CYCLIC AMP CONTROL BY PROSTAGLANDIN E_1 IN NON-PARENCHYMAL LIVER CELLS

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1. Introduction

There is little doubt, despite some contradictory reports, that the liver is a main target of prostaglandin E_1 (PGE_1). The PGE_1-due increase of cyclic-AMP levels occurring when the drug is injected into rats [1] or when it is added to isolated rat liver hepatocytes [2] has been fully accounted for by a stimulatory effect on adenylate cyclase of particulate liver fractions [1] and isolated liver plasma membranes as well [3]. Using liver plasma membranes we have shown that a PGE_1-sensitive cyclase is clearly distinct from glucagon and epinephrine-sensitive cyclases [3]; in several tissues moreover PGE_1 action appears to be best explained by assuming an interaction with a specific receptor that does not share any property with polypeptide hormone or catecholamines receptors [4,5].

In order to distinguish whether PGE_1 has to be considered an intercellular or an intracellular messenger it was important in our opinion to establish whether PGE_1 receptor is on the outer or on the inner layer of the plasma membrane. Experiments carried out in isolated hepatocytes point to an external rather than to an internal localization of PGE_1 receptor, thus favouring a role of PGE_1 as intercellular messenger [4].

The results of experiments reported here, which indicate that PGE_1 dramatically increases cyclic-AMP levels in a discrete hepatocyte population not involving parenchymal cells, give a strong support to the role of PGE_1 as intercellular regulator of cyclic AMP. While this work was in progress Wincek et al. [6] separated Kupffer cells from parenchymal cells after collagenase digestion of the liver and observed that PGE_1-sensitive cyclase is anatomically separated from glucagon sensitive cyclase, the former being present largely in an endothelial rich cellular fraction (and absent from Kupffer cells) and the latter being present just in parenchymal cells.

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2. Materials and methods

2.1. *Determination of cyclic-AMP levels in isolated hepatocytes*

Hepatocytes were isolated according to the Seglen's modification [7] of the Berry and Friend [8] method, using fed male Wistar rats 200–250 g in weight, as previously described [9]. Hepatocytes suspended in Krebs–Ringer bicarbonate buffer (pH 7.4) containing 2.5 mM calcium and 2% bovine serum albumin, at the concentration of 10–20 mg protein/ml, were incubated in test tubes in a shaking bath at 30°C for 5 min. Reactions were blocked by centrifugation at low speed in the cold and by addition of 1 ml of 5% cold TCA to the sediments. On TCA-free supernatants cyclic AMP was assayed according to the method of Brown et al. [10] as previously described [9].

2.2. *Fractionation of isolated hepatocytes*

In a first series of experiments hepatocytes were subjected to the fractionation procedure described by Drochmans et al. [11] employing a continous Ficoll gradient containing EDTA and albumin. After 3 h of centrifugation at 62 500 × *g* most of the cells were found at the bottom of the tube and only a minor

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part was recovered close to the top of the tube. Owing to the low yield and variability of the results we tried the aqueous two-phase system obtained by mixing Dextran T 500 with polyethylene glycol, as described by Albertson [12]. We were unable to achieve a separation of liver cells since all of the material added partitioned at the interface.

Satisfactory results were finally obtained employing centrifugation on discontinuous Ficoll density gradients. Solutions of Ficoll dissolved in Krebs–Ringer containing 2 mM EDTA and 2% albumin were layered in S. W. 25.1 Spinco tubes. The gradient was formed immediately before use by layering 40%, 37%, 30% and 15% Ficoll solutions (5 ml each), 5 ml of hepatocyte suspension in Krebs–Ringer were layered at the top and tubes were centrifuged at 62 500 × *g* for 60 min. Four layers of cells were observed: fraction I between 15 and 30%, fraction II between 30 and 37%, fraction III between 37 and 40% and fraction IV at the bottom of the tube. The fractions were collected by a Pasteur pipette, diluted with Krebs–Ringer and washed free of Ficoll by centrifuging two times at low speed. Hepatocyte fractions were finally suspended in Krebs–Ringer (5–10 mg proteins/ml) and used for assays. A further liver fraction designed 'hepatocyte wash' was obtained by collecting supernatants of whole liver cells suspension centrifuged at 50 × *g* (see ref. [9]).

Prostaglandins were a gift of Dr J. Pike, the Upjohn Co. Kalamazoo, USA. Glucagon (insulin free) was from E. Lilly Co. Indianapolis, USA. Labeled cyclic AMP (27 Ci/mmol) was a product of Radiochemical Centre England. Ficoll was a product of Pharmacia Uppsala, Sweden.

3. Results

The observation that glucagon stimulates about 10-fold adenylate cyclase of liver plasma membranes and increases cyclic AMP levels from 8–15-fold in isolated hepatocytes while PGE_1 stimulates the enzyme 6–8-fold but increases cyclic AMP only 1.5–3-fold [3,4], lead us to investigate whether some change in the liver cellular population distribution occurred passing from the whole organ (from which plasma membrane is isolated) to the suspension of liver cells we generally employed. It turned out that after digestion of the liver with collagenase the mechanically dispersed cells are subjected – as prescribed by the Berry and Friend method [8] – to a low-speed centrifugation which sediments the heavier parenchymal cells leaving most of the non-parenchymal light cells in the supernatant. However a microscopical examination showed that the parenchymal cell-rich sediment contained from 4–6% of non-parenchymal cells, which were on the other hand abundant in the supernatant. Therefore it was important to investigate the effect of glucagon and PGE_1 on relatively homogeneous cellular populations. The use of discontinuous Ficoll density gradient enabled us to isolate parenchymal cells completely free from non-parenchymal elements. When the four gradient fractions were examined at microscope it was observed that the lightest fraction I which constituted 6.2 ± 2.5% of total cellular proteins included non-parenchymal cells only (table 1). In table 1 it is shown that optimal doses of glucagon produced a modest increase of cyclic AMP while the PGE_1-due increase was six fold. Fraction II (13.6 ± 6.5% of total cells) was constituted mainly by parenchymal cells containing less than 2% of non-parenchymal elements. When added to this fraction glucagon very effectively increased cyclic AMP while PGE_1 effect was not significantly different from control. Fraction III (10.0 ± 3.7% of total cells), a homogeneous population of parenchymal cells behaved similarly to fraction II. In the major part of cells sedimenting at the bottom of the tube, basal cyclic AMP levels were lower than those of the lighter fractions and glucagon was less effective probably because part of the cells were damaged.

In fig.1 a dose response study of the effect of glucagon and PGE on parenchymal and non parenchymal cells is reported. The K_a (half-maximal stimulating dose) for glucagon was 2×10^{-8} M a value which compares well with that obtained with plasma membrane adenylate cyclase [4], the K_a for PGE_1 was 2×10^{-7} M, about one order of magnitude lower than that found using plasma membranes [4]. This is not surprising if one considers that liver is constituted by more than 70% of parenchymal cells and it is likely therefore that about 30% of isolated membranes are of non-parenchymal nature. PGE_2 as expected was found to be less effective than PGE_1.

In fig.2 a time-course study depicting glucagon and

Table 1
Cyclic AMP control by glucagon and PGE_1 in hepatocyte fractions isolated by Ficoll density gradient

Gradient fraction	I	II	III	IV	Hepatocyte wash
Control	12.2 ± 2.0 (4)	9.3 ± 2.3 (3)	7.3 ± 1.3 (3)	5.1 ± 1.4 (3)	7.1 ± 1.8 (3)
Glucagon	24.2 ± 4.9 (4)[a]	65.9 ± 25.0 (3)[a]	75.1 ± 12.0 (3)[a]	27.6 ± 3.3 (3)[b]	14.2 ± 2.9 (3)[c]
PGE_1	69.0 ± 14.1 (4)[b]	15.5 ± 3.8 (3)[c]	9.1 ± 1.4 (3)[c]	7.2 ± 2.4 (3)[c]	53.6 ± 18.0 (3)[a]
% of total proteins	6.2 ± 2.5	13.6 ± 6.5	10.0 ± 3.7	70.0 ± 2.6	

For experimental conditions see under Materials and methods. Data are means ± S.E. of the experiments reported in parentheses and represent pmoles cyclic AMP/mg protein. Glucagon was 0.1 μM and PGE_1 2.5 μg/ml.

[a] $p < 0.05$ with respect to control.
[b] $p < 0.005$ with respect to control.
[c] Not significantly different.

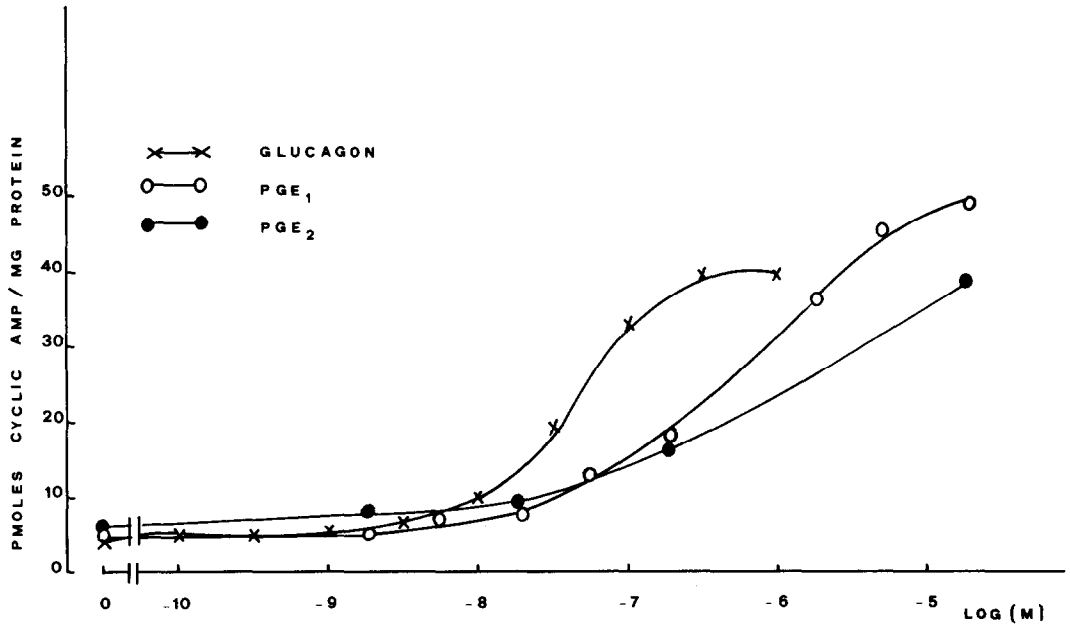

Fig.1. Dose–response curve regarding cyclic AMP control by glucagon and PGE on parenchymal and non parenchymal cells respectively. Incubations were carried out for 5 min at 30°C as described under Materials and methods using standard hepatocyte preparations when glucagon (10^{-10}–10^{-6} M) was tested and the 'hepatocyte wash' constituted mainly by non-parenchymal cells – Kupffer, endothelial cells and erythrocytes – when PGE (2.5×10^{-9} – 2.5×10^{-5} M) were tested. Each point represents the mean of 3 determinations.

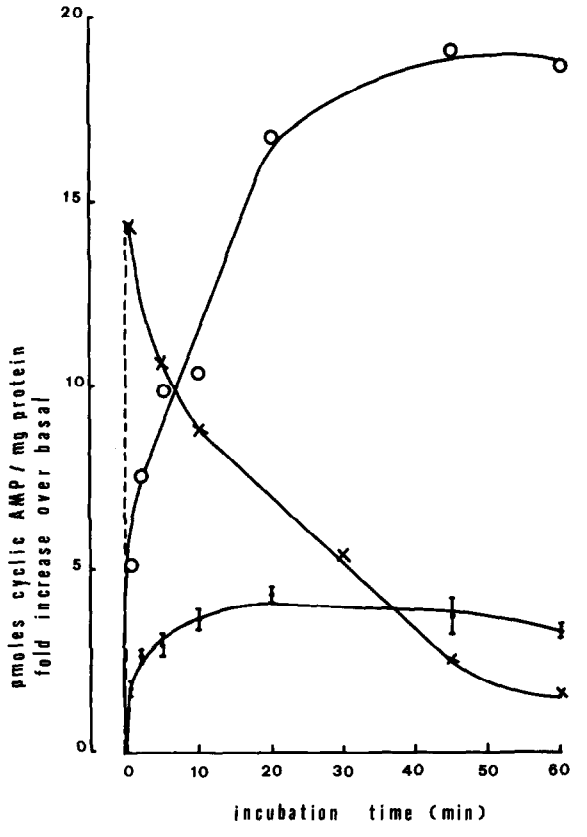

Fig.2. Time–course curves regarding cyclic AMP control by glucagon and PGE_1 on parenchymal and non parenchymal cells respectively. Glucagon effect (1 μM) was tested using standard hepatocyte preparations (X——X). PGE_1 (2.5 μg/ml) was tested on the 'hepatocyte wash' (o——o) as well as on Ficoll density gradient fraction I (•——•). Each point represents the mean of 3 experiments and bars represent S.E. of 6 determinations.

PGE_1 action on hepatocytes is shown. Glucagon at 30°C produced a maximum increase of cyclic AMP yet after 30 s of incubation, afterwards the nucleotide level rapidly declined reaching the control value after 45 min of incubation. PGE_1 was tested on the fraction called hepatocyte wash and on gradient fraction I. In the former case PGE_1 action was less rapid than that due to glucagon, but cyclic AMP increase is dramatic and persistent up to 60 min, the longest incubation time tested. PGE_1 elevated cyclic AMP level about 20-fold when added to hepatocyte wash and about 5-fold when added to fraction I, namely to a relative homogeneous Kupffer cells population. This indicates that the target of PGE_1 action is probably a liver cellular fraction distinct from parenchymal and Kupffer cells.

4. Discussion

The findings reported here, as well as those of Wincek et al. [6] may help to unravel some unexplained findings in the area of the mechanism of PGE_1 action at least in the liver. The decreased sensitivity to PGE_1 observed when comparing adenylate cyclase of liver plasma membrane to cyclic AMP levels of isolated hepatocytes has to be explained by a loss of non-parenchymal, PGE_1-sensitive cells when isolating hepatocytes by currently used techniques.

The preferential action of PGE_1 on non-parenchymal cells may account for the observation that PGE_1, unlike glucagon, does not modify or even inhibit gluconeogenesis in the perfused rat liver [13] or in isolated rat liver cells [14]. In addition the glycogenolytic effect of PGE_1 is considerably less pronounced than that of glucagon [2].

The findings of Wincek et al. [6] are only in part in agreement with ours but it has to be underlined that they assayed effects on adenylate cyclase of particulate fractions while we measured variations of cyclic AMP levels. Although they found a glucagon stimulation of about 10-fold in accordance with our data, they never observed a PGE_1 stimulation over 3–4-fold, which compares with the 20-fold increase we observed using the same hepatocyte wash used by Wincek et al. [6]. Secondly they interpreted the data as indicating that PGE_1-sensitive cyclases are present in endothelial cells as well as in parenchymal cells. Our results on the other hand show that PGE_1 has no significant effect on cyclic AMP of parenchymal cells. While the first discrepancy is difficult to explain, the second one may well be due to the extent of contamination of parenchymal cells. We observed that gradient fractions as III or IV (see table 1) unresponsive to PGE_1 contained less than 1% of non-parenchymal elements. We agree with Wincek et al. that PGE_1 receptor should be present in sinusoids-lining endothelial cells rather than in Kupffer cells.

Since the function of liver endothelial cells is poorly understood [15] it is hazardous to speculate

about the functional significance of the PGE_1 induced cyclic AMP elevation. The fact that cyclic AMP increase persisted for at least 1 h while glucagon effect on parenchymal cells is much more transitory, may suggest that unlike glucagon which behaves mainly as a metabolic regulator, PGE_1 may act as a mitotic regulator, a function that it probably exerts in thymic lymphocytes [16] and in mast cells [17]. Bourne et al. [17] have shown that PGE acting on mast cells are released from other cell types thus substantiating a role of PGE as intercellular messenger. A similar conclusion based on different arguments was reached by Bito [18] who considers prostaglandins as extracellular mediators. It is interesting that Minna and Gilman [19] studying the effect of PGE on a cell line of hepatic origin observed a dramatic increase in cyclic AMP level (69-fold). In addition the concentration and the time of exposure curves were very similar to ours.

Experiments now in progress to find out which kind of liver cells are capable of synthesizing prostaglandins suggest that the biosynthetic activity is restricted to non-parenchymal cells.

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