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Glycogenolytic and antiglycogenolytic prostaglandin E_2 actions in rat hepatocytes are mediated via different signalling pathways

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Prostaglandin E_2 has been reported both to stimulate glycogen-phosphorylase activity (glycogenolytic effect) and to inhibit the glucagon-stimulated glycogen-phosphorylase activity (antiglycogenolytic effect) in rat hepatocytes. It was the purpose of this study to resolve this apparent contradiction and to characterize the signalling pathways and receptor subtypes involved in the opposing prostaglandin E_2 actions.

Prostaglandin E_2 (10 μM) increased glucose output, glycogen-phosphorylase activity and inositol trisphosphate formation in hepatocyte cell culture and/or suspension. In the same systems, prostaglandin E_2 decreased the glucagon-stimulated (1 nM) glycogen-phosphorylase activity and cAMP formation.

The signalling pathway leading to the glycogenolytic effect of PGE_2 was interrupted by incubation of the hepatocytes with 4β-phorbol 12-myristate 13-acetate (100 nM) for 10 min, while the antiglycogenolytic effect of prostaglandin E_2 was not attenuated.

The signalling pathway leading to the antiglycogenolytic effect of prostaglandin E_2 was interrupted by an incubation of cultured hepatocytes with pertussis toxin (100 ng/ml) for 18 h, whereas the glycogenolytic effect of prostaglandin E_2 was enhanced.

The EP_1/EP_3 prostaglandin-E_2-receptor-specific prostaglandin E_2 analogue Sulproston had a stronger glycogenolytic potency than the EP_3 prostaglandin-E_2-receptor-specific prostaglandin E_2 analogue Misoprostol. The antiglycogenolytic potency of both agonists was equal.

It is concluded that the glycogenolytic and the antiglycogenolytic effects of prostaglandin E_2 are mediated via different signalling pathways in hepatocytes possibly involving EP_1 and EP_3 prostaglandin E_2 receptors, respectively.

Prostaglandins have been implicated to participate in cell to cell signal propagation between non-parenchymal and parenchymal cells in the liver. They are synthesized only in non-parenchymal liver cells [1], primarily Kupffer cells, but also in endothelial cells and Ito cells. They are degraded mainly by the parenchymal cells, i.e. hepatocytes [2–6], which also have been shown to possess binding sites for prostaglandins [2, 7–10]. The role of prostaglandins in the regulation of liver metabolism has been discussed controversially in previous studies. The use of cyclooxygenase inhibitors have revealed the involvement of prostaglandins in signalling pathways leading to an increase in glycogenolysis and glucose output elicited by such diverse stimuli as zymosan [11], endotoxins [12], heat-aggregated immunoglobulins [13], peptides of the activated complement system [14–16], platelet-activating factor [17], extracellular nucleotides and nucleosides [18, 19], phorbol esters [20, 21] and stimulation of sympathetic hepatic nerves [22]. Many of these effectors have also been shown to stimulate eicosanoid formation in Kupffer cells. Prostaglandins D_2, E_2 (PGE_2) and $F_{2\alpha}$ enhanced glucose output in perfused liver [23–26] and elevated the glycogen-phosphorylase activity in isolated rat hepatocytes via an increase in inositol 1,4,5-trisphosphate (InsP_3) [27, 28]. In addition, prostaglandins E_2 and $F_{2\alpha}$ have been shown to decrease glucagon-stimulated glycogen-phosphorylase activity and cAMP formation in rat hepatocytes [29–38]. These studies failed to show a stimulatory effect of prostaglandins on basal glycogen-phosphorylase activity and glucose output. The contradicting results have been ascribed to differences in the experimental protocol. It was the purpose of this study to resolve this apparent contradiction and to study the signalling pathways as well as the receptor subtypes involved in the glycogenolytic and antiglycogenolytic PGE_2 actions.

PGE_2 stimulated glycogen-phosphorylase activity both in cell suspension and cell culture (glycogenolytic effect). In the same experimental systems, PGE_2 decreased the glucagon-stimulated glycogen-phosphorylase activity (antiglycogenolytic effect). The glycogenolytic effect was mediated via an

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Abbreviations. PGE_2, prostaglandin E_2; G protein, trimeric GTP-binding protein; InsP_3, inositol 1,4,5-trisphosphate; PMA, 4β-phorbol 12-myristate 13-acetate; EP_1, EP_2, EP_3 receptors, prostaglandin E_2 receptor types 1–3; G_i, inhibitory G protein; G_s, stimulatory G protein.

Enzymes. Glycogen phosphorylase (EC 2.4.1.1); alanine amino transferase (EC 2.6.1.2); glucose dehydrogenase (EC 1.1.1.47); lactate dehydrogenase (EC 1.1.1.27); mutarotase (EC 5.1.3.3).

Note. Dedicated to Professor Dr. Gustav Paumgartner on the occasion of his 60th birthday.

InsP_3-coupled PMA-inhibitable signal chain possibly involving EP_1 prostaglandin-E_2 receptors, whereas the antiglycogenolytic effect was mediated via a G_i-(inhibitory G protein)-linked, pertussis-toxin-sensitive signal chain, possibly involving type 3 prostaglandin-E_2 receptors (EP_3).

MATERIALS AND METHODS

Materials

All chemicals were reagent grade and were from commercial sources. The Merck Glucose System was purchased from Merck. Lactate dehydrogenase, alanine amino transferase, M199 and fetal calf serum were from Boehringer. PGE_2 was from Paesel; the InsP_3 and cAMP assay kit was from Amersham Buchler. Percoll was purchased from Pharmacia. Misoprostol and SC 19220 were gifts of Searle; Sulproston and AH 6809 were gifts of Schering and Glaxo, respectively.

Methods

Male Wistar rats (160–200 g, Winkelmann) were kept on a 12-h day/night rhythm, light from 7 a.m. to 7 p.m., with free access to water and food (standard rat diet, Ssniff). All experiments were begun between 9 a.m. and 10 a.m. Rats were anaesthetized by intraperitoneal injection of pentobarbital (60 mg/kg body mass).

For cell-suspension experiments, hepatocytes were isolated according to Meredith without the use of collagenase [39] to avoid degradation of surface receptors by the proteases during cell preparation. The method was slightly modified. The liver was perfused without recirculation via the portal vein with a Ca^{2+}-free Krebs-Henseleit buffer containing 15 mM glucose, 2 mM lactic acid, 0.2 mM sodium pyruvate and 2 mM EDTA for 30–40 min. The bulk of detritus and non-parenchymal cells was removed by three subsequent washing steps, sedimenting the viable hepatocytes at 50 *g*. Viable hepatocytes were further purified by centrifugation through a gradient with 58% Percoll.

Purified hepatocytes (30 mg/ml, 3×10^6 cells/ml) were suspended in Hepes-buffered saline (20 mM Hepes, 120 mM NaCl, 4,8 mM KCl, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, 2.5 mM $CaCl_2$, pH 7.4). The incubation mixture contained a final concentration of 5 mM glucose, 2 mM lactate and 0.2 mM pyruvate (buffer A). Cells were incubated for 10 min; then PGE_2 (final concentration 10 μM), glucagon (1 nM) or both were added. The incubation was stopped by the addition of trichloroacetic acid after 30 s for the measurement of InsP_3 and after 30 min for the determination of glucose or by freezing in liquid nitrogen after 2 min for the assay of glycogen phosphorylase. For the zero time values, the reactions were terminated immediately before addition of the agonist.

For experiments in cell culture, hepatocytes were isolated either according to Berry and Friend [40] or as described above for cell suspensions according to Meredith [39]. Both methods yielded the same results. Hepatocytes were plated on 9-cm^2 tissue plates (9 mg/plate, 9×10^5 cells/plate) in M199 medium containing 0.5 nM insulin, 100 nM dexamethasone, penicillin/streptomycin (10 mg/ml) and for the first 4 h of culture 4% fetal calf serum. After 24 h in primary culture, the medium was removed and cells were washed three times with buffer A. Cells were incubated for 10 min at 37 °C in 1 ml buffer A; then PGE_2 (final concentration 10 μM), glucagon (1 nM) or both were added. The buffer was removed after 2 min and the plates were frozen in liquid nitrogen.

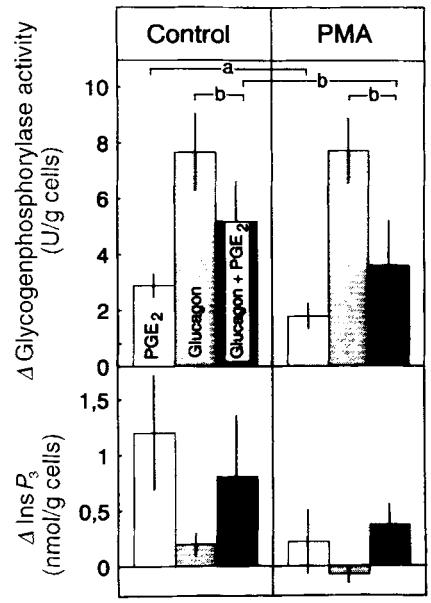

Fig. 1. Inhibition of the glycogenolytic but not the antiglycogenolytic PGE_2 effect by PMA. Hepatocytes (30 mg/ml) were suspended in Hepes-buffered saline, containing 5 mM glucose, 2 mM lactate and 0.2 mM pyruvate. PGE_2 (10 μM), glucagon (1 nM) or both were added after a 10-min initial incubation with or without PMA (100 nM). Samples were stopped in 45% trichloroacetic acid for the determination of InsP_3 at 30 s or frozen in liquid nitrogen at 2 min for the determination of glycogen-phosphorylase activity. The basal levels for glycogen phosphorylase was 4.7 ± 0.4 U/g and InsP_3 was 0.9 ± 0.2 nmol/g. The values are means ± SEM of four experiments. The student's *t*-test for paired values was performed. a, $P<0.01$; b, $P<0.05$.

Glucose was assayed in the hepatocyte supernatants using a commercial test kit (Merck Glucose System) based on the glucose-dehydrogenase method [41]. Lactate was measured using a combined optical test with alanine amino transferase and lactate dehydrogenase in a glutamate buffer (230 mM glutamate, pH 9.5) [42]. Glycogen-phosphorylase activity was determined by a standard assay [43] and protein was determined by the method of Lowry [44]; InsP_3 and cAMP were determined using Amersham radioligand assays.

RESULTS

Glycogenolytic and antiglycogenolytic effect of PGE_2 in hepatocyte suspensions and hepatocyte cultures

Hepatocytes were suspended in Hepes-buffered saline. After a 10-min incubation at 37 °C, the glucose concentration was approximately 5 mM. At this time, buffer (controls), PGE_2, glucagon or PGE_2 plus glucagon were added. Cells released glucose at a basal rate of 0.78 ± 0.08 μmol · min^{-1} · g^{-1}, the basal glycogen-phosphorylase activity was 4.6 μmol · min^{-1} · g $cells^{-1}$, the basal InsP_3 level was 0.9 ± 0.2 nmol · g $cells^{-1}$. PGE_2 enhanced glucose output by 20%, glycogen-phosphorylase activity by 60% and InsP_3 levels by approximately 100% (Fig. 1 left, Table 1). Glucagon increased glucose output by 50% and glycogen-phosphorylase activity by 160%. Glucagon stimulated hepatocytes to take up lactate at a rate of 0.31 μmol · min^{-1} · g^{-1}. It had no

Table 1. Increase in glucose output and lactate uptake by PGE_2, glucagon and PGE_2 plus glucagon. Rat hepatocytes (30 mg in a final volume of 1 ml), that were prepared as described in the Materials and Methods section, were incubated at 37 °C in Hepes-buffered saline, containing approximately 5 mM glucose at the beginning of the experiment. PGE_2, glucagon or both were added to a final concentration of 10 μM and 1 nM, respectively. Glucose and lactate were determined in the supernatant at 1 min and 30 min. The basal rate of glucose output was 0.78 μmol · min^{-1} · g^{-1}, the basal lactate balance was not significantly different from 0, the mean value was −0.087 μmol · min^{-1} · g^{-1}. Values are the means ± SEM. The student's *t*-test was performed for paired values. Positive values indicate release and negative values indicate uptake.

Additions	Increase in glucose output	Increase in lactate uptake
	μmol · min^{-1} · g wet $mass^{-1}$	
PGE_2	0.14 ± 0.02[a,d]	−0.04 ± 0.03[e,d]
Glucagon	0.37 ± 0.02[b]	−0.31 ± 0.04[b]
PGE_2 + glucagon	0.23 ± 0.02[a,c]	−0.14 ± 0.04[a,c]

[a] $P < 0.05$ versus control.
[b] $P < 0.01$ versus control.
[c] $P < 0.05$ versus glucagon-treated samples.
[d] $P < 0.01$ versus glucagon-treated samples.
[e] Not significant.

effect on InsP_3 formation. The glucagon-mediated increases in glucose output, lactate uptake and glycogen-phosphorylase activity were partially inhibited, if PGE_2 was added concomitantly with glucagon (Fig. 1 left, Table 1). InsP_3 formation was not significantly different from samples that were stimulated with PGE_2 alone.

Similar results were obtained in hepatocytes after 24 h of primary culture, yet changes in metabolite concentrations could not be determined because of the unfavourable ratio of cell mass to medium. PGE_2 as well as glucagon increased the glycogen-phosphorylase activity by 50% and 180%, respectively (Fig. 2). Glucagon increased the cAMP level by more than 1000% (from 0.2 to 2.4 nmol/g) but PGE_2 had no effect. Both the glucagon-mediated increase in glycogen-phosphorylase activity and cAMP formation were antagonized if PGE_2 was administered simultaneously with glucagon; the glucagon-stimulated glycogen-phosphorylase activity was lowered to the same activity as with PGE_2 alone, the cAMP level was reduced strongly (Fig. 2 left).

Interruption by 4β-phorbol 12-myristate 13-acetate of the glycogenolytic but not the antiglycogenolytic PGE_2 signal chain

Activation of protein kinase C with the phorbol ester 4β-phorbol 12-myristate 13-acetate (PMA) has been shown to interrupt the signal chains of hormones, that mediate their effects via an increase in InsP_3 and cytosolic free calcium, e.g. noradrenaline or vasopressin [45–49]. Incubation of hepatocytes in suspension for 10 min prior to the experiment with 100 nM PMA, which suffices to activate protein kinase C [45], resulted in a partial reduction of the PGE_2-mediated increase in glycogen-phosphorylase activity and an almost complete reduction in PGE_2-mediated InsP_3 formation (Fig. 1 right). The glucagon-mediated increase in glycogen-phosphorylase activity was not affected by a prior PMA treatment. The antiglycogenolytic PGE_2 effect tended to be more pronounced than in control experiments.

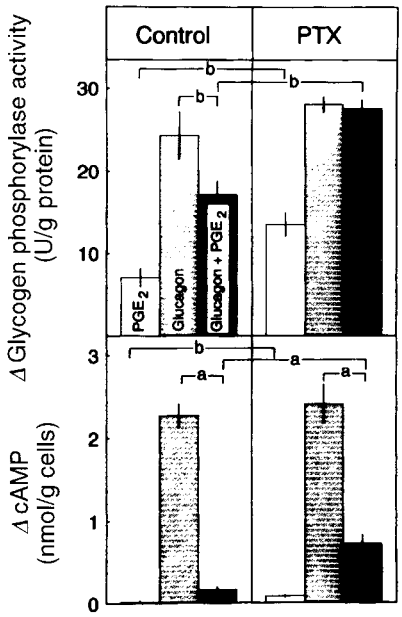

Fig. 2. Inhibition of the antiglycogenolytic but not the glycogenolytic PGE_2 effect by pertussis toxin. Hepatocytes were maintained in primary culture for 24 h in M199 containing 0.5 nM insulin, 100 nM dexamethason and 1% (mass/vol.) penicillin-streptomycin on 3-cm-diameter tissue-culture plates. Pertussis toxin (PTX, 100 ng/ml) was added where indicated for the last 18 h of culturing. For the experiments, cells were washed three times with Hepes-buffered saline, containing 5 mM glucose, 2 mM lactate and 0.2 mM pyruvate. 1 ml of this buffer was added to all plates. After 10 min, PGE_2 (10 μM), glucagon (1 nM) or both were added. The reaction was stopped after 2 min by removing the medium and freezing the plates in liquid nitrogen for the determination of the glycogen-phosphorylase activity or the cAMP concentration. Values are the means ± SEM of four experiments. The student's *t*-test was performed for paired values. a, $P < 0.01$; b, $P < 0.05$.

Interruption by pertussis toxin of the antiglycogenolytic but not the glycogenolytic PGE_2 signal chain

The signal chain leading to the antiglycogenolytic PGE_2 effects had previously been shown to be interrupted by prior treatment of hepatocytes with pertussis toxin [32, 34]. As in these studies, treatment of hepatocytes in primary culture with 100 ng/ml pertussis toxin for 18 h abolished the inhibitory effect of PGE_2 on the glucagon-stimulated glycogen-phosphorylase activity (Fig. 2). The glucagon-mediated increase in cAMP formation, that had been inhibited strongly by PGE_2, was partially restored in cells that had been incubated with pertussis toxin for 18 h prior to the experiment; cAMP levels increased by 350% (from 0.2–0.85 nmol/g) and thus reached approximately 35% of the level of glucagon-stimulated cells. In contrast to the antiglycogenolytic PGE_2 effect, the glycogenolytic PGE_2 effect was not inhibited but rather enhanced by a pertussis toxin treatment; the PGE_2-mediated increase in glycogen-phosphorylase activity was twice as large as in control cells. In cells treated with pertussis toxin, PGE_2 caused a small but significant increase in cAMP formation by 50% (from 0.2–0.3 nmol/g).

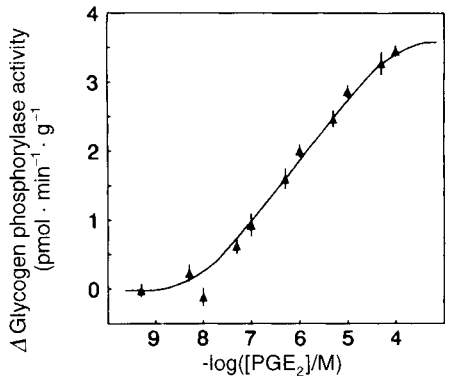

Fig. 3. Dose/response curve of the glycogenolytic PGE_2 effect. Hepatocytes (30 mg/ml) were suspended in Hepes-buffered saline, containing 5 mM glucose, 2 mM lactate and 0.2 mM pyruvate. PGE_2 was added after a 10-min initial incubation to the concentrations indicated. Samples were frozen in liquid nitrogen for the determination of glycogen-phosphorylase activity at 2 min. Values are the means ± SEM of six experiments.

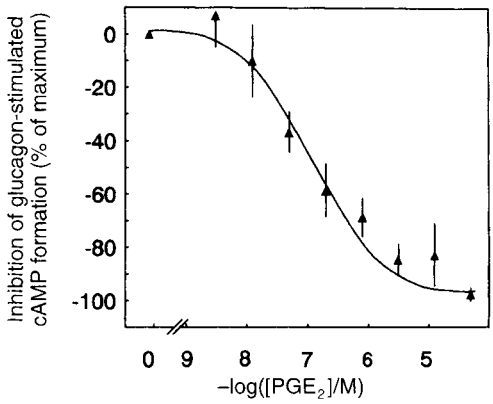

Fig. 4. Dose/response curve of the antiglycogenolytic PGE_2 effect. Hepatocytes were maintained in primary culture for 24 h in M199 containing 0.5 nM insulin, 100 nM dexamethasone and 1% (mass/vol.) penicillin-streptomycin on 3-cm-diameter tissue-culture plates. For the experiments, cells were washed three times with Hepes-buffered saline, containing 5 mM glucose, 2 mM lactate and 0.2 mM pyruvate. 1 ml of this buffer was added to all plates. After 10 min, PGE_2 at the concentration indicated and glucagon (1 nM) were added simultaneously. The reaction was stopped after 2 min by removing the medium and freezing the plates in liquid nitrogen for a later determination of the cAMP concentration in a radioimmunoassay. Values are the means ± SEM of four experiments.

Concentration dependence of the glycogenolytic and antiglycogenolytic PGE_2 response

PGE_2 stimulated glycogen-phosphorylase activity half maximally at concentrations of approximately 0.5 μM (Fig. 3). It also increased InsP_3 significantly at concentrations greater than 1 μM PGE_2 (data not shown); a dose/response curve for the formation of InsP_3 could, however, not be obtained since the results varied too much between the experiments (compare Fig. 1). PGE_2 inhibited the glucagon-dependent increase in glycogen-phosphorylase activity (data not shown) and in the increase cAMP (Fig. 4) at concentrations of 0.1−50 μM. Since the inhibitory PGE_2 effect was clearly greater with cAMP formation (compare Fig. 2), the latter was the better parameter to obtain a dose/response curve (Fig. 4). The glucagon-stimulated cAMP formation

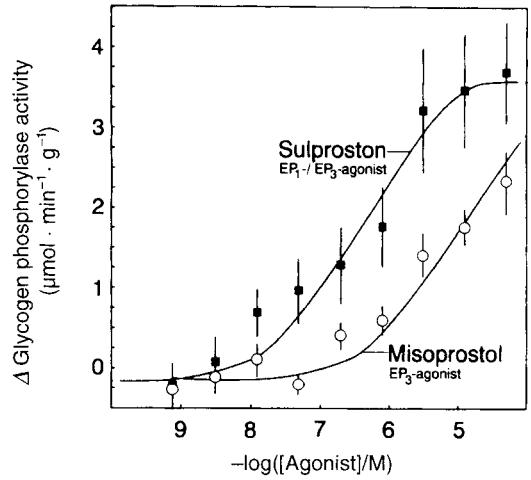

Fig. 5. Dose/response curve of the glycogenolytic effect of the EP_1/EP_3 agonist Sulproston and the EP_3 agonist Misoprostol. Hepatocytes (30 mg/ml) were suspended in Hepes-buffered saline, containing 5 mM glucose, 2 mM lactate and 0.2 mM pyruvate. Sulproston and Misoprostol were added after a 10-min initial incubation to the concentrations indicated. Samples were frozen in liquid nitrogen for the determination of glycogen-phosphorylase activity at 2 min. Values are the means ± SEM of three and six experiments, respectively.

was half-maximally inhibited at PGE_2 concentrations of approximately 0.2 μM. The dose/response curves do not allow to distinguish whether a single or different subtypes of the PGE_2 receptor were involved in the glycogenolytic and the antiglycogenolytic PGE_2 action, because both effects were half maximal at similar concentrations and because the saturation level for the signal chains is not known.

Receptors involved in the glycogenolytic and the antiglycogenolytic PGE_2 actions

So far, three types of PGE_2 receptors, named EP_1, EP_2 and EP_3 receptors, have been described in non-hepatic organs [50, 51]. To assess whether a single PGE_2 receptor type was linked to two different signal chains or whether two different PGE_2 receptor types were responsible for the glycogenolytic and the antiglycogenolytic PGE_2 effects, experiments with the EP_1/EP_3 receptor-specific agonist Sulproston and the EP_3-specific Misoprostol were performed. Both agonists are approximately equipotent on EP_3 receptors [52]. In addition, Sulproston potently stimulates EP_1 receptors and EP_3 receptors in chick ileum [50, 51]. Misoprostol is equipotent with PGE_2 on guinea pig vas deferens EP_3 receptors and is three times less potent on EP_2 receptors of cat trachea [53].

Both Sulproston and Misoprostol stimulated glycogen-phosphorylase activity (Fig. 5), yet the EP_1 and EP_3 agonist Sulproston enhanced glycogen phosphorylase activiy at 10 μM concentrations approximately twice as much as the EP_3 agonist Misoprostol. The half-maximally effective concentration (EC_{50}) of Sulproston for the stimulation of glycogen phosphorylase was approximately tenfold lower than the EC_{50} of Misoprostol. At concentrations that half-maximally stimulated glycogen-phosphorylase activity, Sulproston (0.5 μM) and Misoprostol (3 μM) increased the InsP_3 formation to 152 ± 28% and 166 ± 17%, respectively. Neither Sulproston nor Misoprostol at concentrations up to 50 μM increased cAMP formation. Thus, the glycogenolytic effect was probably mediated via EP_1 receptors.

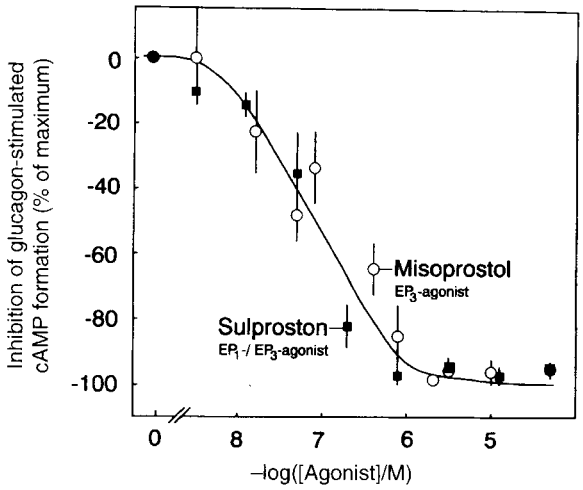

Fig. 6. Dose/response curve of the antiglycogenolytic effect of the EP_1/EP_3 agonist Sulproston and the EP_3 agonist Misoprostol. Hepatocytes were maintained in primary culture for 24 h in M199 containing 0.5 nM insulin, 100 nM dexamethasone and 1% (mass/vol.) penicillin-streptomycin on 3-cm-diameter tissue-culture plates. For the experiments, cells were washed three times with Hepes-buffered saline containing 5 mM glucose, 2 mM lactate and 0.2 mM pyruvate. 1 ml of this buffer was added to all plates. After 10 min, Sulproston or Misoprostol at the concentration indicated and glucagon (1 nM) were added simultaneously. The reaction was stopped after 2 min by removing the medium and freezing the plates in liquid nitrogen for a later determination of the cAMP concentration in a radioimmunoassay. Values are the means ±SEM of three experiments.

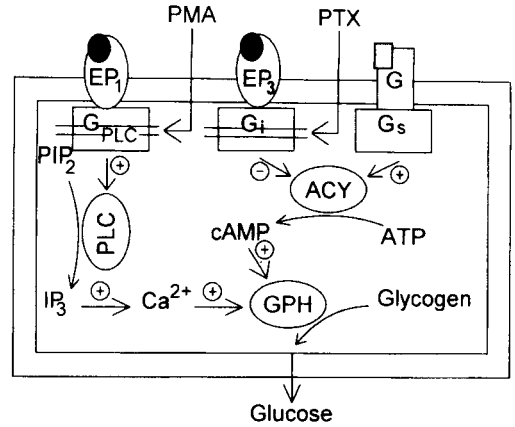

Fig. 7. Hypothetical signal chains for the glycogenolytic and antiglycogenolytic PGE_2 actions. Glycogenolytic signal chain: EP_1, PGE_2 receptor of the subtype 1; PMA, 4β-phorbol 12-myristate 13-acetate; G_{PLC}, phospholipase-C-linked G protein; PIP_2, phosphatidyl-inositol 4,5-bisphosphate; PLC, phospholipase C; IP_3, inositol 1,4,5-trisphosphate. Antiglycogenolytic signal chain: EP_3, PGE_2 receptor of the subtype 3; G, glucagon receptor; PTX, pertussis toxin; G_i, inhibitory G protein; G_s, stimulatory G protein; ACY, adenylate cylase; GPH, glycogen phosphorylase.

Sulproston and Misoprostol also inhibited the glucagon-stimulated cAMP formation with a similar EC_{50} value of approximately 0.1 μM (Fig. 6). Since both substances can act as EP_3 agonists, the antiglycogenolytic effect was most likely mediated via EP_3 receptors.

To further corroborate these data, the EP_1-receptor-selective antagonists AH 6809 and SC 19220 were used. However, AH 6809 had a strong intrinsic activity; it stimulated glycogen-phosphorylase activity twofold over basal rates and had no antiglycogenolytic effect on the glucagon-stimulated glycogen-phosphorylase activity (data not shown). SC 19220 neither inhibited the glycogenolytic nor the antiglycogenolytic PGE_2 effect if added at concentrations up to approximately 10 μM as a stock solution in ethanol (data not shown). Since this drug is extremely insoluble in water and the addition of higher concentrations of organic solvents is prohibitive in cell-suspension experiments, the effective concentration of SC 19220 might have been much lower. Thus, the lack of an inhibition by this inhibitor does not argue against the involvement of EP_1 receptors.

DISCUSSION

Glycogenolytic and antiglycogenolytic PGE_2 actions in the same experimental system

The glycogenolytic and antiglycogenolytic actions of PGE_2 have previously been observed in different experimental systems. The glycogenolytic effect has either been shown in rat liver perfused with glucose-free [23, 25, 26] or glucose-containing medium [24] or in hepatocyte suspensions with glucose-free media [27, 28]. In these investigations, an increase either in glucose output or glycogen-phosphorylase *a* activity was determined. The antiglycogenolytic effect has been demonstrated in cell culture or suspension with media containing 5 mM glucose and no substrates for gluconeogenesis [29–38]. In these studies, either the glycogenolytic rate was determined as the release into the medium of glucose or [^{14}C]glucose from labelled glycogen, or the decrease in the glucagon-induced cAMP formation was measured. Differences in the experimental set up, i.e. perfusion and cell suspension with glucose-free media versus culture with glucose-containing media have previously been used [37] to explain the lack of glycogenolytic PGE_2 effects in the latter studies with cultures [29–38]. A slight PGE_2-mediated increase in cAMP has previously been described in hepatocytes in the presence of the cAMP-phosphodiesterase inhibitor isobutyl methyl xanthine together with an inhibition of the glucagon-mediated cAMP formation [32]. A potentiation of the glucagon-mediated glucose output at low (10^{-9} M) concentrations of PGE_2 and an attenuation of the glucagon-mediated glucose output at high (10^{-6} M) concentrations of PGE_2 in cell suspension in a glucose-free and lactate free medium has also been reported [28].

In the present investigation, both the glycogenolytic and antiglycogenolytic actions of PGE_2 have been observed in hepatocyte suspensions and cultures with media containing 5 mM glucose, 2 mM lactate and 0.2 mM pyruvate by measuring glycogen-phosphorylase activity. Moreover, in suspensions an increase by PGE_2 in basal glucose release and a decrease by PGE_2 in the glucagon-stimulated glucose release into the medium could be demonstrated. In addition to the attenuation of the glucagon-stimulated glycogenolysis, PGE_2 seemed to decrease the glucose output from the hepatocytes by diminishing the glucagon-stimulated lactate uptake, which can be used as a rough estimate for gluconeogenesis. Both the glycogenolytic and antiglycogenolytic PGE_2 effects were half maximal at concentrations of 0.5 μM exceeding by at least one order of magnitude the systemic or portal concentrations of 0.5–1 nM. However, local prostaglandin concentrations of 5–10 μM may be reached in the proximity of hepatocytes due to the local production and secretion into the narrow space of Disse by non-parenchymal liver cells [10].

Involvement of different signal chains

The glycogenolytic PGE_2 effect was mediated via an InsP_3-dependent signal chain. It could be interrupted by a short-term activation of protein kinase C with the phorbol ester PMA (Fig. 7, compare Fig. 1). This interruption by PMA has been demonstrated previously for other InsP_3-dependent signal chains such as noradrenaline, vasopressin, phenylephrine [45–49] and prostaglandins [54–56]. Activation of protein kinase C neither interfered with the cAMP-dependent signal chain of glucagon nor with the antiglycogenolytic PGE_2 signal chain.

The antiglycogenolytic PGE_2 effect was mediated via a decrease in cAMP. It could be inhibited by an incubation of hepatocytes with pertussis toxin (Fig. 7, compare Fig. 2). This is in agreement with previously published data [32, 34]. By contrast, the glycogenolytic PGE_2 effect was rather enhanced. Thus, the antiglycogenolytic effect but not the glycogenolytic effect was mediated via a pertussis-toxin-inhibitable G_i protein.

Treatment with pertussis toxin abolished the inhibition by PGE_2 of the glucagon-stimulated glycogen-phosphorylase activity entirely, whereas it reduced the inhibition by PGE_2 of the glucagon-stimulated cAMP formation only partially. Hence, a submaximal increase (35%) in cAMP formation already yielded a maximal activation of glycogen phosphorylase. This lack of a strict correlation of cAMP formation and activation of glycogen phosphorylase, where cAMP apparently is produced in excess, is a well-known phenomenon [57, 58] which is also observed with the second messenger InsP_3 [59].

The enhancement of the glycogenolytic PGE_2 effect by pertussis toxin might be explained in two ways. (a) Pertussis toxin might have diminished an inhibition by PGE_2 of the glycogenolytic of the phospholipase-C-linked PGE_2 signal chain. This inhibition might be mediated via a pertussis toxin sensitive phospholipase-C-like G_i protein as has been described for endothelin in brain [60] and heart [61]. This hypothesis cannot be tested at the level of the second-messenger InsP_3, since the increases in InsP_3 formation seems not to correlate strictly with the activation of glycogen phosphorylase [27, 59]. (b) Alternatively, pertussis toxin might abrogate a PGE_2-enhanced block via a G_i protein of a stimulatory G-protein (G_s)-linked glycogenolytic PGE_2 signal chain. The latter view is supported by the fact that PGE_2 elicited a small but significant increase in cAMP formation only in presence of pertussis toxin (Fig. 2) indicating that the partial inactivation of a G_i protein revealed the presence a G_s-linked PGE_2 receptor signal chain normally obscured by the predominance of G_i action. This is in agreement with a previous finding, that a small PGE_2-mediated increase in cAMP formation was potentiated by initial treatment of hepatocytes with pertussis toxin [32].

Involvement of different receptor types

PGE_2 receptors have been subdivided into three classes, EP_1, EP_2 and EP_3 receptors [50, 51], according to the relative potency of PGE_2 analogues in various non-hepatic tissues and the signal chain they use. The existence of a fourth EP-receptor subtype linked to G_s has been suggested [62] and two isoforms of the EP_3 receptor have been cloned [63]. Receptors designated EP_1 receptors operate via phospholipase C and InsP_3 [64], EP_2 receptors via a G_s-mediated increase in cAMP and EP_3 receptors via a G_i-mediated decrease in cAMP [65]. Since the glycogenolytic effect of PGE_2 could be mimicked best by Sulproston, that acts both as an EP_1 and EP_3 agonist, whereas the EP_3-selective Misoprostol was clearly less effective, it seems that the glycogenolytic effect of PGE_2 was mediated via EP_1 receptors (Fig. 7, compare Fig. 5). This is in agreement with the observed increase in InsP_3 (Fig. 1).

The antiglycogenolytic PGE_2 effect was mimicked equally well by Sulproston and Misoprostol, which have a similar affinity to EP_3 receptors [52]. The antiglycogenolytic PGE_2 effect thus seems to be mediated by EP_3 receptors (Fig. 7, compare Fig. 6). This is in agreement with the observed pertussis-toxin-sensitive decrease in cAMP formation (Fig. 2).

Conclusion

The data presented in this study favour the hypothesis that hepatocytes possess two PGE_2 receptor subtypes. These subtypes are an EP_1 receptor that stimulates glycogenolysis via a PMA-sensitive, pertussis-toxin-insensitive InsP_3-linked signal chain and an EP_3 receptor that inhibits the glucagon-stimulated glycogenolysis via a pertussis-toxin-sensitive G_i protein.

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