The effect of carbacyclin, a prostaglandin analogue, on adenylate cyclase activity in platelet membranes

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The effect of carbacyclin, a chemically stable analogue of prostacyclin, on the activity of adenylate cyclase in platelet membranes was measured, and compared with the effect of PGE_1. When GTP was added in concentrations up to 10 μM the activation of adenylate cyclase by carbacyclin was increased, whereas higher concentrations of GTP were inhibitory. The addition of a non-hydrolysable analogue of GDP, guanosine 5'-[β-thio]diphosphate (GDP[βS]) resulted in a dose-dependent inhibition of adenylate cyclase activation by carbacyclin; this inhibition was relieved by adding increased amounts of GTP.

Carbacyclin *PGE_1* *Adenylate cyclase* *Platelet membrane* *GTP* *GDP[βS]*

1. INTRODUCTION

Agents which raise cyclic AMP levels in platelets also inhibit platelet aggregation. This is consistent with the potent anti-aggregatory action of prostacyclin (PGI_2) a prostaglandin produced by the vascular wall which activates adenylate cyclase and raises cyclic AMP levels in platelets [1,2]. Comparisons have been carried out on the action of PGI_2 and PGE_1 on inhibition of platelet aggregation, on changes in cyclic AMP levels [1,2] and on adenylate cyclase activity [2] and it seems likely that PGI_2 may act on the same receptor as PGE_1 in platelets [3].

The experimental use of PGI_2 is complicated by its instability at physiological temperature and pH. The development of carbacyclin, a chemically stable analogue of prostacyclin has recently led to detailed studies on the mechanism of inhibition of platelet aggregation, both in vivo and in vitro [4].

We have here examined the action of carbacyclin on adenylate cyclase activation in platelet membranes and the role of GTP in this activation. A comparison has been made with the action of PGE_1 in this system.

Abbreviation: GDP[βS], guanosine 5'-[β-thio]diphosphate

2. MATERIALS AND METHODS

Carbacyclin synthesized as in [5], was kindly supplied by Dr B.J.R. Whittle, Wellcome Research Laboratories (Langley Court, Beckenham, Kent) and PGE_1 was purchased from Sigma (Poole, Dorset). Stock solutions of carbacyclin and of PGE_1 were made up in absolute ethanol and stored at $-20°C$. When required they were diluted with water, added to the adenylate cyclase assay cocktail at pH 7.4, and kept in ice until the assay was started. GDP[βS] was purchased from Boehringer (Lewes, East Sussex).

Membranes were prepared from platelet concentrates as in [6]. As reported, the dose-response curves to PGE_1 for adenylate cyclase activity were similar for membranes from platelet concentrates and from fresh human platelets. Adenylate cyclase activity was assayed as in [7]. The assay contained 25 mM Tris-HCl buffer (pH 7.4), 0.1 mM ATP, 1 μCi [α-^{32}P]ATP, 0.1 mM cyclic AMP, 10 mM $MgCl_2$, 1 mM dithiothreitol, 5 mM phosphocreatine and 5 units of creatine kinase. Platelet membranes (20–40 μg protein) were incubated for 10 min at 37°C in the assay (vol. 0.1 ml). The activity of adenylate cyclase was linear for at least 10 min under all conditions.

Protein was determined as in [8].

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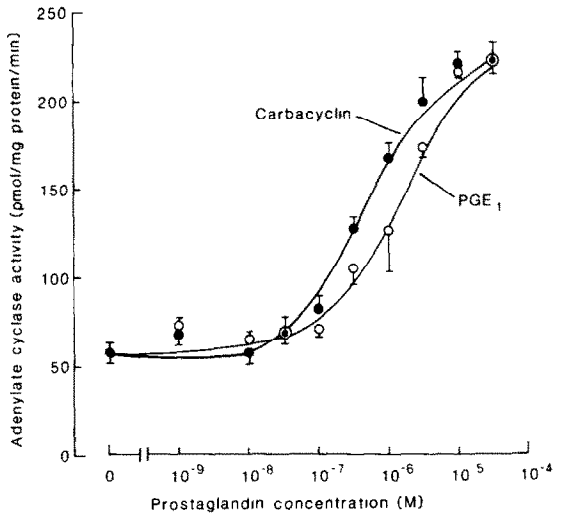

Fig.1. Dose-response relation of platelet membranes to carbacyclin and PGE_1. Platelet membranes (22 μg protein/assay) were incubated for 10 min at 37°C with GTP (10 μM) and with PGE_1 (○) or carbacyclin (●). Adenylate cyclase activity was determined as described in section 2. Each point is the mean value from 3 separate incubations ± SE.

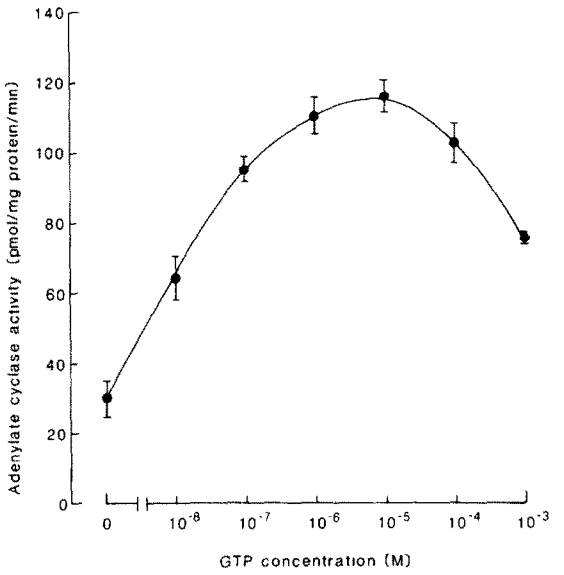

Fig.2. Effect of increasing concentrations of GTP on carbacyclin-stimulated adenylate cyclase activity. Platelet membranes (37 μg protein/assay) were incubated for 10 min at 37°C with carbacyclin (1 μM) and GTP at the concentrations indicated. Basal activity in the absence of any additions was 19 ± 0.8 pmol·mg protein^{-1}·min^{-1}. Each point is the mean value from 3 separate incubations ± SE.

3. RESULTS

A comparison of the dose-response relation of adenylate cyclase in platelet membranes to carbacyclin and to PGE_1 in the presence of GTP (10 μM) is shown in fig.1. The dose-response curves were very similar, with the curve for carbacyclin slightly to the left of that for PGE_1. The maximal levels of adenylate cyclase activity were the same for both carbacyclin and PGE_1. Fig.2 shows the effect of adding increasing amounts of GTP to platelet membranes incubated with carbacyclin (1 μM). The adenylate cyclase activity increased as the concentration of added GTP was increased, with maximal activity observed at 10 μM GTP. Higher concentrations of GTP (100 μM and mM) resulted in some inhibition.

The effect of GTP on the stimulation of adenylate cyclase was further examined using GDP[βS], a non-hydrolysable analogue of GDP which competes with GTP and other guanine nucleotides and thus inhibits adenylate cyclase [9].

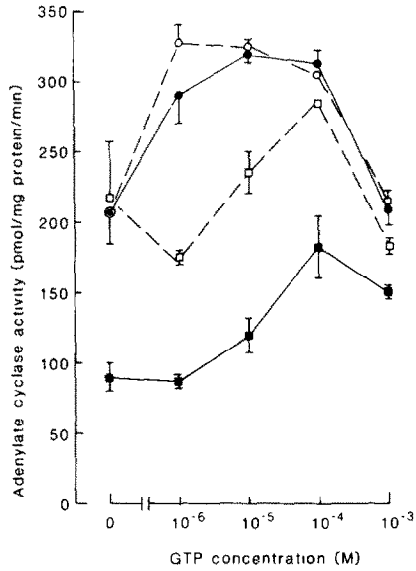

Fig.3. Effect of GDP[βS] and GTP on carbacyclin-stimulated adenylate cyclase activity. Platelet membranes (20 μg protein/assay) were incubated for 10 min at 37°C with carbacyclin (0.1 μM) and GTP at the concentrations indicated. GDP[βS] concentrations were: (○) none; (●) 10 μM; (□) 0.1 M; (■) 1 mM. Basal activity (no additions) was 81.1 ± 5.0 pmol·mg protein^{-1}·min^{-1}. Each point is the mean of 3 separate incubations ± SE.

Platelet membranes were incubated with carbacyclin (0.1 μM) and the amount of added GTP and GDP[βS] systematically varied (fig.3). Whereas the addition of 10 μM GDP[βS] had little effect on adenylate cyclase activity, the addition of 0.1 mM GDP[βS] and 1 mM GDP[βS] progressively inhibited activation.

4. DISCUSSION

The dose-response curves of adenylate cyclase to carbacyclin and to PGE_1 were very similar. There was also a concentration-dependent effect of GTP on the activation of adenylate cyclase by carbacyclin. The role of GTP was further defined using GDP[βS], which resulted in a dose-dependent inhibition of the carbacyclin activation of adenylate cyclase; this was comparable to the inhibitory effect of GDP[βS] on the activation of adenylate cyclase by PGE_1 [6].

Carbacyclin has been shown to be rather more potent (about 2-fold) than PGE_1 in the inhibition of ADP-induced aggregation of human platelets in vitro [4]. However, we here found little difference between the dose-response curves of adenylate cyclase activated by carbacyclin and PGE_1, and the maximum stimulation was the same. It appears that the differential effects of carbacyclin and PGE_1 on inhibition of platelet aggregation are not related to different degrees of activation of adenylate cyclase by these compounds. We can speculate that there is a further point of regulation of cyclic AMP levels, possibly by phosphodiesterase, and that in intact platelets different levels of cyclic AMP may result from stimulation by carbacyclin and PGE_1. This effect has been described in platelets stimulated by PGI_2 and PGE_1, in which PGI_2 had a much higher potency (10-fold) than PGE_1 in inhibition of platelet aggregation, and a more marked difference between the levels of cyclic AMP produced by PGI_2 and PGE_1, but in which the adenylate cyclase activation was the same for both prostaglandins [2].

We conclude that carbacyclin and PGE_1 activate adenylate cyclase to a similar maximal extent in platelet membranes, and that the effect is mediated by GTP.

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REFERENCES

[1] Tateson, J.E., Moncada, S. and Vane, J.R. (1977) Prostaglandins, 13, 389–397.
[2] Gorman, R.R., Bunting, S. and Miller, O.V. (1977) Prostaglandins 13, 377–388.
[3] Whittle, B.J.R., Moncada, S. and Vane, R.R. (1978) Prostaglandins 16, 373–388.
[4] Whittle, B.J.R., Moncada, S., Whiting, F. and Vane, J.R. (1980) Prostaglandins 19, 605–627.
[5] Morton, D.R., Bundy, G.L. and Nishizawa, E.E. (1979) in: Prostacyclin (Vane, J.R. and Bergstrom, S. eds) Raven Press, New York.
[6] Stein, J.M. and Martin, B.R. (1983) Biochem. J. 214, 231–234.
[7] Salomon, Y., Londos, C. and Rodbell, M. (1974) Anal. Biochem. 58, 541–548.
[8] Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275.
[9] Eckstein, F., Cassel, D., Levkovitz, H., Lowe, M. and Selinger, Z. (1979) J. Biol. Chem. 254, 9829–9834.