Vol. 118, No. 2, 1984 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
January 30, 1984 Pages 555-560

THE MODE OF ACTION OF CHLORIDE ON RABBIT HEART ADENYLATE CYCLASE

S.E. Harding, L. Leclerc and P. Harris

Cardiothoracic Institute, University of London
2, Beaumont Street, London, W1N 2DX, UK

Received October 21, 1983

The mechanism by which chloride stimulates adenylate cyclase was investigated. Depletion of GDP increased basal adenylate cyclase activity and reduced the stimulation by isoprenaline. Restoration of bound GDP partially reversed these effects. Chloride stimulated cyclase activity by the same proportion in control, GDP-depleted and GDP-restored preparations, as did Gpp(NH)p. Fluoride increased adenylate cyclase activity to the same final level in both GDP-depleted and GDP-restored membranes; addition of Gpp(NH)p as well as fluoride had no further effect. Solubilisation of adenylate cyclase reduced the stimulatory effect of Gpp(NH)p only slightly, but greatly attenuated the activation by chloride. We conclude that chloride does not stimulate cyclase activity by an action on GDP exchange. Activation by chloride may be due to a disrupting or chaotropic effect on membrane/protein interactions.

In the previous paper it was shown that the adenylate cyclase of rabbit heart sarcolemma was strongly stimulated by KCl, and that the stimulation was due to the chloride anion rather than the cation. The activating effect of KCl was accompanied by a reduction in responsiveness to the beta agonist, isoprenaline. In this paper we investigate the possible mechanisms underlying the action of chloride.

The β-receptor is coupled to the catalytic unit of adenylate cyclase by a guanine-nucleotide binding protein (G-protein) (1, 2). When GTP is bound to this protein it activates the cyclase. The G-protein has an associated GTP-ase activity, which hydrolyses bound GTP to GDP, and hence reduces cyclase activity (3, 4, 5, 6, 7). It is thought that the main effect of β-stimulation is to release GDP from the G-protein and allow further GTP binding (5, 6, 8).

We first investigated whether chloride was enhancing GDP release in our preparation. Such an action at a single site could explain why chloride both stimulated the cyclase and reduced the effect of β-agonists. To do this we compared the effect of chloride on control,

0006-291X/84 $1.50

GDP-depleted and GDP-restored membranes. GDP-depletion was achieved by preincubating the membrane with isoprenaline and GMP, and then washing. This method has been used to release bound GDP from the adenylate cyclase of turkey and pigeon erythrocyte membranes (6, 8, 9, 10, 11). GDP-loading was carried out by incubating GDP-depleted preparations with saturating amounts of GDP, again followed by washing (8).

We also examined the possibility that chloride acts indirectly on the system by altering the interaction of the cyclase proteins with the plasma membrane.

METHODS

L-isoprenaline, GMP, GDP, Gpp(NH)p and Na-cholate were obtained from Sigma. Preparation of the sarcolemma was as described in the previous paper.

Pretreatment of membrane for removal and restoration of GDP (8): Approximately 9 mg sarcolemmal protein in TD buffer (10 mM Tris acetate, 2 mM dithiothreitol, pH 7.5) was thawed from -80°C. One-third was set aside as a control; the remainder was made up with 50 μM isoprenaline, 1 mM ascorbate and 1 mM GMP. Control and treated membranes were incubated at 37°C for 30 min, then washed by dilution in TD buffer and centrifugation at 43,000 g for 10 min at 4°C. Pellets were resuspended in TD. Treated membranes were divided in two, and half made up with 10-4M GDP. All membranes were left at room temperature for 5 min, then washed as before. Pellets were resuspended in TD buffer.

Solubilisation of adenylate cyclase (12): Membranes were thawed from -80°C, centrifuged at 10,000 g for 5 min and resuspended in cholate buffer [0.1 M HEPES, 1 mM EDTA, 1 mM dithiothreitol, 2 mM $MgCl_2$, 0.6 M $(NH_4)_2SO_4$, 15 mM Na-cholate (pH 8.0)] at a concentration of 5 mg/ml protein. After stirring for 1 h on ice, the mixture was centrifuged at 200,000 g for 1 h and the supernatant diluted x15 with assay medium. Adenylate cyclase was assayed as described in the previous paper, except that creatine phosphate and creatine kinase were omitted from the medium, and that the assay time was 10 min. Omission of the regenerating system did not affect the linearity of cAMP production with time up to 10 min. 200 μl was removed into the acetate stopping buffer, and cAMP was measured as before. Protein was measured using Coomaisie blue (13). Results are the mean ± S.E.M. of 4 experiments.

RESULTS

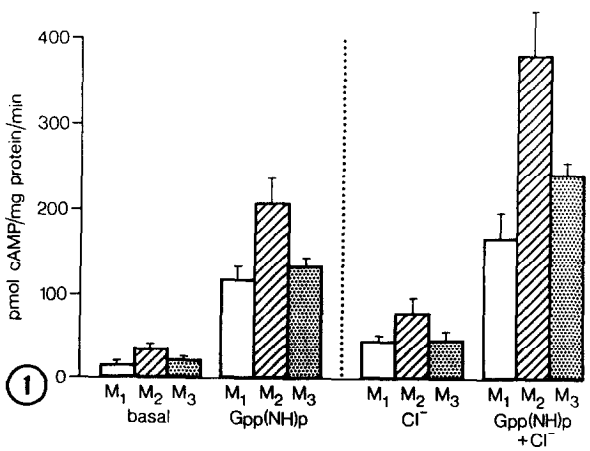
Chloride and Gpp(NH)p on GDP-depleted and GDP-restored membranes (Fig. 1). Basal activity of isoprenaline+GMP treated (GDP-depleted) membranes was significantly higher than that of control ($p > 0.05$). In GDP-restored membranes, activity was lower than in GDP-depleted ($p > 0.05$) and slightly, but not significantly, higher than in control. Gpp(NH)p caused substantial activation in all membranes, and the percentage increase was similar in each of the three. Chloride also stimulated all three membranes,

and, as with Gpp(NH)p, the proportional increase was similar in each case. The effects of Gpp(NH)p and chloride were more than additive.

Isoprenaline on GDP-depleted and GDP-restored membranes (Fig.2). Stimulation by isoprenaline was reduced in GDP-depleted membranes compared with control. The increase was reduced from 156 ± 57 pmols/mg protein/min (248% above basal) to 70 ± 16 pmols/mg/min (36% above basal). Replacement of GDP partially restored stimulation by isoprenaline to 110 ± 30 pmols/mg/min (101% above basal).

Fluoride and Gpp(NH)p on GDP-depleted and GDP-restored membranes (Fig.3). As before, basal and Gpp(NH)p-stimulated activities were lowest in control and highest in GDP-depleted membranes. However, with fluoride, activities were similar in all three membranes. Control membranes were the lowest, but an analysis of variance failed to show a significant difference. Addition of Gpp(NH)p as well as fluoride had little further effect on the treated membranes, but increased control activity to the same final level as in the treated.

Solubilised adenylate cyclase plus KCl and Gpp(NH)p (Fig.4). Basal activity was reduced by solubilisation from 70 ± 12 to 33 ± 3 pmols/min/mg protein. The stimulatory effect of Gpp(NH)p was reduced from 304% in the normal to 218% in the solubilised



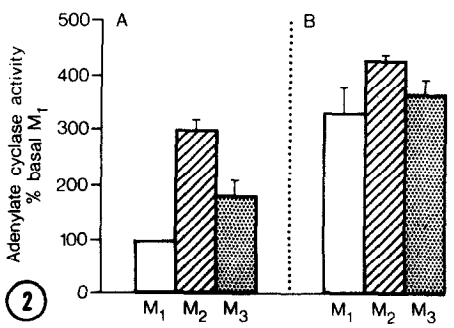


Figure 1. The effect of 0.1 mM Gpp(NH)p and 100 mM chloride on control (M1), GDP-depleted (M2), and GDP-restored (M3), membranes.

Figure 2. Control (M1), GDP-depleted (M2), and GDP-restored (M3), membranes with (B) and without (A) 0.1 mM isoprenaline.

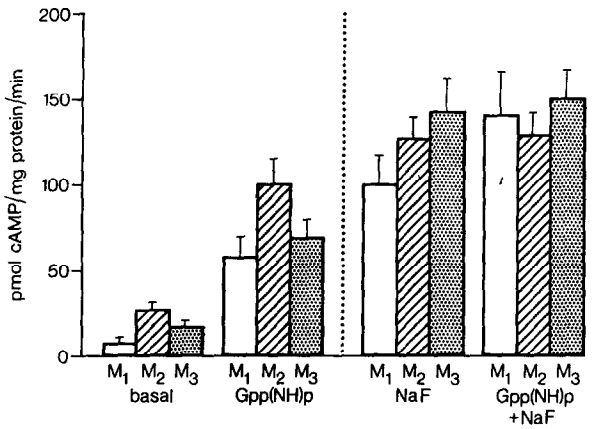


Figure 3. Effect of 0.1 mM Gpp(NH)p and 5 mM NaF on control (M1), GDP-depleted (M2), and GDP-restored (M3), membranes.

preparation. The effect of KCl was greatly attenuated by solubilisation, falling from 300% of basal to 125%. The more-than additive effects of chloride and Gpp(NH)p were still evident.

DISCUSSION

Membranes which had been treated to remove GDP showed a higher basal activity than controls. Activity could be returned towards control levels by a second treatment to restore bound GDP. Similar results have been found in pigeon and turkey erythrocytes (9, 10). This suggests that the GDP-free G-protein can support greater adenylate cyclase activity

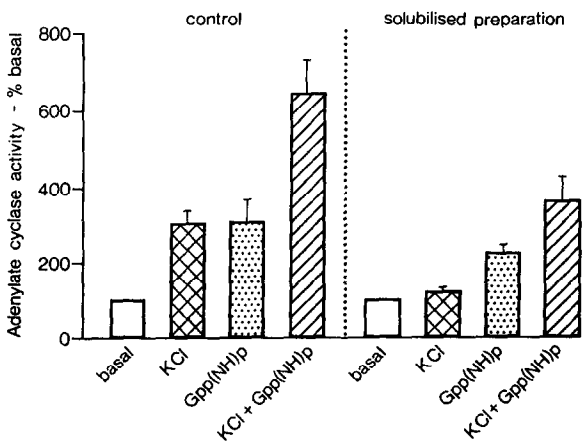


Figure 4. 100 mM KCl and 0.1 mM Gpp(NH)p on control and solubilised adenylate cyclase. Expressed as a percentage of basal activity of control or solubilised preparations.

than the GDP-bound species, and that GTP binding to the G-protein is not an absolute requirement for cyclase activation in the heart. This view is in agreement with the model of cyclase activation proposed by Iyengar and others (14) and explains why hormones activate the cyclase in the absence of GTP in various systems (9, 15, 16, 17, 18). Isoprenaline stimulation was reduced in the GDP-depleted preparation, a result consistent with the hypothesis that β-receptor agonists have their effect by releasing GDP from the G-protein. Re-addition of bound GDP partially restored isoprenaline stimulation.

The stimulation caused by the treatment to remove GDP was multiplicative with that due to Gpp(NH)p. This would be consistent with a preferential binding of Gpp(NH)p to the GDP-free G-protein. However, there was stimulation of basal activity by Gpp(NH)p, which implies that a proportion of the G-protein is not bound to GDP in the basal state. Fluoride was able to stimulate the cyclase to the same final level in GDP-depleted and GDP-restored membranes. Similar findings were obtained in the turkey erythrocyte (8). Addition of Gpp(NH)p to these fluoride-stimulated preparations did not produce any further activation. Fluoride-stimulated activity was lower in control membranes than in GDP-depleted or restored ones, and addition of Gpp(NH)p produced a slight stimulation, bringing control activity into line with that of the other two. This suggests that Gpp(NH)p and fluoride produce the same conformational change, but that, unlike Gpp(NH)p, fluoride can stimulate whether or not the G-protein is bound to GDP.

Like Gpp(NH)p, chloride gave a similar percentage stimulation in control, GDP-depleted or GDP-restored membranes. It is, therefore, unlikely that chloride acts by releasing GDP from the G-protein. These results are in agreement with those of Tkachuk et al. (19) who showed that excess GDP abolished stimulation by isoprenaline, but enhanced that by chloride.

Solubilisation of adenylate cyclase, however, reduced activation by KCl considerably, while retaining a major part of Gpp(NH)p stimulation. Chloride activation, therefore, requires that adenylate cyclase is embedded in the plasma membrane, and so

may be due to a chaotropic effect on the protein or surrounding lipids. This property of anions has been put forward as an explanation for their stimulatory effect on the cyclases of pig kidney medulla and rat liver (20, 21).

ACKNOWLEDGEMENTS

This work was supported by the Medical Research Council.

REFERENCES

1. Limbird, L.E. (1981) Biochem. J. 195, 1-13.
2. Swillens, S. and Dumont, J.E. (1980) Life Sci. 27, 1013-1028.
3. Cassel, D. and Selinger, Z. (1976) Biochim. Biophys. Acta 452, 538-551.
4. Cassel, D. and Selinger, Z. (1977) Biochem. Biophys. Res. Commun. 77, 868-873.
5. Cassel, D. and Selinger, Z. (1978) Proc. Natl. Acad. Sci. 75, 4155-4159.
6. Pike, L.J. and Lefkowitz, R.J. (1981) J. Biol. Chem. 256, 2207-2212.
7. Salomon, Y. and Rodbell, M. (1975) J. Biol. Chem. 250, 7245-7250.
8. Lad, P.M., Nielsen, T.B., Preston, M.S. and Rodbell, M. (1980) J. Biol. Chem. 255, 988-995.
9. Abramowitz, J., Iyengar, R. and Birnbaumer, L. (1980) J. Biol. Chem. 255, 8259-8265.
10. Pfeuffer, T. and Helmreich, E.J.M. (1975) J. Biol. Chem. 250, 867-876.
11. Swillens, S., Juvent, M. and Dumont, J.E. (1979) FEBS Letts. 108, 365-368.
12. Ross, E.M. (1981) J. Biol. Chem. 256, 1949-1953.
13. Bradford, M.M. (1976) Anal. Biochem. 72, 248-254.
14. Iyengar, R., Abramowitz, J., Bordelon-Riser, M., Blume, A.J. and Birnbaumer, L. (1980) J. Biol. Chem. 255, 10312-10321.
15. Fricke, R.F., Queener, S.F. and Clark, C.M.Jr. (1980) J. Mol. Cell. Cardiol. 12, 595-608.
16. Lyndon, N.B., Young, J.L. and Stansfield, D.A. (1981) Biochem. J. 198, 631-638.
17. Snyder, F.F. and Carter, R.J. (1979) Biochem. J. 183, 589-594.
18. Steer, M.L. and Wood, A. (1981) J. Biol. Chem. 256, 9990-9993.
19. Tkachuk, V.A., Avdonin, P.V. and Panchenko, M.P. (1981) Biokhimiya 46, 333-341.
20. Roy, C., Le Bars, N-C. and Jard, S. (1977) Eur. J. Biochem. 78, 325-332.
21. Johnson, R.A., Pilkis, G.S. and Hamet, P. (1975) J. Biol. Chem. 250, 6599-6607.