would be expected to have effects similar to those described here for ibuprofen, meclofenamate and indomethacin.

While the present study does not provide evidence supporting a role of endogenous prostaglandins in regulatory hepatic glucose metabolism, the results should not be interpreted to rule out such a role. The ability of liver to synthesize (Morita & Murota, 1978; Hewertson *et al.*, 1984) and rapidly degrade (Garrity *et al.*, 1984) prostaglandins, the presence of a PGE specific receptor on liver plasma membranes (Robertson *et al.*, 1980; Rice *et al.*, 1981) and the actions of exogenous PGE on hepatic glycogenolysis (Brass *et al.*, 1984, Brass & Garrity, 1985) all suggest a potential physiological role for PGE in the regulation of hepatic metabolism. The isolated, suspended hepatocyte system may not be optimal for demonstrating the action of endogenous prostaglandins. The cell density in the suspension is very low as compared to the intact organ and this might dilute the effective concentration of any prostaglandins formed. Additionally, the incubation conditions may not be optimal for prostaglandin synthesis, or accurately reflect the balance seen *in vivo* between prostaglandin synthesis and breakdown. Finally, the cells are removed from their natural cellular environment found in the intact organ *in vivo* which

might remove a local source of prostaglandins independent of the hepatocytes. Further work is in progress to examine these possibilities.

In summary, nonsteroidal anti-inflammatory drugs which are carboxylic acids can act directly on hepatocytes to increase the rate of glycogenolysis. This effect appears to be independent of cyclo-oxygenase inhibition, and is consistent with the suggestion that these drugs interfere with hepatic intracellular calcium sequestration.

The authors thank Stewart A. Metz, M.D. for his helpful comments on this manuscript. The technical assistance of Linda Schalch, Phoebe Tsai and Marilee Kao is gratefully acknowledged. This work was supported in part by a Feasibility Grant from the American Diabetes Association and by grant BRSG-05357 awarded by the Bio-medical Research Grant Program, Division of Research Resources, NIH.

References

BERRY, M.N. & FRIEND, D.S. (1969). High-yield preparation of isolated rat liver parenchymal cells. *J. cell Biol.*, **43,** 506–520.

BRASS, E.P., GARRITY, M.J. & ROBERTSON, R.P. (1984). Inhibition of glucagon-stimulated hepatic glycogenolysis by E-series prostaglandins. *FEBS Lett.*, **169,** 293–296.

BRASS, E.P. & GARRITY, M.J. (1985). Effect of E-series prostaglandins on cyclic AMP-dependent and -independent hormone-stimulated glycogenolysis in hepatocytes. *Diabetes*, **34,** 291–294.

BURCH, R.M., WISE, W.C. & HALUSHKA, P.V. (1983). Prostaglandin-independent inhibition of calcium transport by nonsteroidal anti-inflammatory drugs: Differential effects of carboxylic acids and piroxicam. *J. Pharmac. exp. Ther.*, **227,** 84–91.

DORFLINGER, L.J., ALBERT, P.J., WILLIAMS, A.T. & BEHRMAN, H.R. (1984). Calcium is an inhibitor of luteinizing hormone-sensitive adenylate cyclase in the luteal cell. *Endocrinology.*, **114,** 1208–1215.

EXTON, J.H. (1982). Regulation of carbohydrate metabolism by cyclic nucleotides. In *Cyclic Nucleotides II, Handb. Exp. Pharmac.*, vol. 58, ed. Kehabian, J.W. & Nathanson, J.A., pp. 3–88. Berlin and Heidelberg: Springer-Verlag.

GANGULI, S., SPERLING, M.A., FRAME, C. & CHRISTENSEN, R. (1979). Inhibition of glucagon-induced hepatic glucose production by indomethacin. *Am. J. Physiol.*, **236,** E358–E365.

GARRISON, J.C. & HAYNES, R.C., JR. (1973). Hormonal control of glycogenolysis and gluconeogenesis in isolated rat liver cells. *J. biol. Chem.*, **248,** 5333–5343.

GARRITY, M.J., BRASS, E.P. & ROBERTSON, R.P. (1984). Kinetics of prostaglandin E metabolism in isolated hepatocytes. *Biochim. biophys. Acta*, **796,** 136–141.

GRINDE, B. & ICHIHARA, A. (1983). Effects of prostaglandins and divalent cations on cAMP production in isolated rat hepatocytes. *Exp. Cell Res.*, **148,** 163–172.

HASSID, A. (1982). Regulation of prostaglandin biosynthesis in cultured cells. *Am. J. Physiol.*, **243,** C205–C211.

HEWERTSON, S., McDONALD-GIBSON, R.G. & SLATER, T.F. (1984). The arachidonic acid cascade in rat liver. *Biochem. Soc. Trans.*, **12,** 835–837.

HJELM, M. & DEVERDIER, C.H. (1963). A methodological study of the enzymatic determination of glucose in blood. *Scand. J. clin. Invest.*, **15,** 415–428.

METZ, S.A. (1981). Anti-inflammatory drugs as inhibitors of prostaglandin synthesis in man. *Med. Clinics North Amer.*, **65,** 713–758.

MILLER, J.D., GANGULI, S. & SPERLING, M.A. (1983). Prostaglandin synthesis inhibitors impair hepatic glucose production in response to glucagon and epinephrine stimulation. *Diabetes*, **32,** 439–444.

MORITA, I. & MUROTA, S. (1978). Prostaglandin-synthesizing system in rat liver. *Eur. J. Biochem.*, **90,** 441–449.

NORTHOVER, B.J. (1973). Effect of anti-inflammatory drugs on the binding of calcium to cellular membranes in various human and guinea-pig tissue. *Br. J. Pharmac.*, **48,** 496–504.

NORTHOVER, B.J. (1977). Effect of indomethacin and related drugs on the calcium ion-dependent secretion of lysosomal and other enzymes by neutrophil polymorphonuclear leukocytes *in vitro*. *Br. J. Pharmac.*, **59,** 253–259.

RICE, M.G., McRAE, J.R., STORM, D.R. & ROBERTSON, R.P. (1981). Up-regulation of hepatic prostaglandin E receptors *in vivo* induced by prostaglandin synthesis inhibitors. *Am. J. Physiol.*, **241,** E291–E297.

ROBERTSON, R.P., WESTCOTT, K.R., STORM, D.R. & RICE, M.G. (1980). Down-regulation *in vivo* of PGE receptors and adenylate cyclase stimulation. *Am. J. Physiol.*, **239,** E75–E80.

SALOMON, Y., LONDOS, C. & RODBELL, M. (1974). A highly sensitive adenylate cyclase assay. *Anal. Biochem.*, **58,** 541–548.

THURSTON, J.H., POLLOCK, P.G., WARREN, S.K. & JONES, E.M. (1970). Reduced brain glucose with normal plasma glucose in salicylate poisoning. *J. clin. Invest.*, **49,** 2139–2145.

VANE, J.R. (1971). Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. *Nature, New Biol.*, **234,** 231–238.

(Received April 3, 1985.
Revised May 30, 1985.
Accepted June 11, 1985.)