SOLUBILIZED PROSTAGLANDIN BINDING COMPONENTS

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1. Introduction

The prostaglandins are a group of hormones, derived from essential fatty acids, which exhibit a broad spectrum of pharmacological effects as diverse as the inhibition of platelet aggregation and the regulation of intestinal fluid secretion [1]. Prostaglandins, and likewise, catecholamines and glucagon, act on the cell by elevating the intracellular level of cAMP [2,3]. The precise mechanism by which cAMP formation is stimulated by hormone still remains to be elucidated, despite the great amount of work which has been carried out on this system in recent years [3]. Nevertheless, it is clear that two distinct events occur; binding of hormone to its receptor and the subsequent stimulation of adenylate cyclase.

Since the floating receptor hypothesis was first proposed [4], the non-identity of receptor and adenylate cyclase has been proved by a variety of techniques [5–9]. Undoubtedly, it is the complexity of the system which has frustrated our understanding of it and this being the case, it would clearly be advantageous to examine each component separately, removed from its membraneous milieu, since the membrane itself can also influence the properties of integral components [10,11].

Here we report the solubilization of prostaglandin E_1 (PGE_1)-binding components from cultured mammalian cells.

2. Experimental

2.1. *Materials*

[5,6-^{3}H]PGE_1 (50 Ci/mmol) and [^{14}C]protein hydrolysate (57 mCi/matom carbon) were purchased from the Radiochemical Centre, Amersham; guanylyl-imido-diphosphate (Gpp(NH)p), Lubrol PX, neuraminidase (type V), PGE_1 and phospholipase C (type X) from Sigma; trypsin from Difco. All other chemicals were of analytical grade. The ultrafiltration equipment is the product of the Amicon Corporation.

2.2. *Preparation of homogenate*

P388 mouse lymphatic leukaemia cells were grown and homogenised as in [12], except that 0.05 μCi/ml [^{14}C]protein hydrolysate was included in the growth medium, 4 days before harvesting the cells, as a membrane marker. The homogenate was stored under liquid nitrogen, aliquots being taken when required and resuspended in buffer (10 mM Tris–HCl, pH 7.6 at 20°C, 0.15 M NaCl) following centrifugation at 40 000 × *g* for 20 min (4°C).

2.3. *Solubilization of the PGE_1-binding components*

Homogenate (1 mg protein by the Lowry method [13]) was incubated in 0.27 ml buffer for 30 min at 30°C together with 2 mM $MgCl_2$, 0.15 μM [5,6-^{3}H]PGE_1 and any other component, as described below. All subsequent steps were carried out at 4°C.

The suspension was eluted through a column (8.6 × 1.2 cm) of Sephadex G-50 Fine with buffer, the void volume was collected and centrifuged at 40 000 × *g* for 20 min. The pellet was resuspended in 0.6 ml buffer, to which 0.3 ml 0.15% (w/v) Lubrol PX in buffer was added subsequently. This suspension was incubated for 30 min, during which time it was periodically agitated on a vortex mixer, then centrifuged at 60 000 × *g* for 20 min.

The supernatant was concentrated by ultrafiltration in a Centriflo CF-25 cone for 15 min at 800 × *g*. The concentrate, 200 μl, was applied to a column

(18.2 X 0.8 cm) of Sepharose CL-6B, pre-equilibrated with buffer containing 0.05% (w/v) Lubrol PX and eluted at a 9 ml/h flow rate. 200 μl fractions were collected directly into plastic mini scintillation vials to which 2.4 ml Triton–toluene fluor containing PPO and POPOP was added.

2.4. *Liquid scintillation counting*

Samples were counted in a Packard model 3385 liquid scintillation spectrometer for 20 min each, using the preset channels for ^{3}H and ^{14}C dual labelling. Counting efficiencies were 21% and 57%, whilst backgrounds were 7 cpm and 6 cpm for ^{3}H and ^{14}C, respectively; 47% of the ^{14}C cpm spilt over into the ^{3}H channel. Results were converted to dpm by an off-line computer.

3. Results

Prostaglandin E_1 binding was assayed by incubating P388 homogenate at 30°C with labelled PGE_1. Free hormone was removed by Sephadex chromatography and the homogenate was then treated with 0.05% Lubrol PX, a concentration found to be optimal for the solubilization of active adenylate cyclase from P388 (unpublished data). The solubilized material was concentrated, then fractionated on Sepharose CL-6B with complete recovery of the applied radioactivity. This method is reproducible and a typical example is given in fig.1. Specific binding is represented by the difference (not shown) of the total and non-specific binding.

Non-specific binding was only 15% of the total and

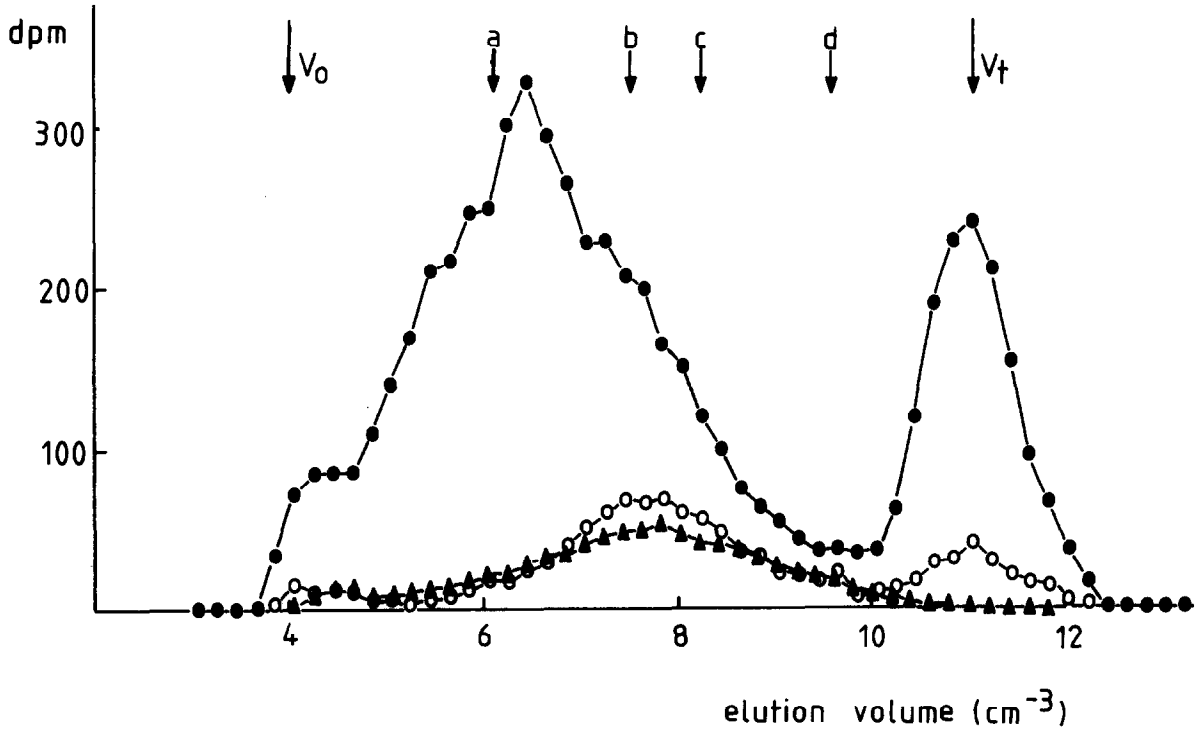

Fig.1. Fractionation of PGE_1-binding components. [5,6-^{3}H]PGE_1 was bound to P388 homogenate, which was then solubilized and fractionated as described in section 2. Total binding (●–●) was assayed for in the absence of unlabelled PGE_1, whilst non-specific binding (○–○) included 37 μM unlabelled PGE_1 in the incubation mixture. Fractionation of the ^{14}C marker label is also shown (▲–▲). Molecular weight markers were: (a) thyroglobulin, 67 X 10^4; (b) aldolase, 14 X 10^4; (c) haemoglobin, 6 X 10^4; (d) cytochrome *c*, 12 X 10^3.

was distinguishable from specific binding on account of their different elution volumes. This level of non-specific binding was made possible by the use of Sephadex chromatography, which gave a 12-fold enhancement in the ratio of specific/non-specific binding. Reaction of the non-specific binding was thought to be due to both removal of free [5,6-^{3}H]PGE_1 and to the partial purification of plasma membrane, since much of the homogenate was unable to pass through the Sephadex column.

The ^{14}C-marker label (fig.1) was presumably incorporated into lipid, as it co-migrated with the non-specific PGE_1-binding component.

Dissociation of bound [5,6-^{3}H]PGE_1, during and after solubilization, accounts for the label eluting in the total volume fraction. Prior to solubilization, no free label could be detected. ^{14}C was never detected in the total volume fraction.

Treatment of the homogenate with trypsin, phospholipase C or neuraminidase, during the incubation with [5,6-^{3}H]PGE_1 resulted in a reduction in the molecular weight of the specific PGE_1-binding component. Solubilization and fractionation of the membrane with 0.45% Lubrol reduced the molecular weights of both the specific and non-specific PGE_1-binding components (data not shown). The increased Lubrol concentration did not significantly improve the yield of bound PGE_1, apparently because the rate of dissociation was also enhanced [8].

The presence of 0.1 mM Gpp(NH)p in the PGE_1-binding incubation caused the complete loss of specific PGE_1 binding, resulting in an elution profile identical to that for non-specific PGE_1 binding (fig.1).

4. Discussion

Evidence that the observed specific [5,6-^{3}H]PGE_1 binding is indeed binding to the bona fide receptor, is that the K_d for binding and the K_a for adenylate cyclase activation by PGE_1, in particulate preparations of P388, are of the same order, i.e., 40 nm [12,14]. The receptor density is 0.4 pmol/mg membrane protein [14], which corresponds to 2000 receptors/cell. Moreover, we have shown here that specific binding is completely abolished in the presence of 0.1 mM Gpp(NH)p. GTP and its analogues are known to reduce the receptor affinity for agonists, whilst having no effect on antagonist binding [15–18].

The prostaglandins, being derived from fatty acids, are an enigmatic group of hormones. It has been suggested that the hormone receptor site is constituted by the receptor/membrane lipid interface [19,20] and if this is indeed the case, one could envisage the membrane lipids playing a predominant role in the PGE_1 receptor. This report, however, shows that it is possible to solubilize specific PGE_1-binding components in the same manner as the β-adrenergic receptor [6,8,21], the gonadotropin receptor [22] and the glucagon receptor [23]. Furthermore, the apparent molecular weight of the specific PGE_1-binding components, although somewhat heterogeneous, lies within the same range as that of other solubilized receptors [7,21,22], namely $2–5 \times 10^5$. Perhaps this is not surprising, when one considers that these receptors have to satisfy presumably stringent conditions in order to couple satisfactorily with adenylate cyclase.

The effect of trypsin, phospholipase C, neuraminidase and 0.45% Lubrol on the reduction of the PGE_1-binding components' molecular weight range is probably due to the breakdown of an aggregate of PGE_1 receptor, membrane lipid and protein; resistance of specific PGE_1 binding to trypsin action suggests that the receptor is buried within the plasma membrane, inaccessible to proteolytic attack.

In conclusion, we have shown that specific PGE_1-binding components, assayed with an agonist, can be solubilized from cultured mammalian cells. Within the limits of our current knowledge, these components are very similar to those other receptors which interact with adenylate cyclase.

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