Volume 295, number 1,2,3, 89–92 FEBS 10513 December 1991

PGE_2 regulates cholecystokinin-octapeptide (CCK-8)-stimulated Cl^- conductance in isolated zymogen granules from rat pancreas

Thorsten Plusczyk, Albrecht Piiper and Irene Schulz

Max-Planck-Institut für Biophysik, Kennedyallee 70, 6000 Frankfurt am Main 70, Germany

Received 21 October 1991

In this study we have examined the effects of prostaglandin E_2 (PGE_2), the cyclooxygenase inhibitor, indomethacin, and a protein kinase A inhibitor (PKA-I) on the Cl^- conductance in isolated zymogen granules (ZG) from cholecystokinin octapeptide (CCK-8) pre-stimulated pancreatic acini. The Cl^- conductance in isolated ZG from CCK-8 pre-stimulated rat pancreatic acini increases with increasing CCK-8 concentrations and decreases at supramaximal CCK-8 concentrations. The basal and CCK-8-stimulated Cl^- conductance in ZG is inhibited by pretreatment of acini with PGE_2 (10^{-6} M). This PGE_2-induced inhibition is abolished in the presence of PKA-I (20 U/ml). Furthermore, pretreatment of acini with indomethacin (10^{-5} M) or PKA-I (20 U/ml) abolishes the decrease in the Cl^- conductance at supramaximal CCK-8 concentrations (10^{-9} M). We conclude that the inhibition of the Cl^- conductance in isolated ZG at high CCK-8 concentrations is mediated by an enhanced production of PGE_2, and that PGE_2 operates by stimulating adenylate cyclase (AC) with a consequent rise in cAMP and activation of PKA.

Signal transduction; Indomethacin; Protein kinase A inhibitor

1. INTRODUCTION

Hormonally stimulated enzyme secretion from pancreatic acini is activated mainly by two signalling pathways. Cholecystokinin (CCK)- or acetylcholine (ACh)-receptor-mediated stimulation of phospholipase C (PLC) results in a rise of both inositol 1,4,5-trisphosphate (IP_3) and diacylglycerol (DG), which leads to Ca^{2+}- mobilisation [1] and activation of proteinkinase C (PKC) [2], respectively. Secretin and vasoactive intestinal polypeptide (VIP) stimulate adenylate cyclase (AC) with a subsequent rise in cAMP and activation of protein kinase A (PKA) [3,4]. As previously postulated, a hormonally regulated Cl^- conductance present in pancreatic ZG is involved in 'flushing out' the granular content during exocytosis [5–8]. Both the Cl^- conductance and the enzyme secretion from isolated pancreatic acini, which had been stimulated at various concentrations of cholecystokinin octapeptide (CCK-8), increased with increasing CCK-8 concentrations and decreased at high CCK-8 concentrations [8]. Stimulation with high CCK-8 concentrations (>10^{-10} M) leads to a supramaximal stimulation of PLC, causing an 'overproduction' of IP_3 and DG [9]. This led us to suppose that products of DG metabolization, like arachidonic acid (AA) and/or prostaglandin E_2 (PGE_2), could be responsible for the inhibition at high CCK-8 concentrations. For the exocrine rat pancreas, Mössner et al. have shown that PGE_2 inhibits CCK-8 stimulated enzyme secretion [10].

If a close relationship exists between activation of both the Cl^- conductance in ZG and the enzyme secretion from pancreatic acini, PGE_2 should also affect CCK-8 stimulated Cl^- conductance in ZG. We have therefore determined the Cl^- conductance in ZG isolated from pancreatic acini, which had been pre-stimulated with CCK-8 in the presence or absence of PGE_2 and the cyclooxygenase inhibitor indomethacin. Since PGE_2 has been shown to regulate AC [11], we have examined the effect of the protein kinase A-inhibitor (PKA-I) on the Cl^- conductance in ZG from CCK-8 and PGE_2-pretreated acini. Our data show that PGE_2 inhibits the basal and CCK-8-stimulated Cl^- conductance in isolated ZG and that both indomethacin and PKA-I abolish the decrease in the Cl^- conductance of ZG isolated from acini, which had been pre-stimulated with high concentrations of CCK-8. We conclude that the inhibition of the Cl^- conductance in isolated ZG at high CCK-8 concentrations is mediated by an enhanced production of PGE_2, and that PGE_2 operates by a separate signal transduction pathway involving stimulation of AC with a consequent rise in cellular cAMP and activation of PKA.

2. MATERIALS AND METHODS

2.1. *Materials*

Collagenase type III, bovine serum albumin (BSA, fatty acid-free), (*N*-morpholino) propane sulfonic acid (MOPS), ethylene glycol bis-(β-aminoethyl ether) *N,N,N',N'*-tetraacetic acid (EGTA), digitonin, phenylmethylsulfonyl fluoride (PMSF), cholecystokinin-octapeptide

Correspondence address: I. Schulz, Max-Planck-Institut für Biophysik, Kennedyallee 70, 6000 Frankfurt a.M. 70, Germany.

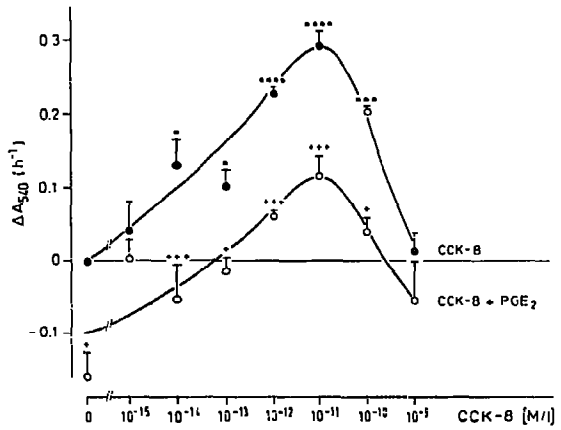

Fig. 1. Effects of pretreating acinar cells with CCK-8 (10^{-9}–10^{-14} M (●) and PGE_2 (10^{-6} M) (○) on lysis rates of isolated zymogen granules (ZG). Following incubation of isolated acini with indicated concentrations of CCK-8 alone or in the presence of PGE_2, ZG are prepared on a Percoll-gradient. The lysis of isolated ZG from pretreated acini is induced by valinomycin and expressed as the difference (h^{-1}) ΔA_{540} nm from ZG lysis rates of untreated acini. Each point represents the mean value ± SE from 3–8 separated experiments. (*) level of significance as compared to the lysis rates in ZG from unstimulated acini. (+) level of significant difference between pre-stimulation with CCK-8 alone and pre-stimulation with CCK-8 in the presence of PGE_2. *P < 0.05; ***P < 0.005; ****P < 0.001; ^{+}P < 0.05; ^{+++}P < 0.005.

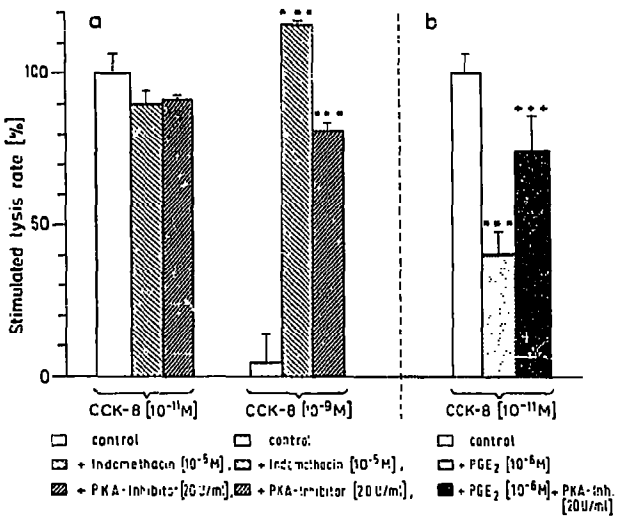

Fig. 2. Stimulated lysis rates of isolated zymogen granules (ZG) from intact and permeabilized pancreatic acini pretreated with CCK-8, PGE_2, indomethacin and a protein kinase A inhibitor. Experimental conditions are as described in Fig. 1. Stimulated lysis rates of isolated ZG are expressed as percentages of the maximal CCK-8 (10^{-11} M)-stimulated lysis rate (100%: ΔA_{540} nm = 0.294 ± 0.019 h^{-1}). Columns represent mean values ± SE of 3–8 separate experiments. (*) significant difference to the control (i.e. to the lysis rates observed by pre-stimulation with CCK-8 (10^{-11} M or 10^{-9} M)). (+) significant difference between the effect of CCK-8 (10^{-11} M) + PGE_2 (10^{-6} M) and CCK-8 (10^{-11} M) + PGE_2 (10^{-6} M) + PKA-I (20 U/ml). ***P < 0.005; ^{+++}P < 0.005.

(CCK-8), prostaglandin E_2 (PGE_2), valinomycin, protein kinase A inhibitor (type II from bovine heart and rabbit sequence) and indomethacin were obtained from Sigma (St. Louis, MO, USA).

2.2. *Preparation of pancreatic acini*

The preparation of pancreatic acini by collagenase digestion was performed as described previously [6,8]. Pancreata from 4–6 male Wistar rats, which had been starved overnight, were trimmed free from fat, lymph nodes and connective tissue, minced and suspended in 10 ml fresh Krebs-Ringer-HEPES (KRH) digestion buffer containing in mM: NaCl 145; KCl 4.7; $MgCl_2$ 2; $CaCl_2$ 2; HEPES 10 (pH 7.4 adjusted with Tris); glucose 1.5; trypsin inhibitor 0.1 g/l; BSA 2 g/l. Collagenase digestion was carried out in KRH buffer by two steps at 37°C, under continuous supply of O_2. In the first step the tissue fragments were treated with 1500 U collagenase for 15 min and in the second step with 2500 U for 45 min.

2.3. *Incubation of acini and isolation of zymogen granules (ZG)*

Isolated intact acini were stimulated with CCK-8 in 35 or 50 ml KRH buffer for 20 min in the presence or absence of PGE_2 or of indomethacin. Stimulation and permeabilization of acini was done in 10 ml medium containing in mM; KCl 130; $MgSO_4$ 2; KH_2PO_4 1.2; $CaCl_2$ 0.1; glucose 15; HEPES 20 (pH 7.4/Tris); trypsin inhibitor 0.1 mg/ml; BSA 2 g/l; digitonin 10 μg/ml; in the presence or absence of CCK-8, PGE_2 and PKA-I. Following incubation of acini, zymogen granules (ZG) were isolated as described previously [5]. Briefly, isolated acini were homogenized in a Parr 'bomb' (Parr Instruments, Moline, IL, USA), pressurized to 250 psi in 15 ml homogenisation buffer which contained in mM: sucrose 250; $MgSO_4$ 0.1; EGTA 0.1; PMSF 0.2; BSA (fatty acid-free) 0.1%; MOPS 50 (pH 7.0/Tris). Density gradient centrifugation was performed in a medium which contained in mM; sucrose 250; MES 100 (pH 5.5, Tris); $MgSO_4$ 0.1; EGTA 4; PMSF 0.2; BSA (fatty acid free 0.1%); and 40% Percoll; at 20 000 × g for 20 min in a fixed angle rotor (Beckmann Ti 60). ZG formed a distinct white band near the bottom of the gradient. The granules were collected with a Pasteur pipette, resuspended in 'wash' buffer containing in mM: sucrose 320; MOPS 1 (pH 7.0/Tris); $MgSO_4$ 0.1; EGTA 1; and centrifuged for 15 min at 1000 × g. Purified ZG were resuspended in 250–500 μl homogenisation buffer.

2.4. *Measurement of Cl^- permeability in zymogen granules (ZG)*

The Cl^- permeability in isolated ZG was determined as described previously [8] in a Hitachi spectrophotometer U 2000 at 540 nm under continuous stirring at 37°C. Aliquots of ZG were added to a 'KCl buffer' which contained in mM: KCl 150; EGTA 1; $MgSO_4$ 0.2; HEPES 20 (pH 7.0/Tris) until a final absorbance (A) of 0.2 was obtained. Following the addition of the K^+ ionophore, valinomycin, the decrease in absorbance was measured for the initial 2 min. Since in the presence of valinomycin the K^+ permeability is no longer rate-limiting, the decrease in absorbance due to lysis of ZG is a measure for the Cl^- conductance in ZG membranes.

2.5. *Statistical analysis*

All results are expressed as means ± SE from at least three experiments. Statistical analysis was performed using Student's paired t-test.

3. RESULTS

3.1. *Effects of PGE_2 on the Cl^- conductance in ZG isolated from unstimulated and CCK-8-stimulated pancreatic acini*

Similar to what has been shown previously [8], the rate of ZG lysis as a measure for the Cl^- conductance in ZG isolated from CCK-8-pre-stimulated pancreatic acini reaches a maximum at 10^{-11} M CCK-8 (ΔA 0.294

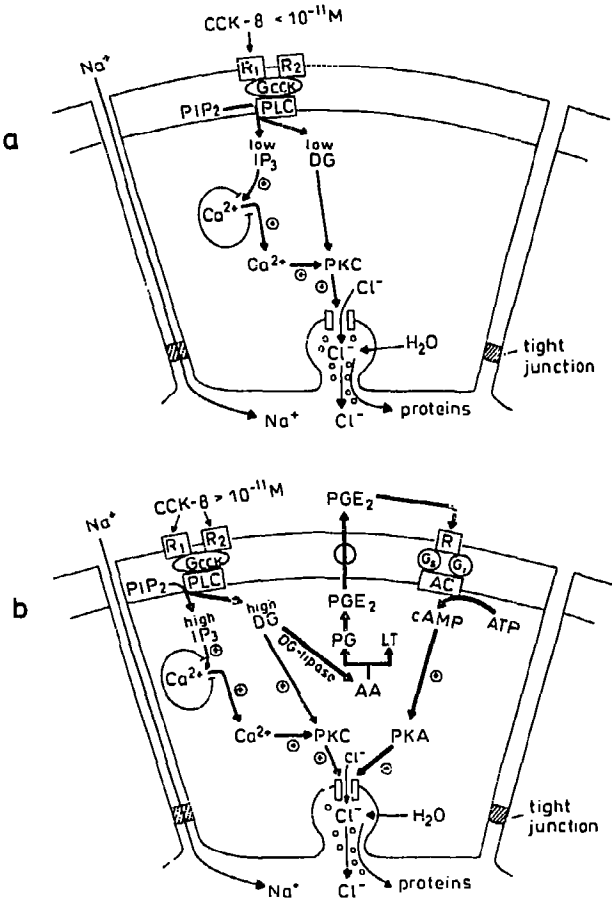

Fig. 3. Model for CCK-8-regulated Cl^- conductance in zymogen granules of pancreatic acinar cells. For explanation see text.

$\pm$ 0.019 h^{-1}; $P < 0.001$) and decreases at higher CCK-8 concentrations (Fig. 1) [8]. Pretreatment of pancreatic acini with various concentrations of CCK-8 (10^{-9}–10^{-15} M) in the presence of PGE_2 (10^{-6} M) leads to a decrease in CCK-8-induced Cl^- conductance, which is significant between 10^{-11}–10^{-14} M CCK-8 (Fig. 1). At CCK-8 10^{-11} M, PGE_2 (10^{-6} M) causes a decrease of CCK-8-stimulated Cl^- conductance in isolated ZG from ΔA 0.294 $\pm$ 0.019 h^{-1} to ΔA 0.117 $\pm$ 0.029 h^{-1} ($P < 0.005$). The basal Cl^- conductance in isolated ZG decreases in the presence of PGE_2 (10^{-6} M) by ΔA 0.160 $\pm$ 0.034 h^{-1} ($P < 0.02$) (Fig. 1). Direct addition of PGE_2 to isolated ZG has no effect on the Cl^- conductance (data not shown).

3.2. *Effects of indomethacin on the Cl^- conductance in isolated ZG from CCK-8-stimulated pancreatic acini*

To examine whether the decrease in the Cl^- conductance of ZG isolated from pancreatic acini, which had been pre-stimulated with supramaximal concentrations of CCK-8, could be mediated by an enhanced synthesis of PGE_2, we have blocked the PGE_2 production by indomethacin, an inhibitor of the cyclooxygenase. The basal Cl^- conductance is not altered in ZG isolated from pancreatic acini pre-incubated with indomethacin (10^{-5} M) (data not shown). The Cl^- conductance in isolated ZG from acini, which had been pre-stimulated with the maximal effective CCK-8 concentration (10^{-11} M) in the presence of indomethacin (10^{-5} M) is not significantly different (ΔA 0.262 $\pm$ 0.025 h^{-1}) as compared to the Cl^- conductance in isolated ZG from acini pre-stimulated with CCK-8 (10^{-11} M) alone (ΔA 0.294 $\pm$ 0.019 h^{-1}) (Fig. 2a). But in acini pre-stimulated with supramaximal concentrations of CCK-8 (10^{-9} M) in the presence of indomethacin (10^{-5} M) the Cl^- conductance is significantly increased (ΔA 0.342 $\pm$ 0.04 h^{-1} ; $P < 0.005$) as compared to that induced by stimulation with CCK-8 (10^{-9} M) alone (ΔA 0.0126 $\pm$ 0.024 h^{-1}) (Fig. 2a).

3.3. *Effects of a protein kinase A inhibitor on the Cl^- conductance in ZG isolated from pancreatic acini pre-stimulated with CCK-8 in the presence and absence of PGE_2*

To test whether stimulation of PKA, which inhibits the Cl^- conductance in isolated ZG [6], might be involved in the effects of PGE_2, we have examined the protein kinase A inhibitor (PKA-I) on the Cl^- conductance in ZG from CCK-8 and PGE_2-pretreated acini. Treatment of permeabilized acini with PKA-I (20 U/ml) did not alter either the basal Cl^- conductance (data not shown) or the maximal stimulated Cl^- conductance at CCK-8 10^{-11} M (Fig. 2a). At supramaximal CCK-8 concentrations (10^{-9} M), PKA-I (20 U/ml) caused an increase in the Cl^- conduction from ΔA 0.0126 $\pm$ 0.024 h^{-1} with CCK-8 (10^{-9} M) alone to ΔA 0.237 $\pm$ 0.022 h^{-1}, $P < 0.005$ (Fig. 2b). When acini had been pre-stimulated with CCK-8 (10^{-11} M) in the presence of both PGE_2 (10^{-6} M) and PKA-I (20 U/ml) the Cl^- conductance was significantly increased (ΔA 0.214 $\pm$ 0.042 h^{-1}) as compared to that induced by stimulation with CCK-8 (10^{-11} M) plus PGE_2 (10^{-6} M) alone (ΔA 0.117 $\pm$ 0.029 h^{-1}, $P < 0.005$) (Fig. 2b). Permeabilization of isolated acini has no effect on stimulated lysis rates of isolated ZG.

4. DISCUSSION

We had recently shown that both the Cl^- conductance in ZG isolated from pancreatic acini, which had been pre-stimulated with various concentrations of CCK-8, and the CCK-8-induced enzyme secretion from isolated acini increased with increasing CCK-8 concentrations and decreased at high CCK-8 concentrations [8]. Stimulation with high concentrations of CCK-8 leads to supramaximal activation of PLC causing an overproduction of IP_3 and DG [9]. High amounts of DG could be metabolized to AA via diacylglycerol lipase [12] or could increase the AA level by inhibiting reincorporation of AA into membrane phospholipids

(reacylation) by DG-induced PKC activation [13]. The increase of free intracellular AA levels causes an enhanced synthesis of leukotrienes and prostaglandins [14]. For the exocrine mouse pancreas it has been shown that hormonally induced breakdown of phosphatidylinositol provides AA for PGE_2 synthesis [15] that stimulates enzyme secretion [16]. PGs act as local 'hormones', which are liberated to the extracellular fluid and bind to the cell surfaces [11]. Receptors for PGE_2 are present on parietal cells [17–19], platelets [20,21] and pancreatic acinar cells [12]. Binding of PGE_2 to parietal cells abolishes histamine stimulated acid secretion by PGE_2-induced inhibition of AC and consequent cAMP formation [17]. Binding of PGE_2 to platelets, however, causes an activation of AC with a subsequent rise of cAMP [22,23]. For the exocrine rat pancreas it has been shown that PGE_2 inhibits secretagogue-induced enzyme secretion [12]. Hormonal activation of the Cl^- conductance in isolated pancreatic ZG is closely related to enzyme secretion, but the increase in the Cl^- conductance occurs at much lower CCK-8 concentrations than necessary for stimulating enzyme secretion [8]. This could indicate that the conditions for measuring the Cl^- conductance in isolated ZG in the cuvette do not reflect the conditions for ZG present in intact acini so that the dose–response relation for both enzyme secretion from intact acini and isolated ZG are not quite comparable. However, if they were the same, this would indicate that the regulation of the Cl^- conductance is not the limiting step for enzyme secretion.

Since indomethacin abolishes the decrease of the Cl^- conductance at high- but has no effect at low CCK-8 concentrations (Fig. 2a), we conclude that the decrease in the Cl^- conductance at high CCK-8 concentrations could be due to enhanced endogenous synthesis of PGE_2. We assume that PGE_2 operates by a separate signal transduction pathway involving stimulation of AC with consequent activation of cAMP-dependent PKA, since PKA-I abolishes the decrease in the Cl^- conductance due to exogenously added PGE_2 as well as to supramaximal CCK-8 stimulation. Activation of PKA might be responsible for the inhibitory effect of PGE_2 on Cl^- conductance in isolated ZG. This conclusion is confirmed by a previous study showing that direct addition of PKA to isolated ZG inhibits the Cl^- conductance in isolated ZG [6].

In summary, we propose the following model for the CCK-8-stimulated Cl^- conductance in isolated pancreatic ZG (Fig. 3a,b): Low concentrations of CCK-8 bind to a high-affinity CCK-receptor (R_1) and stimulate PLC via a GTP-binding protein (G_{CCK}) [24]. Stimulation of PLC results in the production of low amounts of IP_3, DG and activation of PKC, which cause activation of the Cl^- conductance present in the membrane of ZG. At supramaximal concentrations, CCK-8 binds to the high- as well as to the low-affinity CCK-receptor (R_2) [25], which are coupled to PLC, too. Additional occupation of R_2 leads to supramaximal stimulation of PLC, causing large IP_3 and DG production. High amounts of DG could be metabolized to AA. The increase in AA levels within the acini enhances the synthesis of PGE_2, which is liberated to the extracellular fluid and binds to specific receptors on the acinar cells. Binding of PGE_2 inhibits the Cl^- conductance in ZG by stimulating AC with a consequent rise in cAMP and activation of PKA.

Acknowledgements: This work was supported by the Deutsche Forschungsgemeinschaft (Pl 160/1-1).

REFERENCES

[1] Streb, H., Irvine, R.F., Berridge, M.J. and Schulz, I. (1983) Nature 306, 67–69.
[2] Castagna, M., Takai, M., Kaibuchi, K., Sano, K., Kikkawa, U. and Nishizuka, Y. (1982) J. Biol. Chem. 257, 7847–7851.
[3] Robbrecht, P., Coulon, T.P. and Gardner, J.D. (1976) J. Biol. Chem. 251, 4635–4639.
[4] Taylor, S.S., Bubis, J., Toner-Webb, J., Saraswat, L.D., First, E.A., Buechler, J.A., Knighton, D.R. and Sowadski, J. (1988) FASEB J. 2, 2677–2685.
[5] Gasser, K.W., Di Dominco, J. and Hopfer, U. (1988) Am. J. Physiol. 254, G93–G99.
[6] Fuller, C.M., Deetjen, H.H., Piiper, A. and Schulz, I. (1989) Pflügers Arch. 415, 29–36.
[7] De Lisle, R.C., Schulz, I., Tyrakowski, T., Haase, W. and Hopfer, U. (1984) Am. J. Physiol. 246, G411–G418.
[8] Piiper, A., Plusczyk, T., Eckhardt, L. and Schulz, I. (1991) Eur. J. Biochem. 197, 391–398.
[9] Matozaki, T., Göke, B., Tsunoda, Y., Rodriguez, M., Martinez, J. and Williams, J.A. (1990) J. Biol. Chem. 264, 14729–14734.
[10] Mössner, J., Seeknus, R., Spiekermann, G.M., Sommer, C., Biernat, M., Bahnsen, H. and Fischbach, W. (1991) Am. J. Physiol. 260, G711–G719.
[11] Streyer, L. (1988) Biochemistry, third edition, pp. 991–992, Freeman, New York.
[12] Exton, J.H. (1990) J. Biol. Chem. 265, 1–4.
[13] Fuse, I. and Tai, H-H. (1989) J. Biol. Chem. 264, 3890–3895.
[14] Burgoyne, R.D. and Morgan, A. (1990) Trends Biochem. Sci. 15, 365–366.
[15] Marshall, P.J., Boatman, D.E. and Hokin, L.E. (1981) J. Biol. Chem. 256, 844–847.
[16] Marshall, P.J., Dixon, J.F. and Hokin, L.E. (1980) Proc. Natl. Acad. Sci. USA 77, 3292–3296.
[17] Chen, M.C.Y., Amirian, D.A., Toomey, M.J. and Soll, A.H. (1988) Gastroenterology 94, 1121–1129.
[18] Seidler, U., Beinborn, M. and Sewing, K.-F. (1989) Gastroenterology 96, 314–320.
[19] Soll, A.H. (1980) J. Clin. Invest. 65, 1222–1229.
[20] McDonald, J.W.D. and Stuart, R.K. (1974) J. Lab. Clin. Med. 84, 111–121.
[21] Gorman, R.R (1974) Prostaglandins 6, 542–547.
[22] Wolfe, S.M. and Shulman, N.R. (1969) Biochem. Biophys. Res. Commun. 35, 265–272.
[23] Salzman, E.W., Kensler, P.C. and Levine, L. (1972) Ann. N.Y. Acad. Sci. 201, 61–71.
[24] Yu, D.H., Huang, S.C., Wank, S.A., Mantey, S., Gardner, D.J. and Jensen, R.T. (1990) Am. J. Physiol. 258, G86–G95.
[25] Jensen, R.T., Lemp, G.F. and Gardener, D.J. (1982) J. Biol. Chem. 257, 5554–5559.