THE MONOVALENT ANIONS CHLORIDE AND AZIDE AS POTENT ACTIVATORS OF NaF- AND p(NH)ppG-STIMULATION OF PANCREATIC ADENYLATE CYCLASE

Michal SVOBODA and Jean CHRISTOPHE

Department of Biochemistry and Nutrition, Medical School, Université libre de Bruxelles, Bld. Waterloo 115, 1000 Brussels, Belgium

Received 20 December 1977

1. Introduction

The activation of adenylate cyclase by monovalent anions other than fluoride was first reported in 1974 [1,2] and further documented recently [3,4] using liver and kidney-medulla plasma membranes. Variations in techniques and in the magnitude of the effects warrant additional studies on other adenylate cyclase systems. In the present study NaCl and NaN_3 used at maximally effective concentration were found to activate the adenylate cyclase of rat pancreatic plasma membranes 1.3–2.0-fold under basal conditions, and under stimulation by secretin, the C-terminal octapeptide of cholecystokinin-pancreozymin (OC-PZ) and GTP, or by each peptide offered in combination with GTP or p(NH)ppG. Stimulation of adenylate cyclase by maximally effective concentrations of p(NH)ppG alone or by NaF was enhanced by NaCl or NaN_3 to a greater degree than was observed in the above-mentioned cases. The 5.1-fold anionic activation of p(NH)ppG-stimulated adenylate cyclase was due to an increase in V_{max}. The 3.4-fold anionic activation of NaF stimulation resulted from an increase in app. V_{max} and a lower app. K_m. It is concluded that the amplitude of the activating effects of the monovalent anions Cl^- and N_3^- are highly dependent on the configuration of the adenylate cyclase system.

2. Materials and methods

Rat pancreatic plasma membranes were prepared as described [5] and stored under nitrogen in a medium containing inter alia 20 mM Tris–Cl buffer (pH 7.4). Prior to use, the membrane material was thawed and freed of chloride by two alternate centrifugations (5 min, 50 000 × *g*) and suspensionded in a 20 mM Na glycyl-glycine buffer (pH 7.4).

Adenylate cyclase activity was determined at 37°C in a medium containing 20 mM Na glycyl-glycine, 5 mM $(CH_3COO)_2$ Mg, 0.5 mM EGTA, 1 mM theophylline, 1 mM cyclic AMP, 0.5 mM [α-^{32}P]ATP (1 μCi/assay), and an ATP-regenerating system comprised of 10 mM phospho(enol)pyruvate and 30 μg/ml pyruvate kianse. The pH was adjusted to 7.4 at 25°C. The reaction was initiated by the addition of plasma membranes and the assay was conducted in 0.06 ml final vol. The reaction was stopped by adding 0.5 ml 0.1% sodium dodecylsulfate solution containing 1 mM ATP and 0.5 mM cyclic [8-^{3}H]AMP (20 000 cpm/assay in order to determine the recovery of cyclic AMP). The purification of the cyclic AMP formed was achieved by sequential chromatography on columns of Dowex cation-exchange resin and aluminium oxide [6].

3. Results

In line with [5,7], rat pancreatic adenylate cyclase was stimulated by GTP, p(NH)ppG, NaF, secretin and the C-terminal octapeptide of pancreozymin (OC-PZ). The effect of each peptide was markedly enhanced by both guanyl nucleotides (table 1). A medium enriched with 100 mM NaCl induced a significant but modest 1.3–2.0-fold activation in basal activity and in the GTP- or peptide-stimulated cyclase activity. A

Table 1
Activating effects of 100 mM NaCl on basal and stimulated adenylate cyclase activity
(pmol cyclic AMP formed · min^{-1} · mg $protein^{-1}$)

Additions	No NaCl	NaCl (100 mM)	Rel. act.
Basal (6)	7 ± 1	14 ± 2	2.00
10 μM GTP (6)	25 ± 5	44 ± 4	1.76
10 μM p(NH)ppG (9)	49 ± 4	251 ± 8	5.12[a]
10 mM NaF (7)	155 ± 25	524 ± 53	3.38[a]
1 μM secretin (4)	18 ± 4	34 ± 1	1.89
1 μM OC-PZ (4)	79 ± 14	133 ± 10	1.68
1 μM secretin and 10 μM GTP (3)	354 ± 27	457 ± 42	1.29
1 μM secretin and 10 μM p(NH)ppG (3)	342 ± 27	547 ± 44	1.60
1 μM OC-PZ and 10 μM GTP (5)	395 ± 21	564 ± 20	1.43
1 μM OC-PZ and 10 μM p(NH)ppG (3)	394 ± 17	634 ± 19	1.61

[a] Activating effects significantly higher ($p < 0.02$) than that exerted by NaCl on basal activity

Rat pancreatic plasma membranes were incubated for 7 min at 37°C in the adenylate cyclase medium described in section 2. The results are the means ± SEM of 3–9 experiments performed in duplicate (no. expts in parentheses)

similar activation by NaCl was observed when guanyl nucleotides and peptides were used in combination. The activating effects of NaCl on p(NH)ppG- and NaF-stimulation were much greater than those observed above (a 5.1-fold and 3.4-fold elevation, respectively). Thus, the effects of NaCl permitted to discriminate two groups of stimulatory conditions (table 1). In other experiments (not shown), the activating effects of NaN_3, another form of monovalent anion, were comparable to those of NaCl, i.e., significantly greater in the presence of p(NH)ppG alone or NaF than under the other conditions tested.

Dose–effect curves of NaCl and NaN_3 on p(NH)ppG- (fig.1A) and NaF- (fig.1B) stimulated

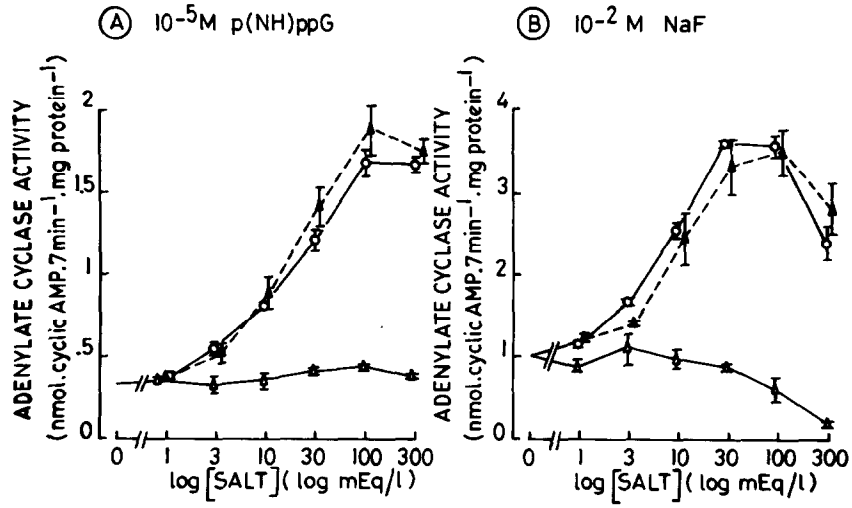

Fig.1. Dose response curves of adenylate cyclase activation by NaCl (–○–), NaN_3 (–▲–) and Na_2SO_4 (–△–) in the presence of 10 μM p(NH)ppG (A) or 10 mM NaF (B). Rat pancreatic plasma membranes were incubated for 7 min at 37°C. Results are the means ± SEM of 3 experiments performed in duplicate.

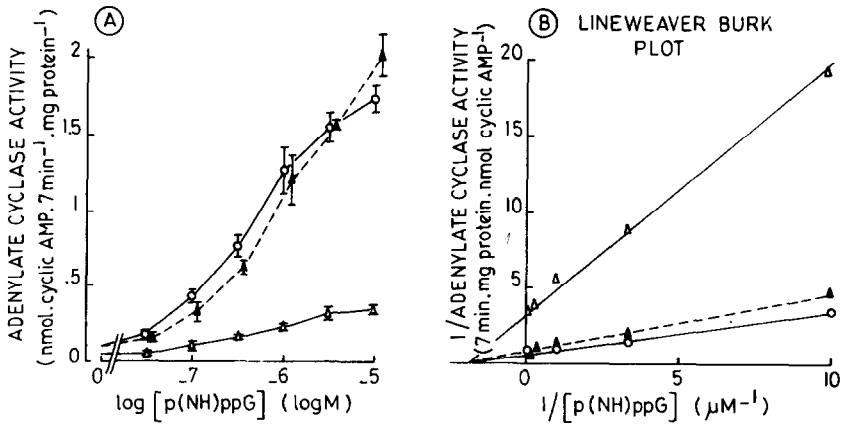

Fig.2. (A) Dose response curves of adenylate cyclase stimulation by p(NH)ppG without (–△–) or with 0.1 M NaCl (–○–) or 0.1 M NaN_3 (–▲–). Membranes were incubated for 7 min at 37°C. Results are the means ± SEM of 3 experiments performed in duplicate. (B) Same results presented as Lineweaver-Burk plots, after correction for basal activity and lag period.

adenylate cyclase activity were compared to that of sodium sulfate to take into account ionic strength and Na^+ concentration. The action of NaCl and NaN_3 could be detected at a 3 mM concentration and was half-maximal for both monovalent anions at identical concentrations (20 mM for p(NH)ppG-stimulation and 10 mM for NaF-stimulation). By contrast, sodium sulfate exerted negligible activating effect on p(NH)ppG-stimulated adenylate cyclase, and was even inhibitory above 30 mM on the NaF-stimulated enzyme. Both NaCl and NaN_3 increased the app. V_{max} of p(NH)ppG-stimulated adenylate cyclase without modifying the app. K_m (fig.2). For NaF-stimulated adenylate cyclase (fig.3A) the effects of NaCl and

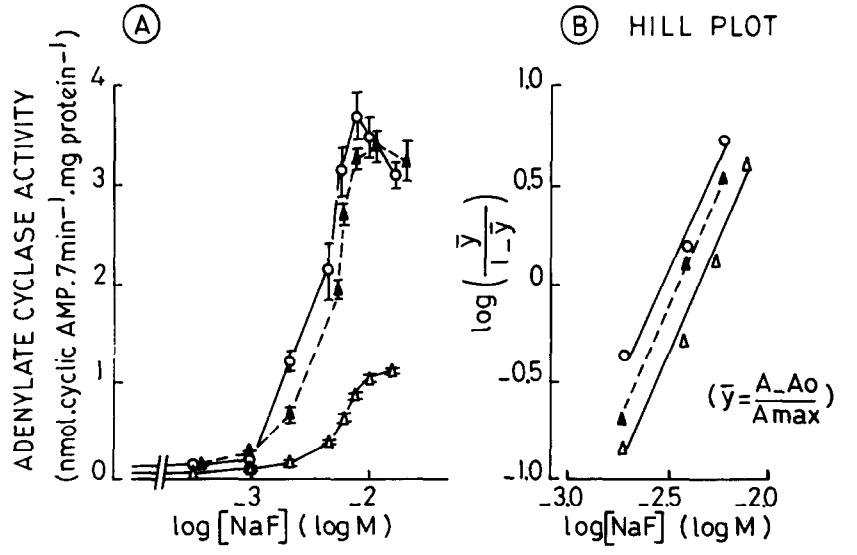

Fig.3. (A) Dose response curves of adenylate cyclase stimulation by NaF without (–△–) or with 0.03 M NaCl (–○–) or 0.03 M NaN_3 (–▲–). Membranes were incubated for 7 min at 37°C. Results are the means ± SEM of 3 experiments performed in duplicate. (B) Same results presented as Hill plots, after correction for basal activity A_0.

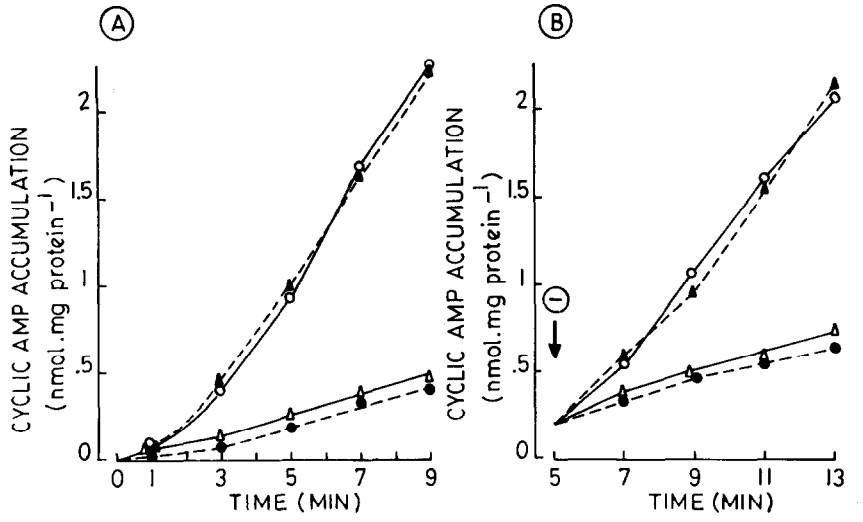

Fig.4. Time course of the activating effects of three monovalent anions on the stimulation of adenylate cyclase by p(NH)ppG. All incubations were performed in the standard assay medium enriched with 10 μM p(NH)ppG, and without (–●–) or with 0.1 M NaCl (–○–), 0.1 M NaN_3 (–▲–), or 0.1 N Na_2SO_4 (–△–). In (A) the anions and p(NH)ppG were added simultaneously at time 0. In (B) p(NH)ppG was added at time 0 and the anions (–) at time 5 min (↓). Results from 2 experiments.

NaN_3, when tested at an optimal 30 mM concentration (fig.1B), consisted in an increase in app.V_{max} and a decrease in app. K_m. A Hill plot (fig.3B) illustrates this decrease in K_m, and shows the preservation under NaCl and NaN_3 of the striking positive cooperative effect exerted by NaF on adenylate cyclase stimulation (Hill coefficient 2.4).

The time course of adenylate cyclase stimulation showed (fig.4) that the 2 min lag period observed with p(NH)ppG was only slightly reduced by the anions. On the other hand, NaCl and NaN_3 caused an immediate response when added to the fully p(NH)ppG-stimulated adenylate cyclase.

4. Discussion

The 1.3–2.0-fold maximal activation of adenylate cyclase observed with NaCl and NaN_3 under several states including basal state confirmed a general effect of Cl^- and N_3^- but not of SO_4^{2-} [1–4]. The concentrations of active anions found to be effective were lower than those used in [4] and were similar to those used in [3]. This discrepancy might be due to the absence of Tris–HCl in our medium rather than to tissue differences [4]. The mechanism of activation might be the result of changes in the catalytic unit and/or the membrane fluidity (in such a way as to increase the accessibility of the substrate). Alterations in the stability constant of the ionic species [8] might also influence the rate of catalysis [4,8]. Conceivably, among the numerous cations and anions of the adenylate cyclase medium (H^+, Na^+, Mg^{2+}, NH_4^+, theophylline$^+$, ATP^{4-}, cyclic AMP^-, CH_3-COO^-, SO_4^{2-}, phospho(enol)pyruvate^{3-}, $EGTA^{4-}$, glycyl-glycine$^-$ and ionic species resulting from their combination), Cl^- and N_3^- might modify the stability and relative proportions of free Mg^{2+}, free ATP, or the ATP–Mg complex, all components known to influence the activity of adenylate cyclase [9,10].

Our major finding was the capacity of NaCl and NaN_3 to exert specifically high activating effects on p(NH)ppG- and NaF-stimulated adenylate cyclase. The absence of effects of these anions on the rate of stimulation of adenylate cyclase by p(NH)ppG and the immediate nature of their activating effect on the enzyme fully stimulated by p(NH)ppG suggest that anionic effects were possible only after the cyclase system was in that specific configuration induced by the stable nucleotide. This configuration was clearly distinct from that elicited by a combination of p(NH)ppG and hormone peptide, for it was

characterized by a much lower anionic sensitivity.

NaCl and NaN_3 activated the NaF-stimulation of adenylate cyclase by another mechanism including an increase in app. V_{max}, a decrease in app. K_m, and no modification of the positive cooperativity of adenylate cyclase stimulation by NaF. This resulted perhaps from an increased concentration in F^-, the apparently active ionic form required in fluoride stimulation [11,12]. Indeed in the absence of NaCl and NaN_3 and in the presence of NaF, Mg^{2+} deriving from 5 mM $(CH_3COO)_2$ Mg might favor the formation of MgF^+ and MgF_2 at the expense of free F^- and free Mg^{2+} [13]. Such variations in ionic concentrations might control the apparent positive cooperativity developing during stimulation of pancreatic adenylate cyclase with increasing concentration of NaF^+ (fig.3B). The monovalent anion perchlorate reduces the stability constant of MgF [8] and it is likely that Cl^- and N_3^- might possess similar properties. If so, the decrease in app. K_m of NaF activation by monovalent anions might well result from an increase in the F^- : total fluoride ratio, itself secondary to a reduction of the MgF^+ stability constant. In line with the preceding considerations, the increase in app. V_{max} of NaF-stimulation could be explained by a modification of the interaction of NaF with the enzyme.

In conclusion, the present data suggest that the activation of adenylate cyclase by the monovalent anions Cl^- and N_3^- depends on the conformation of the system and might therefore serve as a probe for changes in conformation.

Acknowledgements

We thank Professor V. Mutt (Karolinska Institutet, Stockholm) for the generous gift of natural porcine secretin and M. Ondetti (Squibb Institute for Medical Research, NJ) for his valuable gift of OC-PZ. This work was supported by Grant RO-IAM-17010 from the National Institutes of Health (USA) and by Grant No. 20 403 from the Belgian Medical Scientific Research Fund.

References

[1] Kalish, M. I., Pineyro, M. A., Cooper, B. and Gregerman, R. I. (1974) Biochem. Biophys. Res. Commun. 61, 781–787.
[2] Rahmanian, M. and Jarett, L. (1974) Biochem. Biophys. Res. Commun. 61, 1051–1056.
[3] Johnson, R. A., Pilkis, S. J. and Hamet, P. (1975) J. Biol. Chem. 250, 6599–6607.
[4] Roy, C., Le Bars, N. C. and Jard, S. (1977) Eur. J. Biochem. 78, 325–332.
[5] Svoboda, M., Robberecht, P., Camus, J., Deschodt-Lanckman, M. and Christophe, J. (1976) Eur. J. Biochem. 69, 185–193.
[6] Salomon, Y., Londos, C. and Rodbell, M. (1974) Anal. Biochem. 58, 541–548.
[7] Svoboda, M., Robberecht, P., Camus, J., Deschodt-Lanckman, M. and Christophe, J. (1978) Eur. J. Biochem. in press.
[8] Sillen, L. G. and Martell, A. E. (1964) Stability constants of metal-ion complexes, The Chemical Society, London.
[9] Garbers, D. L. and Johnson, R. A. (1975) J. Biol. Chem. 250, 8449–8456.
[10] Londos, C. and Preston, M. S. (1977) J. Biol. Chem. 252, 5957–5961.
[11] Drummond, G. I. and Duncan, L. (1970) J. Biol. Chem. 245, 976–983.
[12] Perkins, J. P. (1973) in: Advances in Cyclic Nucleotide Research (Greengard, P. and Robinson, G. A. eds) Vol. 3, pp. 1–64, Raven Press, New York.
[13] Maguire, M. E., Sturgill, T. W. and Gilman, A. G. (1975) Metabolism 24, 287–299.