Novel role for prostaglandin E₂ in fish hepatocytes: regulation of glucose metabolism

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Abstract

Prostaglandin E₂ (PGE₂) potently activated glycogenolysis and gluconeogenesis in isolated rockfish (Sebastes caurinus) hepatocytes. The average degree of activation for glycogenolysis was 6·4 ± 0·67-fold (mean ± s.e.m.; n = 37), and could be as much as 19-fold. Analysis of dose–concentration relationships between glycogenolytic actions and PGE₂ concentrations yielded an EC₅₀ around 120 nM in hepatocyte suspensions and 2 nM for hepatocytes immobilized on perfusion columns. For the activation of gluconeogenesis (1·74 ± 0·14-fold; n = 10), the EC₅₀ for suspensions was 60 nM. Intracellular targets for PGE₂ actions are adenyl cyclase, protein kinase A and glycogen phosphorylase. Concentrations of cAMP increased with increasing concentrations of PGE₂, and peaked within 2 min of hormone application. In the presence of the phosphodiesterase inhibitor, isobutyl-3-methylxanthine, peak height was increased and peak duration extended. The protein kinase A inhibitor, Rp-cAMPS, counteracted the activation of glycogenolysis by PGE₂, implying that the adenyl cyclase/protein kinase A pathway is the most important, if not exclusive, route of message transduction. PGE₂ activated plasma membrane adenyl cyclase and hepatocyte glycogen phosphorylase in a dose-dependent manner. The effects were specific for PGE₂; smaller degrees of activation of glycogenolysis were noted for PGE₁, 11-deoxy PGE₁, 19-R-hydroxy-PGE₂, and prostaglandins of the A, B and F₁₂-series. The selective EP₂-receptor agonist, butaprost, was as effective as PGE₂, suggesting that rockfish liver contains prostaglandin receptors pharmacologically related to the EP₂ receptors of non-hepatic tissues of mammals. Rockfish hepatocytes quickly degraded added PGE₂ (t₁/₂ = 17–26 min). A similar ability to degrade PGE₂ has been noted in catfish (Ameiurus nebulosus) hepatocytes, but no glycogenolytic or gluconeogenic actions of the hormone are noted for this species.

We conclude that PGE₂ is an important metabolic hormone in fish liver, with cAMP-mediated actions on glycogen and glucose metabolism, and probably other pathways regulated by cAMP and protein kinase A. The constant presence of EP₂-like receptors is a unique feature of the fish liver, with interesting implications for function and evolution of prostaglandin receptors in vertebrates.

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Introduction

Hormones derived from arachidonate fulfill important endocrine and paracrine functions. Of these hormones, prostaglandins (PGs) are known to regulate a multitude of different cellular functions in mammals, with liver among the target tissues. Liver had been identified as a site of action for PGs, not least because of its ability to bind (Garrity et al. 1989), and degrade, prostaglandins (Okumura et al. 1985), but also because of the function of PGs in liver regeneration, DNA synthesis and hepatocyte proliferation (Refsnes et al. 1994). In addition to regulating hepatic blood flow, PGs exert cytoprotective effects and control glycogen metabolism in liver (Okumura et al. 1993, Püschel et al. 1993). Mammalian liver parenchymal cells are potentially exposed to diverse prostaglandins, contributed either by non-parenchymal cells (Kupffer cells, epithelial cells, stellate cells) or remote cells (splanchnic organs) via plasma, or from endogenous sources (Wernze et al. 1986).

Special attention has been devoted to the part played by prostaglandins of the E series in regulating hepatic intermediary metabolism (Garrity et al. 1984, Okumura et al. 1985, Püschel & Christ 1994). With the focus on prostaglandin E₂ (PGE₂), some controversy has arisen over its apparent opposing actions on hepatic glycogenolysis. Conversely, the hormone is believed to activate the breakdown of glycogen, mediated via calcium, inositol triphosphate (IP₃) and, possibly, cAMP systems, resulting in the activation of glycogen phosphorylase through phosphorylation, and leading to increases in hepatocyte glucose output (Mine et al. 1990). In addition, the ability of PGE₂ to curtail or even block the glucagon- or epinephrine-dependent activation of glycogenolysis has attracted as...
much attention (Brass et al. 1988, Püschel et al. 1993). Different, not mutually exclusive, pathways mediate the multifaceted actions of PGE₂. In mammals, four types of G-protein linked prostaglandin E (EP) receptors have been defined by molecular and functional techniques: EP₁ receptors activate intracellular Ca²⁺ and the IP₃ system, EP₃ inhibits adenyl cyclase via pertussis-toxin sensitive inhibitory G proteins, and EP₂- and EP₄-subtype receptors lead to the activation of adenyl cyclase involving Gₛ (Ichikawa et al. 1996). Although molecular evidence is equivocal (Ichikawa et al. 1996), the mammalian liver appears to contain mainly EP₁ and EP₃ receptors, with EP₁ receptors involved in the PGE₂-dependent activation of glycogenolysis, and EP₃ receptors accounting for the antiglycogenolytic effects of PGE₂ on glucagon- or epinephrine-stimulated glycogen breakdown (Püschel et al. 1993). Some recent evidence supports the notion that EP₂ and EP₄ subtypes of receptors can be induced in hepatocytes (Fennekohl et al. 2000).

In fishes, prostaglandins and active prostaglandin turnover are found in numerous cells and tissues (Mustafa & Srivastava 1989), including macrophages (Pettitt et al. 1991), red blood cells (Cagen et al. 1983) and oocytes (Stacey & Goetz 1982). Prostaglandins have important roles in ovulation and egg maturation (Stacey & Goetz 1982), but no information is available on direct or indirect metabolic actions of this group of hormones. However, large differences from mammalian systems are to be expected. For instance, in contrast to mammalian red blood cells, the nucleated erythrocytes of fishes possess a significant ability to synthesize prostaglandins (Cagen et al. 1983), resulting in prostaglandin availability in any well-perfused organ, including the liver. Further, fish liver microarchitecture is distinguished by the absence of cells with macrophage-type characteristics and a high percentage (>96%) of parenchymal cells (Hampton et al. 1989), largely ruling out accessory sources as sources of hepatic prostaglandins. Finally, and again in contrast to mammalian systems, fish liver glycogen is comparatively inert and only partially depleted by a short fast (1 or 2 days) (Gutiérrez & Navarro 1995). Hence, as we have previously shown, glycogenolysis is a pathway reacting sensitively and reproducibly to added hormones (Mommsen & Moon 1990, Moon et al. 1999) and therefore can serve as a useful tool with which to probe hormonal effects on metabolism in vitro.

Using fish hepatocytes, we show here for the first time that fish liver is an important target for prostaglandins of the E series, especially PGE₂. This prostanoïd accelerates glycogenolysis from endogenous glycogen, gluconeogenesis from three-carbon precursors, and activity of adenyl cyclase of isolated liver membranes. We also present data on the involvement of cAMP as short-term intracellular messenger, tentative identification of EP₂-like receptor activity in fish liver, degradation kinetics of PGE₂, target glycogen phosphorylase and the role of protein kinase A in PGE₂-dependent activation of rockfish (Sebastes caurinus) hepatocytes.

Materials and Methods

Copper rockfish (Sebastes caurinus, Teleostei: Scorpaenidae) were caught by hook and line in Georgia Strait, BC, and maintained in a 4500-litre tank (salinity 2-9%) at 12 °C for up to 3 years, under natural light conditions. They were fed chopped herring, squid or smelt weekly. Hepatocytes were isolated from fish of both sexes (200–500 g) by in vitro collagenase perfusion (Danulat & Mommsen 1990). Briefly, fish were killed by stunning and cervical dislocation. The liver was excised and cannulated via two of the portal veins using PE90 tubing. The tissue was washed with modified Hanks’ medium (Mommsen et al. 1994) for up to 20 min at 20 °C and then digested with collagenase (0-01%; type IV, Sigma), until the first cells were noticed in the perfusion medium (<1 h). Digestion medium was recirculated after about 10 min. After digestion, the liver was lightly chopped with a razor blade, suspended in cold isolation medium and passed through two successive filters (254 μm and 64 μm). Cells were collected by centrifugation (90 g, 4 min, 20 °C) and washed at least three times in 10 volumes of modified Hanks’ medium, supplemented with 2% defatted bovine serum albumin (BSA) and 1·5 mM CaCl₂ at pH 7·63 (Hanks’-BSA). Hepatocytes were pretested for their glycogenolytic response to 10 nM and 100 nM PGE₂, preparations yielding an activation of glycogenolysis exceeding twofold that of controls were used in the experiments. After an additional wash, cell concentrations were adjusted to 150–200 mg cell weight per ml.

Incubations contained 50 μl cell suspension and medium and hormones in a final volume of 100 μl in microcentrifuge tubes. Cells were agitated every 10 min and incubated at 20 °C; reactions were terminated by adding 10 μl 35% perchloric acid (PCA). Time-course studies were performed in batches, with removal and acidification of 200 μl aliquots at timed intervals. Samples were centrifuged and 10–15 μl samples of supernatant were assayed enzymatically for glucose with hexokinase and glucose-6-phosphate dehydrogenase and NAD as the chromogen. Assays were performed in microtiter plates in a total volume of 210–260 μl, using external and internal standards for each plate. For gluconeogenesis, cells were incubated with lactate/pyruvate (10 mM/2 mM) and control rates without exogenous carbon sources were subtracted from the total glucose production rates. In rockfish cells given lactate and pyruvate, gluconeogenesis accounts for up to 50% of total glucose production (Danulat & Mommsen 1990).

Hepatocyte perfusion columns were prepared as described elsewhere (Ottolenghi et al. 1994), using 3 ml minicolumns (BioRad, Mississauga, Canada) packed with
cells suspended in BioGel P4 and 0·5 ml BioGel P2 (BioRad), on inflow and outflow sides. Columns containing 80–120 mg of cell wet mass were perfused with Hanks’–BSA. Flow was adjusted to about 200 µl/min, and 1·5–3 min samples were collected. Up to eight columns assembled from the same cell preparation were perfused concurrently. Effluent glucose was determined enzymatically on 50–100 µl aliquots as above. Column contents were homogenized in 1 volume buffer and assayed for malate dehydrogenase activity for back-calculation of cell concentration.

Membranes were isolated after liver or hepatocyte homogenization in isotonic, buffered sucrose, followed by sucrose-gradient centrifugation (Mommsen & Mojsov 1998). Crude membranes were taken up in 5 mM Tris, pH 7·4 and stored at −80 °C for up to 1 month. Proteins were determined by a bicinchoninic acid technique (BioRad) with BSA as reference. Adenylyl cyclase activity was assayed in duplicate following the method of Fabbri et al. (1992); the volume was reduced to 100 µl and 30–100 µg of membrane protein was used per assay.

Activity and activation of glycogen phosphorylase were assayed as outlined elsewhere (Moon et al. 1999). PGE2 and cAMP were measured with enzyme immunoassay kits from Cayman Chemical, Ann Arbor, MI, USA, or Amersham. PCA samples were neutralized and diluted with phosphate buffer; samples were acetylated before cAMP analysis, except for adenylyl cyclase assays.

Biochemicals were supplied by Cayman Chemical, Roche or Sigma. Rp-cAMPS was purchased from Biolog (La Jolla, CA, USA). Bovine glucagon was obtained from CalBiochem (La Jolla, CA, USA), and butaprost was kindly donated by Dr M P Kotick, Bayer Research Center (West Haven, CT, USA).

Agonist stock solutions (10–30 mM) were prepared in ethanol, stored at −20 °C and diluted with assay or perfusion medium just before use. Controls for ethanol were included in the experimental design, but discontinued after it was ascertained that the solvent did not induce metabolic changes in the rockfish. DMSO could not be used as a solvent, because it increases the endogenous rate of glycogenolysis, largely masking hormonal effects.

Statistical analysis

All data are presented as mean ± s.e.m. Because of the block design for the experiments and substantial fish-to-fish variability, samples were normalized to internal standards for each preparation. ANOVA of arc-sine or log-transformed data were used for statistical analysis, followed by Student–Newman–Keul’s test. In some cases, paired t-test or orthogonal design was used. In all cases, P<0·05 was considered significant.

Results

Activation of glycogenolysis and gluconeogenesis

Prostaglandin E2 potently activated glycogenolysis in rockfish hepatocytes. In the absence of exogenous gluconeogenic substrates, exposed hepatocytes released increasing amounts of glucose derived from endogenous glycogen (Fig. 1). This effect was detectable within 2 min of application of the hormone and, under the given experimental conditions, was transient and subsided after about 30–45 min. The rate of glucose release reached an early peak at around 10 min, followed by a decrease (Fig. 1B). About 45–60 min after application of the hormone, depending on the dose, the cells resumed the control rate of glucose production. The EC50 for PGE2-dependent glycogenolysis is in the high nanomolar range, and somewhat dependent on the season, with summer experiments providing the lowest values (data not shown). Such
seasonality in response is not unique to the rockfish, but has been shown for other teleostean models (Mommsen & Moon 1990). It should be noted that our fish were maintained at a constant 12 °C, but under natural light conditions.

The degree of activation of glycogenolysis depended on the hormone concentration (Fig. 2A) and differed between various prostaglandins and analogues (Fig. 2B, Table 1), with PGE₂ eliciting the most consistent and strongest activation among the PGs. We selected two agonists to establish concentration–effect relationships. Misoprostol, a general EP₂ and EP₄ agonist, was more potent than PGE₂, in that the maximum rate of glycogenolytic activation was slightly greater for PGE₂ (P < 0.05), albeit without significant differences in EC₅₀. The effectiveness of the specific EP₂ agonist butaprost was indistinguishable from that for PGE₂ (Fig. 2B).

The response to PGE₂ varied from 1·2-fold to 19-fold activation of glycogenolysis, with an average of 6·4 ± 0·67-fold (mean ± s.e.m.; n = 37), which is similar to that noted for bovine glucagon (6·67 ± 1·05-fold; n = 28). PGE₂ also activates hepatocyte glycogenolysis in quillback rockfish (Sebastes pinniger), but not in two species of freshwater catfish (Ameiurus nebulosus and A. melas) (T P Mommsen and T W Moon, unpublished observations). Freshly isolated copper rockfish hepatocytes (n = 85) were in a transient negative glycogen balance, with an average rate of glucose production of 8·39 ± 0·67 μmol/g packed cells per h at room temperature.

When cells were supplied with lactate and pyruvate as substrates, PGE₂ also enhanced the rate of gluconeogenesis above the control rate (Fig. 2A). At 1·74 ± 0·14-fold (n = 10), the overall degree of activation was considerably smaller than that for glycogenolysis (P < 0.05; paired t-test), whereas the EC₅₀ (60 nM) of the concentration–response curve was slightly left-shifted compared with glycogenolysis (120 nM; Fig. 2). We did not analyze the effects of other prostaglandins on gluconegenic flux.

### Table 1 Glycogenolytic activation in rockfish hepatocytes, exposed to different prostaglandins at 10 μM. Results are expressed as a percentage ± s.e.m. of the concurrent response to 10 μM PGE₂ (number of independent determinations). Rates were measured over 30 min, against the appropriate solvent controls. For rates with 19-R-hydroxy PGE₂, see Fig. 8.

<table>
<thead>
<tr>
<th>Prostaglandin</th>
<th>Activation</th>
<th>n</th>
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<tbody>
<tr>
<td>PGF₂₁α</td>
<td>16·6 ± 6·5</td>
<td>(3)</td>
</tr>
<tr>
<td>Misoprostol</td>
<td>125·9 ± 44</td>
<td>(6)</td>
</tr>
<tr>
<td>Butaprost (methylster)</td>
<td>95·9 ± 25</td>
<td>(8)</td>
</tr>
<tr>
<td>16,16-Dimethyl-PGE₂</td>
<td>92·5 ± 47</td>
<td>(5)</td>
</tr>
<tr>
<td>17-Phenyl-trinor-PGE₂</td>
<td>44·1</td>
<td>(1)</td>
</tr>
<tr>
<td>PGE₁</td>
<td>72·7 ± 7·1</td>
<td>(4)</td>
</tr>
<tr>
<td>PGA₁</td>
<td>31·7 ± 11·7</td>
<td>(4)</td>
</tr>
<tr>
<td>PGA₂</td>
<td>6·6 ± 2·1</td>
<td>(3)</td>
</tr>
<tr>
<td>PGB₁</td>
<td>10·7</td>
<td>(1)</td>
</tr>
<tr>
<td>PGB₂</td>
<td>18·7 ± 2·8</td>
<td>(3)</td>
</tr>
<tr>
<td>PGD₂</td>
<td>11·3 ± 4·6</td>
<td>(3)</td>
</tr>
<tr>
<td>PGE₂₂</td>
<td>16·6 ± 6·5</td>
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In cases where n ≥ 3, rates are statistically different (P < 0·05).
PGE2 degradation

Rockfish liver cells rapidly degraded added PGE2 into a form that failed to cross-react with a monoclonal antibody against PGE2 (Fig. 3). The effective in vitro half-life is between 17 and 27 min, somewhat dependent on the added hormone concentration, and without doubt also a function of the number of cells used in the assays. We tested degradation only at a fixed cell concentration of 75 mg cell wet mass per ml. The maximum activation of glycogenolysis is generally smaller with other prostaglandins than with PGE2, even at relatively high concentrations, at which degradation should exert less of an influence on the effective hormone concentration. Therefore, we can exclude even more rapid PG turnover as a factor explaining decreased responses. Interestingly, PGE2 is degraded just as rapidly in hepatocytes isolated from a freshwater catfish (A. nebulosus), a species in which PGE2 exerts no glycogenolytic action (data not shown). This non-responsiveness is unique to PGE2, as catfish hepatocytes react sensitively to glucagon and other glycogenolytic hormones (Mommsen & Moon 1990).

Perfusion compared with static incubation

Because rockfish hepatocytes degraded added PGE2 quickly (Fig. 3), we wondered whether the relationships presented in Fig. 2 were skewed as a result of constant removal of the hormone. We therefore assessed the concentration–response relationship for PGE2 with hepatocytes immobilized in perifusion columns, in which cells are exposed to a continually renewing source of hormone. After an initial control period, cells were perifused with hormone, followed by an extended wash-out phase (Fig. 4). To minimize temporal effects, columns packed with cells from the same cell preparation were run concurrently. The ‘effectiveness’ of PGE2 varied, depending on whether...
the data were analyzed for PGE$_2$-dependent glucose peak height or total glucose produced area under the curve (Fig. 5). The resulting curves were significantly different from each other and also different from the curve for PGE$_2$ determined for the same cells in parallel under static conditions. EC$_{50}$ was lowest for peak height at 2 nM and considerably greater for static incubations (Figs 2 and 5).

**Message transduction**

PGE$_2$ activates glycogenolysis largely via a cAMP-mediated mechanism, with adenylyl cyclase and glycogen phosphorylase as readily identified intracellular targets. Intracellular concentrations of cAMP increased rapidly, reaching a maximum within 2 min of hormone application, followed by a decline (Fig. 6). As expected, the concurrent presence of the phosphodiesterase inhibitor, isobutyl-3-methylxanthine (IBMX), led to larger accumulation of cAMP than with hormone alone. The presence of IBMX largely prevented the time-dependent decay in the intracellular message, without affecting the rate of cAMP production. As in the case of the metabolic output, cAMP accumulation was dependent on the concentration of PGE$_2$, with an EC$_{50}$ in the range 85–150 nM. IBMX also changed the metabolic output of the cells. In its presence, the baseline rate of glycogenolysis was increased (data not shown), as was the specific response to PGE$_2$ (Fig. 1), confirming the results presented in Fig. 6. Conversely, in the presence of Rp-cAMPS, a competitive inhibitor of protein kinase A, significant decreases in the glycogenolytic response to PGE$_2$ in hepatocytes were noted (Fig. 1), substantiating the contention that PGE$_2$-dependent hepatocyte responses are predominantly cAMP-linked.

**Adenylyl cyclase and glycogen phosphorylase**

PGE$_2$ enhanced the activity of adenylyl cyclase in membranes prepared from fresh rockfish liver or isolated hepatocytes, in a concentration-dependent manner. A significantly greater degree of activation was reached in membranes isolated from fresh liver tissue (Fig. 7). However, no difference was noted in the sensitivity of the two membrane preparations to PGE$_2$, as the EC$_{50}$ values for PGE$_2$ were not significantly different. The stimulation of adenylyl cyclase and increased production of cAMP led to the activation of glycogen phosphorylase; PGE$_2$ brought about a conformational shift of this enzyme into its active a-form in a concentration-dependent manner (Fig. 8).

11-deoxy-PGE$_1$ and 19-R-hydroxy-PGE$_2$ also stimulated activation of glycogen phosphorylase, although to a smaller extent than PGE$_2$. 
Receptor type

The physiological response to PGE2 in rockfish liver is mediated by a prostaglandin receptor functionally resembling the mammalian EP2 receptor subtype. As the stimulation of glycogenolysis by the EP2-receptor-specific agonist, butaprost, was indistinguishable from that of PGE2 (Fig. 2B), we conclude that the majority of receptors in rockfish liver are pharmacologically comparable to mammalian EP2-type receptors.

Discussion

The rapid and powerful stimulation of glycogenolysis and gluconeogenesis in rockfish liver by PGE2 suggests a direct metabolic, and perhaps even endocrine, role for PGE2. Although glycogenolysis and control of other metabolic pathways fall into the sphere of PGE2 influence in mammals, such a direct and immediate role of PGE2 appears to be a novel observation for a non-mammalian vertebrate. PGE2 may regulate glucose output by the fish liver, similar to hormones such as glucagon, glucagon-like peptide (GLP), epinephrine or vasoactive peptides. Just like PGE2, these hormones also target the cAMP-dependent cascade, involving protein kinase A and glycogen phosphorylase. However, distinct and subtle differences exist. Glycogenolysis is activated in rat liver cells, but not as reproducibly or quickly as in rockfish liver. Also, in the rat, EP1 (IP3 and Ca2+) and EP3 (Gi) receptor subtypes alter the rate of glycogenolysis, whereas we consistently identified Gs-mediated activation of adenylyl cyclase as responsible in rockfish liver. In contrast to GLP-1 in fish liver (Mommsen & Mojsov 1998), PGE2-dependent glycogenolysis does not appear to undergo homologous desensitization, as it does in mammalian liver (Ichikawa et al. 1996). Finally, we can predict that PGE2 and other glycogenolytic and gluconeogenic hormones exert additive effects on these different pathways.

PGE2 is the most effective glycogenolytic natural prostaglandin, followed by PGE1, then PGB2 and PGF2α. Structural analogs and agonists, such as 16,16-dimethyl-PGE2, 19-R-hydroxy PGE2 and butaprost are as potent as PGE2. Misoprostol is slightly more potent than PGE2. Control rate

The degree of activation of glycogenolysis strongly depends on the control rate of glucose production. The control rate, in turn, is artificially increased during the preparation of hepatocytes and subsides slowly after cells have been put into primary culture (Mommsen et al. 1994). This initial activation may be a stress response of the liver cells, not mediated by epinephrine. It artificially decreases the degree of activation by PGE2 and suggests
that our data present a nadir of potential activation by the hormone. Similarly, the increased control rate may also account for the relatively smaller activation of adenylyl cyclase in membranes isolated from hepatocytes than from liver pieces (Fig. 6), although EC50 values were not significantly different for the two different membrane preparations.

As IBMX and Rp-cAMPS exert opposing effects on the control rate of glucose production (Fig. 1), the appreciable control rate appears in its turn to be under the control of cAMP/protein kinase A, and its magnitude is probably an artifact of the cell isolation procedure. Over time, basal rates of glucose production decreased, in parallel with the degree of phosphorylation of glycogen phosphorylase. This also implies that the rates of cAMP turnover in fish liver cells must be quite high, and that longer-term effects of cAMP are likely to be pronounced. If maintained in suspension for longer periods or if put into primary culture, fish hepatocytes generally cease to be in negative glycolysis balance, rates of endogenous glycolysis reach very low levels, and de novo synthesis of glycogen occurs (M Vijayan & A Takemura, unpublished observations).

Degradation

Hepatocytes in perifusion columns and thus exposed to renewing concentrations of PGE2 are about six times more sensitive to the hormone than cells kept in static suspension. Active PGE2 degradation by hepatocytes in suspension may account for some of the difference, an effect compounded by the relatively long incubation time required for the accurate measurement of glucose production in suspensions. As shown above, glucose production rates reached a peak within the first 10-min interval after hormone administration, whereas the cAMP peak was already reached within 2 min, followed by a decline. The perifusions more closely resemble the in vivo situation, and PGE2 degradation will be relevant to the down-stream hepatocytes alone.

Signal transduction

The signal transduction pathway for the glycogenolytic effect involves adenylyl cyclase, cAMP and protein kinase A, leading to increased phosphorylation and activation of glycogen phosphorylase. To date, hormones including epinephrine, glucagon and vasoactive peptides have been shown to accelerate the phosphorylation of fish glycogen phosphorylase into the active α-form, and we now can add PGE2 to this list. Peak activation rates are similar for most agonists used, probably reflecting the maximum activation ability for glycogen phosphorylase. However, large differences exist in the half-maximal concentration of agonists required (Fig. 2).

Our results with the phosphodiesterase inhibitor, IBMX, showing an immediate increase in response in peak height and duration (Figs 1 and 5) and with Rp-cAMPS, an inhibitory cAMP analog, resulting in a marked decrease in glycogenolytic response, imply an almost exclusive involvement of protein kinase A. Because of the central role of cAMP in the liver, we expect that any pathway regulated by protein kinase A will be affected. For fish liver, this includes negative effects on lipid biosynthesis, glycolysis and glycogen synthesis, and positive effects on lipolysis, glycogenolysis and gluconeogenesis (Moon 1998).

Mechanisms of action – gluconeogenesis

As shown for glucagon and GLP-1, flux through the gluconeogenic pathway in fish liver cells is primarily controlled at the level of pyruvate kinase, and only in the longer term through induction of phosphoenolpyruvate carboxykinase (PEPCK) (Foster & Moon 1990). Because of the involvement of cAMP in PGE2 message transduction, long-term effects in the rockfish probably differ from those in mammals, in which PGE2 counteracts glucagon-dependent increases in PEPCK mRNA (Pischel & Christ 1994). For the fish, we expect positive effects of PGE2 on PEPCK transcription. We have recently cloned and sequenced several fish PEPCKs (H L Booth, E R Busby & T P Mønnessen, unpublished observations) and now have the tools to examine hormone-dependent induction of hepatic PEPCK. Also, cAMP responsive element binding proteins (CREBs) have already been described for fish genes (Tsai et al. 2000), but knowledge is still fairly limited, yet interactions between PGs and CREBs in mammalian liver are common (Rudnick et al. 2001). Short-term activation of gluconeogenesis as noted in the rockfish could be based on increased transport of lactate/pyruvate and decreased futile cycling through protein kinase-dependent inactivation of pyruvate kinase (Mønnessen & Moon 1990). The EC50 values for glyco-genolysis and gluconeogenesis differ significantly in rockfish hepatocytes, possibly indicating post-receptor dichotomy in message transduction routes for the two pathways. The perceived discrepancy in the efficacy and potency of PGE2-dependent activation of glycogenolysis compared with that of gluconeogenesis is not unique to PGE2, as it is also found for glucagon or GLP-1 (Mønnessen & Moon 1990).

Receptors

Under normal conditions, rat parenchymal cells do not express EP1- or EP2-type receptors, and hence receptors leading to PGE2-dependent increases in cAMP are restricted to the Kupffer cells (Boie et al. 1997). However, these receptor mRNAs are inducible in the parenchymal cells (Fennekohl et al. 2000), possibly explaining the inconsistent observations on EP2 receptor transcripts in the rat liver (Katsuyama et al. 1995). Two features set the
rockfish liver apart from the situation in mammals. First, fish liver is almost devoid of Kupffer-like cells, with parenchymal cells accounting for at least 85% of the liver mass (Hampton et al. 1989) – compared with 60% in rodents – and in excess of 95% in our isolated hepatocyte preparations, excluding non-parenchymal cells as candidate targets. Secondly, rockfish appear to be unique in the ability to express EP2-like receptors constitutively, as all liver cell preparations responding to glycogenolytic hormones such as glucagon or GLP-1 also respond to PGE2. Therefore, the apparent-nonresponsiveness of hepatocytes from other teleosts to PGE2 may be related to a temporal absence of EP2-like receptors, and specific receptors may be inducible.

Three observations support our idea of the presence of EP2-like PGE2 receptors in the rockfish liver: the effects of PGE2 are accompanied by large increases in cAMP; the cells respond equally well to butaprost, a selective EP2-agonist, as to PGE2; the response to PGE2 does not undergo the rapid desensitization that is the hallmark of mammalian EP4-subtype receptors. Finally, comparative analysis of PGE2 responses with EP3- and EP4-selective PGE2 agonists in rockfish enterocytes implies the presence of EP1- and EP2-like receptors in this tissue. The fact that butaprost showed only a fraction of the effectiveness of PGE2 in rockfish enterocytes (data not shown) validates our use of alleged receptor-specific agonists and our conclusions concerning EP2-like receptors in the liver.

Our conclusions on receptor type are based on pharmacological similarities with those of mammals. We have initial Western blots showing cross-reactivity of two or three rockfish liver proteins with antibodies raised against mammalian EP2 receptors. However, we have not yet been successful in isolating these proteins or in developing specific primers for RT-PCR. Either way, characterization of these fish receptors might provide an interesting window on molecular and functional evolution of EP-type receptors in vertebrates that cover a much longer evolutionary history than mammals.

Physiological implications

The rockfish liver will be exposed to prostaglandins, including PGE2, yet we are uncertain about actual PGE2 concentrations. Potentially, PGE2 can affect the rockfish liver by different routes: endogenous production by hepatocytes, contributions of invading macrophages, or release from other tissues and from circulating red cells. Therefore, we suggest an important endocrine role for PGE2 in rockfish, in addition to its known paracrine actions. As Gs-dependent EP2-like receptors are involved, PGE2 will affect other protein kinase A targets, including transient enzyme phosphorylation and longer-term nuclear actions. Also, we can predict substantial cross-talk with other hormones activating this transduction system. The significantly different EC50 values for glycogenolysis and gluconeogenesis imply that post-receptor events do not follow a simple pattern. Finally, regulation of carbohydrate metabolism represents just one of the multifaceted actions of PGE2 in fish liver.

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