Stimulation of Hormone-Responsive Adenylate Cyclase Activity by a Factor Present in the Cell Cytosol

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1. Homogenates of whole tissues were shown to contain both intracellular and extracellular factors that affected particulate adenylate cyclase activity *in vitro*. Factors present in the extracellular fluids produced an inhibition of basal, hormone- and fluoride-stimulated enzyme activity but factors present in the cell cytosol increased hormone-stimulated activity with relatively little effect on basal or fluoride-stimulated enzyme activity. 2. The existence of this cytosol factor or factors was investigated using freshly isolated human platelets, freshly isolated rat hepatocytes, and cultured cells derived from rat osteogenic sarcoma, rat calvaria, mouse melanoma, pig aortic endothelium, human articular cartilage chondrocytes and human bronchial carcinoma (BEN) cells. 3. The stimulation of the hormone response by the cytosol factor ranged from 60 to 890% depending on the tissue of origin of the adenylate cyclase. 4. In each case the behaviour of the factor was similar to the action of GTP on that particular adenylate cyclase preparation. 5. No evidence of tissue or species specificity was found, as cytosols stimulated adenylate cyclase from their own and unrelated tissues to the same degree. 6. In the human platelet, the inclusion of the cytosol in the assay of adenylate cyclase increased the rate of enzyme activity in response to stimulation by prostaglandin E_1 without affecting the amount of prostaglandin E_1 required for half-maximal stimulation or the characteristics of enzyme activation by prostaglandin E_1 .

In the study *in vitro* of hormone-stimulated adenylate cyclase [EC 4.6.1.1., ATP pyrophosphatase (cyclizing)] activity, most investigators have used partially purified washed membrane preparations. This means that factors present in cytosol that may influence enzyme activity *in vivo* have been removed. Preliminary work (Johnson *et al.*, 1979) on supernatants from whole-tissue homogenates showed that these supernatants produced both inhibiting and stimulating effects on adenylate cyclase activity; the stimulating effect was found to reside in the cytosol obtained from several tissues, particularly cultured cell lines (Crawford *et al.*, 1979).

We have therefore studied the effects of cytosol on hormone-stimulated adenylate cyclase in several systems in order to acquire a clearer understanding of the characteristics and possible mode of action

of the factor or factors in cytosol that appear(s) to enhance hormone-stimulated adenylate cyclase activity.

Materials and Methods

Chemicals

[α - 32 P]ATP (0.5–30 Ci/mmol) and cyclic [8- 3 H]AMP (20–30 Ci/mmol) were obtained from The Radiochemical Centre, Amersham, Bucks., U.K. Cyclic AMP as the crystallized free acid, the sodium salts of ATP and GTP, phosphoenolpyruvate (tricyclohexylammonium salt) and pyruvate kinase (EC 2.7.1.40, sp.act. approx. 200 i.u./mg) were obtained from Boehringer Mannheim, London. Bovine serum albumin (fraction V, powder) was from Armour Pharmaceuticals, Eastbourne, Sussex, U.K. All other chemicals were of A.R. grade.

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Hormone preparations

Highly purified bovine parathyroid hormone (code 77/533, potency 2000 units/mg) was a gift from the National Institute of Biological Standards, Holly Hill, Hampstead, London NW3 6RB, U.K. Synthetic salmon calcitonin (4700 units/mg) was a gift from Armour Pharmaceuticals, and prostaglandins E_1 and E_2 were a gift from Dr. J. E. Pike, Upjohn Co. Kalamazoo, MI, U.S.A. Glucagon (1000 units/mg) was obtained from Eli Lilly and Co. Adrenocorticotrophic hormone (porcine grade II, 100000 units/mg) and bovine pituitary thyroid stimulating hormone (1000 units/mg) were obtained from Sigma; adrenaline injection B.P. (adrenaline 1:1000) was obtained from Antigen, Roscrea, Ireland,

Adenylate cyclase preparations

Cells maintained in culture. Adenylate cyclase preparations were made from rat osteogenic sarcoma, bone cells derived from rat calvaria, mouse B16 melanoma cells, pig aortic endothelial cells, human bronchial carcinoma (BEN) cells (Ellison *et al.*, 1975) and human articular cartilage chondrocytes by the following method. Petri dishes (90 cm²) containing monolayer cultures of cells were washed free of culture medium with approx. 10 ml of 150 mM-NaCl. Cells were then scraped off the dish and suspended in 0.5 ml of 10 mM-Tris/HCl (pH 7.4) at 4°C containing 250 mM-sucrose and 1 mM- Na_4 EDTA. Cells were then lysed by rapidly freezing the cell suspension using a mixture of solid CO₂ and ethanol and then thawing it to room temperature. This was repeated twice to produce a lysate of whole cells that was used immediately in some experiments. Particulate preparations were produced by centrifuging the lysate in the SM 24 head of a Sorvall RC2B centrifuge at 5500 *g* for 10 min (r_{av} , 11.02 cm) at 4°C. The pellet was resuspended in buffer (approximately 0.5 ml/Petri dish) and was again centrifuged at 5500 *g* for 10 min to give the preparation referred to as a particulate cell preparation. This preparation was resuspended in buffer (again 0.5 ml/Petri dish used) and used immediately in the assay of adenylate cyclase.

Freshly isolated cells. Human-platelet adenylate cyclase was prepared by mixing fresh human blood with 0.1 vol. of 3.8% sodium citrate/100 mM- Na_4 EDTA. Blood was centrifuged at 200 *g* (r_{av} , 20.8 cm) in a 63302 rotor in an MSE 6L centrifuge for 10 min at 10–15°C and the supernatant was removed and centrifuged at 600 *g* (r_{av} , 20.8 cm) for 10 min at 10–15°C. The pellet was resuspended in the original blood volume of 15 mM-Tris/HCl (pH 7.4)/1 mM- Na_4 EDTA/140 mM-NaCl and centrifuged at 600 *g* (r_{av} , 20.8 cm) for 10 min at 10–15°C. The pellet was resuspended in 50 mM-Tris/HCl (pH 7.4)/250 mM-sucrose/1 mM- Na_4 EDTA (one-tenth of

the original blood volume) and the platelets were lysed by freeze/thawing twice in a mixture of ethanol and solid CO₂ as previously described. Aliquots of this platelet lysate were used immediately in the assay of adenylate cyclase and the remainder of the lysate was centrifuged at 12300 *g* (r_{av} , 11.02 cm) in a Sorvall RC2B centrifuge as previously described for 10 min at 4°C. The pellet was then resuspended in 50 mM-Tris/HCl (pH 7.4)/250 mM-sucrose/1 mM- Na_4 EDTA and centrifuged at 12300 *g* (r_{av} , 11.02 cm) for 10 min at 4°C to give the washed particulate platelet preparation.

Freshly isolated rat hepatocytes were prepared according to the method of Seglen (1975) and particulate preparations from rat hepatocytes and human platelets were used immediately. Rat renal cortex tubules were prepared by the method of Larkins *et al.* (1974).

Particulate preparations derived from whole tissues. Human-thyroid adenylate cyclase was prepared from tissue obtained at surgery. Tissue specimens were processed as soon as possible after removal (usually within 30 min) at 4°C. Tissue was chopped finely with razor blades and suspended in 10 vol. of 20 mM-Tris/HCl (pH 7.4)/250 mM-sucrose. This suspension was then homogenized with a Polytron PCU-2 homogenizer at speed 4 for 3 × 10 s. Further homogenization was carried out with five strokes of a loose Dounce glass pestle homogenizer and the homogenate was then filtered through muslin. Homogenates were centrifuged at 300 *g* (r_{av} , 20.8 cm) using an MSE 6L centrifuge for 10 min at 4°C to remove large particulate material and then at 10000 *g* (r_{av} , 11.02 cm) using a Sorvall RC2B for 15 min at 4°C to yield a particulate preparation used in the assay of adenylate cyclase.

Rat-liver particulate preparations were prepared from male Wistar rats (150–200 g) by the method of Zenser *et al.* (1974). These preparations were used in the assay of adenylate cyclase activity. Supernatant from the first centrifugation of the homogenate was retained for use in some experiments.

Human renal cortex was obtained from the macroscopically normal tissue surrounding renal cell carcinoma removed at surgery. The particulate preparation was prepared as described by Hunt *et al.* (1978). Rat renal-cortex adenylate cyclase was prepared as described by Dawborn *et al.* (1977). Supernatant from the first centrifugation of the homogenate was retained for use in some experiments.

Particulate preparations from rat liver and renal cortex and human thyroid and renal cortex were all stable at –70°C for several months.

Cytosol preparation. Preparations of cytosol were obtained from isolated and cultured cells as follows. Cell lysates were centrifuged at 50000 *g* for

30 min [r_{av} , 11.02 cm (SM 24 head of a Sorvall RC2B centrifuge)]. The supernatant ('50000g cytosol') was then removed and either was used immediately or was stored at -70°C . No change in activity was observed with up to 6 weeks storage. The protein concentration in the preparations was 0.8–2.5 mg/ml.

Adenylate cyclase assay

Adenylate cyclase activity was measured by the conversion of [α - ^{32}P]ATP to cyclic [^{32}P]AMP as described by Hunt *et al.*, (1976). Reaction mixtures (100 μl) contained 25 mM-Tris/HCl (pH 7.8), 1 mM-[α - ^{32}P]ATP (20–200 d.p.m./pmol), 1 mM-cyclic AMP, 2.5 mM-phosphoenolpyruvate, 1.5 i.u. of pyruvate kinase, 130 μg of bovine serum albumin/ml, 30 mM-KCl and 4.5 mM-MgSO₄.

Incubations at 37°C (30°C for liver adenylate cyclase) were started by the addition of the appropriate adenylate cyclase whole cell lysate or particulate preparation and stopped by the addition of 100 μl of 40 mM-ATP and heating at 95°C for 2 min. Cytosol and GTP were added direct to the particulate pellets rather than to the assay because in preliminary experiments the activity found when pellets were resuspended directly in cytosol or GTP and then used to initiate the assay was at least twice as great as when pellets were resuspended in buffer and then added to an assay containing the same concentration of cytosol or GTP.

Cyclic[^{32}P]AMP was isolated according to Salomon *et al.* (1974) and protein was determined by the method of Hartree (1972).

The results shown are of individual experiments representative of the data obtained on a number of occasions. Assays of adenylate cyclase activity were carried out in triplicate and results are expressed as means $\pm$ S.E.M. of the triplicate determinations.

Results

Effects of homogenate supernatant versus cytosol alone on enzyme activity

Studies of whole-liver homogenates showed that although some stimulation of hormone-responsive adenylate cyclase was seen on addition of a homogenate supernatant back to the particulate preparations (Fig. 1a), as the amount of supernatant was increased there was a gradual inhibition of basal, hormone- and fluoride-stimulated activity. However, the addition of cytosol prepared from washed hepatocytes (Fig. 1b) stimulated the hormone-responsive enzyme activity with no effect on basal or fluoride-stimulated enzyme activity. Similar results were found in a study of rat renal-cortex particulate adenylate cyclase: cytosol prepared from isolated renal tubules produced an increase in the parathyroid hormone-stimulated enzyme

Fig. 1. Comparison of the effects of homogenate supernatant and cytosol on rat liver adenylate cyclase activity

Rat liver particulate adenylate cyclase was prepared as described in the Materials and Methods section and the effects of (a) supernatant from the first centrifugation of the liver homogenate (16 μg of protein/ μl) and (b) cytosol prepared from washed liver hepatocytes (1.8 μg of protein/ μl) were compared with respect to their effects on basal (O), glucagon- (10^{-7}M , ●) and fluoride- (10 mM, ■) stimulated enzyme activity. Standard errors are all within the size of the symbols used.

activity but had no effect on basal or fluoride-stimulated enzyme activity; the additional of a homogenate supernatant had a biphasic effect, eventually producing an inhibition of basal, hormone- and fluoride-stimulated enzyme activity. Since the presence of the extracellular fluid in tissue homogenates produced an inhibition of the adenylate cyclase activity, subsequent experiments were carried out using cytosol preparations prepared from isolated cells washed free of extracellular fluid.

Effect of cytosol alone on adenylate cyclase activity

Fig. 2 shows a comparison of adenylate cyclase activity measured in the whole cell lysate with that found in washed particulate preparations of freshly isolated human platelets, cultured rat osteogenic sarcoma cells and cultured mouse melanoma cells. The hormone- or prostaglandin-stimulated enzyme activity was often 5–8 times greater in the whole cell lysate than in the washed particulate preparations of the same tissue.

In the platelet, a comparative study of the characteristics of enzyme activation indicated no significant difference in the amount of prostaglandin E_1 required for half-maximal activation of the enzyme in the lysate ($12.0 \times 10^{-8} \pm 0.3 \times 10^{-8}\text{M}$; mean $\pm$ S.E.M.) and in the washed particulate preparation

Fig. 2. *Comparison of adenylate cyclase activity in whole cell lysates and particulate preparations* Whole cell lysates (●) and particulate preparations (○) from (a) human platelets, (b) cultured rat osteogenic sarcoma cells and (c) cultured mouse melanoma cells were prepared as described in the Materials and Methods section. The response of lysates and particulate preparations to stimulation by the appropriate prostaglandin or hormone was compared. Standard errors are all within the size of symbols used.

($8.4 \times 10^{-8} \pm 3.0 \times 10^{-8}$ M; mean $\pm$ S.E.M., $n = 5$). Similarly, in the platelet there was no significant difference in the time course of activation of the enzyme by prostaglandin E₁ (cyclic AMP production was linear for up to 8 min at 37°C and for up to 10 min at 30°C), in the pH dependency (optimum at pH 8.0) or in the protein dependency (cyclic AMP production was linear with increasing amounts of particulate protein added up to 0.6 mg of protein/ml). The optimum substrate concentrations for the washed particulate preparation were 1 mM-ATP + 4 mM-Mg²⁺ and at this substrate concentration the activity of the lysate was seven times greater than that of the particulate preparation, indicating that substrate concentration is unlikely to explain the difference in the rate of enzyme activity found in the two preparations.

Occurrence of cytosol factor

An examination of several isolated cell preparations showed that the adenylate cyclase activity of whole cell lysates was greater than that found in washed particulate preparations and that this difference in activity could be restored by adding back the cell cytosol to the washed particulate preparation. Cytosol alone had no intrinsic adenylate cyclase activity. A summary of these results is shown in Table 1. In general, the stimulating effect of the cell cytosol was always greater on the hormone-stimulated activity than on the basal or fluoride-stimulated enzyme activity. In some systems cytosol significantly increased the basal and fluoride-stimulated enzyme activity (see text and Table 2) and in others it had no significant effect (Fig. 3).

Ability of unrelated cytosols to stimulate particulate adenylate cyclases

Particulate adenylate cyclase preparations from whole tissue homogenates responded to platelet cytosol with an increase in the hormone-stimulated enzyme activity and comparatively little change in the basal and fluoride-stimulated activities (Table 2). The response of the rat-liver adenylate cyclase to human-platelet cytosol was as great as the response to cytosol derived from the rat liver hepatocytes, and similarly platelet cytosol and rat renal-tubule cytosol were equally effective in stimulating rat renal cortex adenylate cyclase.

The lack of tissue or species specificity of the cytosolic factor or factors and the ability to increase hormone-stimulated rather than basal or fluoride-stimulated enzyme activity suggested that these factors behaved in a similar manner to GTP.

Comparison of the mode of action of cytosols and GTP on adenylate cyclase activity

A comparison of the action of cytosols and of GTP was carried out on adenylate cyclase preparations derived from freshly isolated human platelets, cultured rat osteogenic sarcoma cells and human renal cortex tissue. Fig. 3 shows a comparison of the effects of platelet cytosol and of GTP on the basal, prostaglandin E₁- and fluoride-stimulated activity of platelet particulate adenylate cyclase. Platelet cytosol and GTP both increased the enzyme activity seen in response to a maximum concentration of prostaglandin E₁ (10⁻⁶ M), but had no significant effect on basal or fluoride-stimulated enzyme

Table 1. *Comparison of adenylate cyclase activity in the presence and absence of the cell cytosol*

Freshly isolated cells and cells maintained in culture were used to prepare whole-cell lysates and washed particulate preparations. Particulate preparations were resuspended in either buffer or the original cell cytosol. Details are given in the Materials and Methods section. Incubations contained a concentration of hormone or prostaglandin that produced maximum enzyme activity. Values are expressed as means $\pm$ S.E.M. of three determinations.

Cells	Hormone (concentration)	Adenylate cyclase activity (pmol cyclic AMP/min per mg of particulate protein)		
		Whole cell lysates	Particulate preparation + buffer	Particulate preparation + cytosol
Freshly isolated human platelets	Prostaglandin E ₁ (10 ⁻⁶ M)	225.9 $\pm$ 7.5	134.2 $\pm$ 8.5	214.9 $\pm$ 7.0
Freshly isolated rat hepatocytes	Glucagon (10 ⁻⁷ M)	83.1 $\pm$ 5.6	32.7 $\pm$ 8.9	90.6 $\pm$ 1.3
Cultured rat osteogenic sarcoma cells	Parathyroid hormone (10 ⁻⁵ M)	154.0 $\pm$ 12.0	30.0 $\pm$ 2.0	145.1 $\pm$ 16.0
Cultured rat calvaria bone cells	Parathyroid hormone (10 ⁻⁵ M)	45.0 $\pm$ 4.5	16.2 $\pm$ 1.5	—
Cultured mouse melanoma cells	Adrenocorticotrophic hormone (10000 units/ml)	61.1 $\pm$ 5.3	5.6 $\pm$ 0.3	55.8 $\pm$ 10.8
Cultured pig aortic endothelial cells	Adrenaline (10 ⁻⁵ M)	86.1 $\pm$ 7.6	<0.1	47.9 $\pm$ 4.9
Cultured human articular cartilage chondrocytes	Prostaglandin E ₁ (3 $\times$ 10 ⁻⁶ M)	583.0 $\pm$ 36.0	34.0 $\pm$ 9.0	—
Cultured human bronchial carcinoma (BEN) cells	Calcitonin (10 ⁻⁶ M)	—	30.0 $\pm$ 3.1	82.3 $\pm$ 8.1

Table 2. *Effects of platelet cytosol on particulate adenylate cyclase from unrelated tissues*

Particulate adenylate cyclase preparations were prepared from rat and human tissues as described in the Materials and Methods section and were resuspended either in buffer or in cytosol obtained from human platelets (23 μ g of protein/100 μ l incubation). The adenylate cyclase activity of the two preparations was compared in the presence of buffer alone (basal activity), a concentration of the appropriate hormone or prostaglandin that gave maximal enzyme activity and in the presence of 10 mM-sodium fluoride. Values are means $\pm$ S.E.M. of three determinations.

Tissue	Addition	Adenylate cyclase activity (pmol cyclic AMP/min per mg of particulate protein)	
		Particulate preparation + buffer	Particulate preparation + cytosol
Rat liver	Basal (none)	14.5 $\pm$ 0.4	15.4 $\pm$ 0.6
	Glucagon (10 ⁻⁷ M)	64.5 $\pm$ 7.5	80.1 $\pm$ 4.7
	NaF	85.5 $\pm$ 1.2	65.7 $\pm$ 1.9
Rat renal cortex	Basal (none)	4.3 $\pm$ 1.0	11.3 $\pm$ 1.1
	Parathyroid hormone (5 $\times$ 10 ⁻⁶ M)	38.4 $\pm$ 0.4	63.7 $\pm$ 2.1
	NaF	32.9 $\pm$ 2.1	48.7 $\pm$ 0.4
Human renal cortex	Basal (none)	3.1 $\pm$ 0.1	10.3 $\pm$ 1.6
	Parathyroid hormone (10 ⁻⁶ M)	147.1 $\pm$ 1.5	244.1 $\pm$ 5.7
	NaF	199.6 $\pm$ 2.5	210.5 $\pm$ 8.8
Human non-toxic goitre	Basal (none)	13.9 $\pm$ 1.7	26.6 $\pm$ 2.1
	Thyrotropin (3 $\times$ 10 ⁻⁵ M)	98.9 $\pm$ 2.3	172.8 $\pm$ 2.7
	NaF	215.2 $\pm$ 12.9	184.1 $\pm$ 18.0

activity. Similarly, in the case of particulate adenylate cyclase preparations prepared from cultured rat osteogenic sarcoma cells and human renal cortex, the effects of cytosol and GTP were very similar. In rat osteogenic sarcoma cells, basal adenylate cyclase activity was increased from

5 $\pm$ 0.3 to 44 $\pm$ 4 pmol of cyclic AMP/min per mg of particulate protein by osteogenic sarcoma cytosol and to 31 $\pm$ 2 pmol of cyclic AMP/min per mg of protein by 10⁻⁴M-GTP; prostaglandin E₁-stimulated activity was increased from 47 $\pm$ 7 to 154 $\pm$ 8 pmol/min per mg by cytosol and to 166 $\pm$

Fig. 3. Comparison of the effects of platelet cytosol (a) and of GTP (b) on particulate platelet adenylate cyclase activity. Particulate platelet adenylate cyclase was prepared as described in the Methods section and pellets were resuspended in various concentrations of either platelet cytosol (1.3 µg of protein/µl) or GTP. Basal (○), prostaglandin E₁ (10⁻⁶ M, ●), and fluoride- (10 mM, ■) stimulated enzyme activities were compared. Values are means ± S.E.M. for three determinations.

2 pmol/min per mg by 10⁻⁴ M-GTP; fluoride-stimulated activity was increased from 92 ± 4 to 147 ± 4 pmol/min per mg by cytosol and to 98 ± 3 pmol/min per mg by 10⁻⁴ M-GTP. In the human renal-cortex adenylate cyclase preparation the basal enzyme activity increased from 30 ± 4 to 80 ± 7 pmol/min per mg in the presence of human platelet cytosol and to 46 ± 3 pmol/min per mg in the presence of 10⁻⁴ M-GTP; parathyroid hormone-stimulated enzyme activity was increased from 166 ± 4 to 446 ± 12 pmol/min per mg with cytosol and to 507 ± 10 pmol/min per mg with 10⁻⁴ M-GTP. Theoretical calculations of the concentration of free GTP present in the platelet-cytosol preparation (Fig. 3), based on the data of D'Souza & Glueck (1977), gave a value of 8.7 × 10⁻⁸ M. The effects of maximal doses of cytosol and GTP were not additive in any of the three systems studied.

Discussion

The adenylate cyclase enzyme is thought to consist of three components, the membrane-bound receptor site which undergoes a specific interaction

with a hormone, a nucleotide-regulatory site which is thought to be a protein complex that binds guanine nucleotides, and a membrane-bound catalytic unit that catalyses the conversion of ATP to cyclic AMP. The role of the nucleotide-regulatory site in cyclase activation has been recently reviewed by Rodbell (1978). Only when GTP occupies the nucleotide regulatory site do the receptor and catalytic unit become sensitized for coupling, which is promoted by the binding of hormones to the receptor site. Recent evidence in intact cells supports the hypothesis that GTP is involved in adenylate cyclase activation *in vivo*, (Johnson & Mukku, 1979). However, the location of the nucleotide regulatory protein within the cell is not known and it is possible that under basal conditions such proteins may be loosely located within the cell membrane without any specific attachment to the adenylate cyclase enzyme or may even be in the cell cytosol. It is possible that in the preparation of washed plasma membranes these proteins may be lost.

The present study illustrates that membrane preparations washed free of cytosol exhibit less adenylate cyclase activity than the whole-cell lysates. The study of whole tissue homogenates masks the

stimulating effects of cytosol, however, presumably because of inhibitory effects of the extra-cellular fluid. If cytosols are prepared from whole tissue homogenates (Doberska & Martin, 1977) then they have both stimulatory and inhibitory effects on the adenylate cyclase but if, as in the present study, isolated cell preparations are made from the same tissues and washed free of extra-cellular fluids, then cytosols prepared from these cells will increase hormone-stimulated adenylate cyclase.

The nature of the factor or factors in the cytosol responsible for adenylate cyclase activation has recently been investigated by several groups of workers. Clark (1978) could explain the loss of activity seen in membrane preparations of astrocytoma cells solely in terms of the concentration of free GTP in the cytosol. In our studies the effects of any cytosol on adenylate cyclase activity were always very similar to the effects of GTP. However, preparations of platelet cytosol that could cause maximal enhancement of adenylate cyclase would contain a theoretical concentration of free GTP 100 times smaller than that required for maximal stimulation of the enzyme. In addition, other investigators (Pecker & Hanoune, 1977; Sanders *et al.*, 1977; Doberska & Martin, 1977; Katz *et al.*, 1978; Egan *et al.*, 1978) and we ourselves (Crawford *et al.*, the following paper) have found evidence of a protein factor in the cytosol that appears to act in a similar manner to GTP. The nature of these variously described proteins and whether they act by binding GTP or simply act at the same site as GTP is unknown. Irrespective of the biochemical nature of the factors present in the cytosol, our results support the observation of other workers in demonstrating the existence of adenylate-cyclase-stimulating factors in the cytosols of tissues from many different sources. Furthermore we have now shown that the factor or factors exhibit no obvious species or tissue specificity, because cytosols stimulated adenylate cyclase enzymes from their own and unrelated tissues to the same degree.

The question of whether cytosol should be included in the assay of hormone responsive adenylate cyclase *in vitro*, in order to more accurately reflect the physiological state, merits serious consideration because we have found this factor or factors in every tissue that we have examined and its behaviour is very similar to GTP. Indeed, in certain particulate adenylate cyclase preparations, the response to a hormone may only be seen in the presence of guanine nucleotides. For example, only in the presence of GTP can adrenaline be as efficient as glucagon in activating rat-liver-plasma-membrane adenylate cyclase (Hanoune *et al.*, 1975). In addition, at the concentrations at which it is found in plasma, calcitonin can only activate renal adenylate cyclase in the presence of guanine nucleotides but whether these nucleotides must be free or are bound to a binding protein has not been established. It is possible therefore that the response to certain hormones in some tissues may be lost *in vitro* if the cytosol factor is absent.

In conclusion, the present study presents evidence for the existence of a factor present in the cytosol of many unrelated tissues that is neither tissue nor species specific and behaves as though it were GTP, increasing the hormone-stimulated adenylate cyclase activity but having relatively little effect on basal or fluoride-stimulated enzyme activity. Further work is required to determine the relevance of this factor to normal hormone action *in vivo*, but adenylate cyclase studies carried out *in vitro* in the absence of this factor may fail to detect responses to hormone which may be important *in vivo*. Studies of the nature of the cytosolic factor are reported in a companion paper (Crawford *et al.*, 1980).

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