Vol. 118, No. 2, 1984 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
January 30, 1984 Pages 561-566

THE EFFECT OF ANIONS AND CATIONS ON CARDIAC ADENYLATE CYCLASE: INTERACTIONS WITH ISOPRENALINE AND GTP

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

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

Received October 21, 1983

Anions stimulated rabbit heart adenylate cyclase in the order chloride > phosphate > acetate, while the cations sodium and potassium had little effect. Activation by KCl was multiplicative with that of fluoride. Isoprenaline (10^{-4}M) and GTP(10^{-4}M) stimulated the cyclase independently, and their combined effects were multiplicative. GTP stimulated less than chloride but more than acetate. Activation by chloride or acetate was more than additive with that by GTP. The effect of isoprenaline was reduced in the presence of chloride or acetate, and this reduction was not modified by sodium or potassium. It was concluded that ionic influence may be biologically important in modifying cyclase activity and the extent of beta-receptor stimulation.

It is known that the activity of adenylate cyclase can be modified by the anionic composition of the assay medium. In addition to the specific activator, fluoride, stimulation has been obtained with chloride, thiocyanate, azide and sulphate (1, 2, 3, 4, 5). These effects vary with both species and tissue. The extent of both hormonal and guanine nucleotide activation may also depend on the anions present in the assay. The nature of the effect appears to be different for different hormones (1, 3, 6). This paper describes the effects of chloride, acetate, phosphate, sodium and potassium ions on basal and GTP- and isoprenaline-stimulated adenylate cyclase activity in rabbit heart, and shows the importance of anionic composition for detection of β-receptor effects.

METHODS

A partially purified heart sarcolemmal preparation was prepared from male New Zealand white rabbits (7). The preparation was kept at -80°C for up to 8 weeks without loss of cyclase activity. Adenylate cyclase was assayed for 20 min in a Tris-acetate buffer (25mM, pH 7.5) containing 0.72mM dithiothreitol, 0.32mM EDTA, 15mM Mg-acetate, 10mM theophylline, 5mM Tris-ATP, 5mM Tris creatine phosphate and 2mg.ml creatine kinase. Sarcolemmal protein concentration was in the range 0.05 - 0.2mg/ml. The concentration of Mg^{2+} was chosen to give maximal activity of the enzyme. With low concentrations of Mg^{2+}, ATP became inhibitory above 1mM, but at 15mM Mg^{2+} the activity was equal at ATP concentrations between 0.5 and 5mM. From these and other preliminary studies it was concluded that, under the assay conditions employed here, the

0006-291X/84 $1.50

enzyme was at maximal velocity with respect to ATP. Production of cAMP was linear with a protein concentration up to 0.2 mg.ml and an incubation period up to 30 min. In the presence of isoprenaline, 1 mM ascorbate was added. A preincubation period of 30 min was allowed for GTP. At the end of the assay, 50 µl of the reaction mixture was added to 500 µl acetate buffer (0.1M, pH 4.0) to stop the reaction. After neutralisation with 3M Tris, 2 x 50 µl aliquots were taken for cAMP measurement with the Amersham International assay kit. Protein was measured using Coomaisie blue (8). Adenylate cyclase activity is expressed as a percentage of basal, which was in the region of 130 pmoles/min/mg protein. Results shown are the mean ± S.E.M. of 4-6 experiments, each done in quadruplicate. At least 3 separate membrane preparations were used in each series.

RESULTS

KCl, NaCl, Na-acetate and sucrose (Fig.1)

The addition of KCl, NaCl or Na-acetate at a concentration of 100 mM increased adenylate cyclase activity. The activation was not due simply to osmolarity since 100 mM sucrose did not have the same effect. Stimulation was less with Na-acetate than with NaCl, indicating that the effect of acetate was weaker than that of chloride. Comparison between the two chloride salts showed an equal effect of sodium and potassium.

K^+, Cl- and acetate (Fig.2)

In this group of experiments the effects of 100 mM KCl and K-acetate were compared with those of 100 mM hydrochloric and acetic acids neutralised with Tris. The results show that K^+ has no more stimulatory effect than the Tris used to neutralise the acids. The major

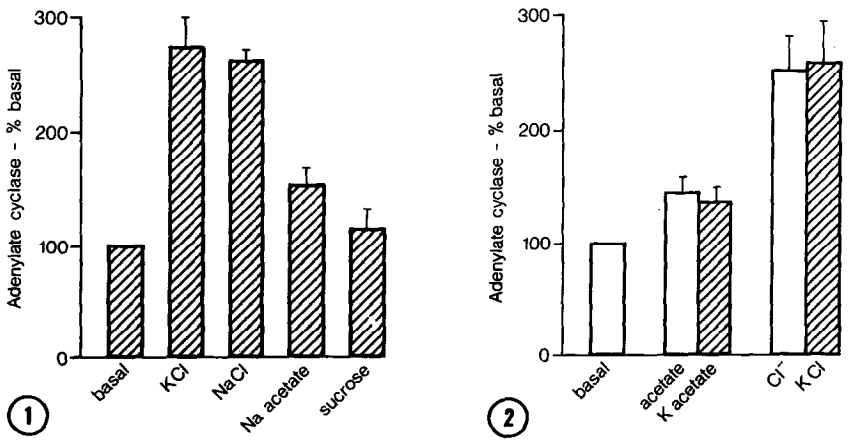
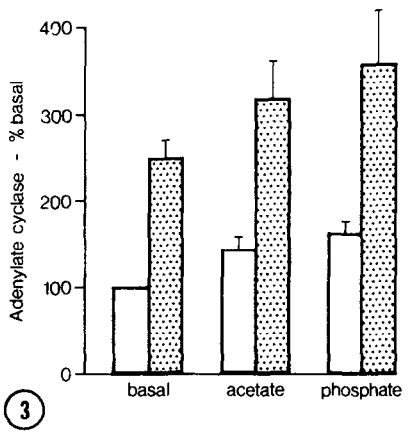


Figure 1. The effect of KCl, NaCl, Na-acetate and sucrose (all at 100 mM) on unstimulated adenylate cyclase activity. n = 5, except Na-acetate where n = 4.

Figure 2. Comparison of the effect of 100 mM KCl and K-acetate with 100 mM hydrochloric and acetic acids buffered with Tris. n = 6.



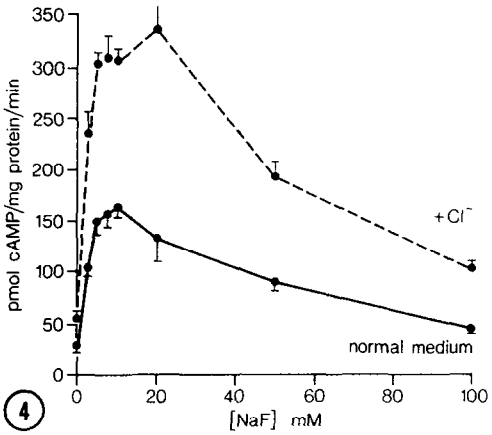


Figure 3. 100mM acetic and orthophosphoric acids buffered with Tris on unstimulated adenylate cyclase activity (open bars), and activity in the presence of 0.1 mM GTP (stippled bars). n = 4.

Figure 4. Effect of NaF on cyclase activity in the presence (dashed line), and absence (unbroken line), of 100mM KCl. n = 3.

part of the increase in adenylate cyclase activity is, therefore, due to the anionic portion of the salt. Since, in the previous group of experiments, the effects of NaCl and KCl were the same, it can also be concluded that Na has little direct effect on cyclase activity at these concentrations.

Acetate and phosphate in the absence and presence of GTP (Fig. 3)

Phosphate stimulated to a greater extent than acetate. GTP caused a large increase in activity. The activity in the presence of both GTP and acetate was more than would be expected by a simple addition of their effects. Similarly the effects of both GTP and phosphate were more than additive.

KCl plus NaF (Fig. 4)

The dose/response curve to NaF was similar to that found by other workers with stimulation reaching a maximum around 5-20mM and declining at higher concentrations (9). In contrast the response to chloride approached an asymptote above 100mM (results not shown). The stimulatory effects of 100mM KCl and NaF were multiplicative at all concentrations of fluoride tested.

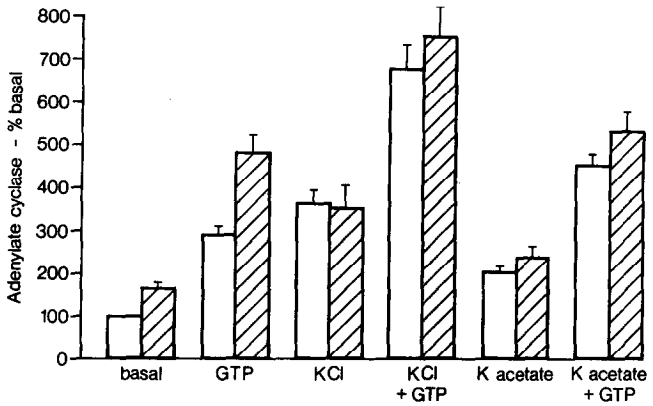


Figure 5. Effect of 0.1 mM GTP, 100 mM KCl and 100 mM K-acetate on unstimulated cyclase activity (open bars), and activity in the presence of 0.1 mM isoprenaline (hatched bars). n = 6.

KCl and K-acetate plus isoprenaline and GTP (Fig. 5)

In the absence of GTP and isoprenaline, the stimulatory effect of chloride was greater than that of acetate, as before. GTP produced a 2.5-3 fold increase in adenylate cyclase activity, an effect rather less than that of 100 mM K-chloride, but greater than that of 100 mM K-acetate. The effects of GTP and KCl or K-acetate were more than additive.

L-isoprenaline caused a 65% increase in cyclase activity both in the absence and presence of GTP, when neither KCl nor K-acetate had been added. Thus the stimulatory effects of GTP and isoprenaline were multiplicative. However, the effect of isoprenaline was reduced or absent when either 100 mM KCl or 100 mM K-acetate were in the assay medium. This reduction in β-receptor activation by the salts was also evident when GTP was present. Further experiments showed that the effect of isoprenaline was the same in 100 mM KCl, 100 mM NaCl and/or in 100 mM HCl buffered with Tris.

DISCUSSION

The present studies confirm the important influence that the ionic composition of the assay medium has on the membrane-bound adenylate cyclase system, the stimulatory effect of chloride being greater than that of GTP. The lack of effect of isoosmolar sucrose shows that the salts were not acting simply by changes in osmolarity. While it is impossible to

rule out some influence of total ionic strength, the large variation in the action of salts of the same molarity indicates that a major part of the effect was due to the specific ion.

By comparing salts of the same cation, the stimulatory order of potency for anions was found to be chloride > phosphate > acetate. Roy and his colleagues also found a higher stimulation by chloride than by acetate on a preparation from the kidney (1).

At the concentration used in these experiments the effects of Na and K were indistinguishable, and 100 mM KCl had the same effect as 100 mM HCl buffered with Tris. It is probable that a major part of the stimulatory action of the salts was due to the anionic moiety. The equivalence of Na and K was also found by Tada (10) up to a concentration of 200 mM, and Katz and his colleagues (2) showed that the stimulatory effects of NaCl and KCl were the same up to a concentration of 400 mM. It is clear that the stimulation by chloride is not due to an action at the fluoride site, since the effects of KCl and NaF are multiplicative.

The increase in activity with GTP was more than additive with that produced by the chloride, acetate or phosphate salts. This suggests that the nucleotide and the anions act at different sites which are located in sequence. The percentage increases in activity caused by isoprenaline was the same on basal and GTP-stimulated activity. Thus the absolute effect of β-agonist was greater in the presence of GTP. Such an amplification is consistent with separate and sequential loci of action of hormone and guanine nucleotide.

The influence of salts on the effect of isoprenaline was unexpected. Chloride and acetate reduced or abolished β-stimulation. These results agree with those of Tkachuk et al. (11), who showed that in rabbit heart membrane preparations both KCl and K-acetate reduced activation by isoprenaline. Katz et al. (12) similarly found that when $MgSO_4$, NaCl, $MgCl_2$ or Na_2SO_4 were present, no further activation of the adenylate cyclase of fat cell ghosts occurred with 10^{-4}M adrenaline.

The plasma concentration of chloride is 115 mM, while that inside the cell is 20 - 30 mM (13). We have shown that variations in chloride concentration within this physiological

range can affect adenylate cyclase activity considerably, and that the stimulatory action of 100mM chloride is greater than the maximum stimulatory action of GTP. In addition, chloride ions can reduce the degree of activation of the cyclase by isoprenaline. These observations raise the possibility that chloride movements could play a role in cyclase regulation in the living cell.

ACKNOWLEDGEMENTS

This work was supported by the Medical Research Council.

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