Volume 242, number 1, 61–64 FEB 06598 December 1988

CCK and gastrin inhibit adenylate cyclase activity through a pertussis toxin-sensitive mechanism in the tumoral rat pancreatic acinar cell line AR 4-2J

Jean Luc Scemama[+], Patrick Robberecht, Magali Waelbroeck, Philippe De Neef, Lucien Pradayrol[+], Nicole Vaysse[+] and Jean Christophe

Department of Biochemistry and Nutrition, Medical School, Université Libre de Bruxelles, 115 Bd of Waterloo, B-1000 Brussels, Belgium and [+] *Unité INSERM U 151, CHU Rangueil, 31054 Toulouse Cédex, France*

Received 12 October 1988

(Thr[28],Nle[31])CCK(23–33) (CCK-9) and gastrin(1–17)$_I$ (gastrin) inhibited adenylate cyclase activity in membranes from the tumoral rat pancreatic acinar cell line AR 4-2J through a *Bordetella* pertussis toxin-sensitive mechanism. This contrasted with the stimulatory effect exerted by CCK-9 on adenylate cyclase activity in membranes from normal rat pancreas. The relative potency of CCK-9, gastrin, and related peptides in inhibiting adenylate cyclase, when confronted with previous evidence, suggests that 'non-selective CCK-gastrin CCK-B receptors' predominating over 'selective CCK-A receptors' in the AR 4-2J cell line, favored the coupling of the first receptors to adenylate cyclase through G_i, while CCK-A receptors capable of stimulating the enzyme through G_s were detected only after *Bordetella* pertussis toxin pretreatment.

Cholecystokinin receptor; Gastrin receptor; Adenylate cyclase; Guanine nucleotide-binding regulatory protein; Pertussis toxin; (Rat pacreas, Tumoral rat pancreatic cell line AR 4-2J)

1. INTRODUCTION

Cholecystokinin (CCK) receptors are heterogeneous: two classes are currently considered based on the relative potency of CCK agonists and antagonists and tissular location. 'Peripheral CCK-A receptors' located in the pancreas and gallbladder smooth muscle show high affinity for CCK but low affinity for gastrin and C-terminal CCK fragments. Such CCK-A receptors in the normal pancreas are likely to exist in two forms: one with high affinity for agonists is coupled to calcium movements, cyclic GMP production and amylase secretion; the second form displays lower affinity for agonists and is coupled to cyclic AMP production [1]. CCK-B receptors, found in preference in the central nervous system, are characterized by high affinity for both CCK, gastrin, and C-terminal gastrin fragments. The second messenger of these receptors is unknown but might involve adenylate cyclase inhibition in the anterior part of the nucleus accumbens of rat where CCK inhibits the cyclic AMP increase provoked by dopamine [2].

Having recently demonstrated the coexistence of CCK-A and CCK-B receptors in the rat cultured tumoral pancreatic acinar cell line AR 4-2J [3], we used this model to investigate the possible coupling of both receptor types to adenylate cyclase through G_s and/or G_i regulatory proteins. Our results demonstrate that CCK, gastrin, and C-terminal gastrin fragments inhibited membrane adenylate cyclase activity and that this inhibition was no longer observed after pretreating the cell line with pertussis toxin. These data represent the first indication of a possible coupling of CCK-B receptors to G_i.

Correspondence address: J. Christophe, Department of Biochemistry and Nutrition, Medical School, Université Libre de Bruxelles, 115 Bd of Waterloo, B-1000 Brussels, Belgium

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/88/$3.50

2. MATERIALS AND METHODS

AR 4-2J cells were grown in Dulbecco's modified Eagle's medium supplemented with 10% (v/v) fetal calf serum, penicillin, streptomycin and fungizone® , in an atmosphere of 5% CO_2 in air at 100% humidity and were used at confluence. Pertussis toxin, prepared as described [4], was kindly given by Dr M. Svoboda (Dept of Biochemistry and Nutrition, Medical School, Université Libre de Bruxelles) and, when used, was incorporated into the culture medium at a concentration of 0.1 μg/ml, 18 h before membrane preparation.

Membranes from control and pertussis toxin-treated AR 4-2J cells were prepared as follows. Cells were harvested with a rubber policeman, pelleted at 200 × g for 5 min then lysed with 1 mM bicarbonate and rapid freezing in liquid nitrogen. After thawing the suspension was centrifuged at 1000 × g for 10 min and the supernatant was recentrifuged at 15000 × g for 10 min. The resulting pellet was suspended in 1 mM bicarbonate in order to obtain a 0.6–1.0 mg protein/ml concentration and tested immediately for adenylate cyclase activity.

A crude preparation of membranes was similarly prepared from rat pancreas homogenized in 1 mM bicarbonate. Adenylate cyclase activity was determined by the Salomon et al. [5] procedure as previously described [6]. Synthetic porcine (Thr^{28},Nle^{31})CCK(25–33), human (Leu^{15})gastrin(2–27) desulfated and gastrin(14–17) were gifts from Professor E. Wünsch (Max-Planck-Institut für Biochemie, Martinsried, FRG); VIP and secretin were gifts from, respectively, Dr D.H. Coy (Dept of Medicine, Tulane University, New Orleans, LA, USA) and Dr W. König (Hoechst Aktiengesellschaft, Frankfurt am Main, FRG). Human gastrin(1–17)$_I$ and pentagastrin were from CRB Laboratories (Cambridge, England). (Thr^{28},Nle^{31})CCK(25–33) and human gastrin(14–17) are referred to as CCK-9 and tetragastrin in the present paper.

3. RESULTS

Adenylate cyclase activity in normal rat pancreatic membranes was stimulated by CCK-9, secretin and VIP in the presence of GTP (fig.1A). Secretin had the highest efficacy. Gastrin at a 10 μM concentration was inactive. A dose-response curve of CCK-9-stimulated adenylate cyclase activity in these membranes indicated a K_{act} value (concentration exerting half-maximal stimulation) of 0.3 μM. Gastrin, pentagastrin and tetragastrin at 10 μM were ineffective per se and unable to inhibit the CCK-9-stimulated adenylate cyclase activity (not shown).

Membranes from AR 4-2J cells showed high adenylate cyclase activity in the presence of GTP alone; secretin and VIP stimulated this activity, 1.5- and 2.6-fold, respectively. In contrast, 1 μM CCK and 1 μM gastrin inhibited the same activity by 30–40% (fig.1B). The dose dependency of adenylate cyclase inhibition for 4 selected CCK

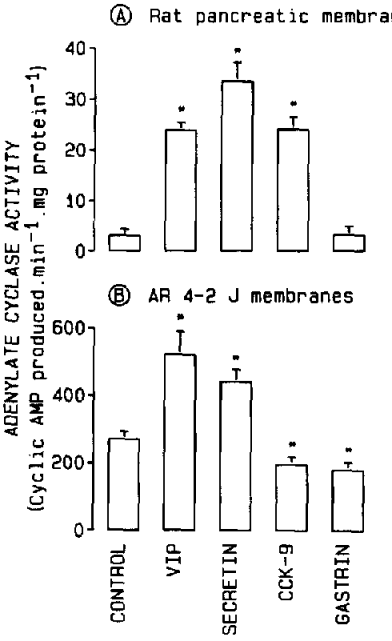

Fig.1. Effects of maximal concentrations (1 μM) of VIP, secretin, CCK-9 and gastrin on adenylate cyclase activity in rat pancreatic membranes (upper panel) and AR 4-2J membranes (lower panel). All assays were performed in the presence of 10 μM GTP. The results were the means ± SE of at least 3 experiments and were expressed in pmol cyclic AMP produced·min^{-1}·mg protein^{-1}.

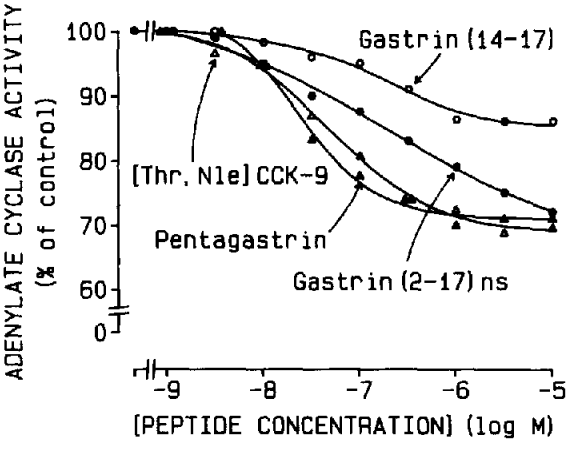

Fig.2. Dose-effect curves of adenylate cyclase inhibition by CCK-9 (△), gastrin(2–17) non-sulfated (●), pentagastrin (▲), and tetragastrin (○) in membranes from AR 4-2J cells. The results were expressed in % of the basal value observed in the presence of 10 μM GTP and were the means of at least 3 experiments.

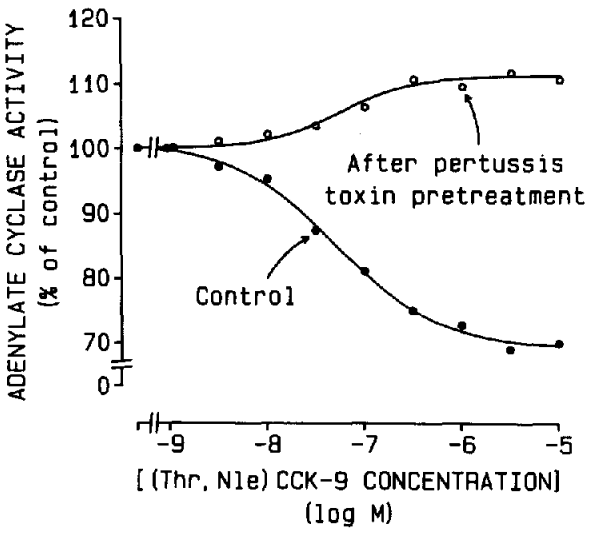

Fig.3. Dose-effect curves of CCK-9 on adenylate cyclase activity in membranes from AR 4-2J cells pretreated (○) or not (●) with *Bordetella* pertussis toxin as indicated in section 2.

agonists and analogues showed the following K_i values (concentration inducing half-maximal inhibition): 20, 300, 10 and 300 nM for, respectively, CCK-9, gastrin(2–17) nonsulfated, pentagastrin, and tetragastrin (fig.2). Pretreating AR 4-2J cells with pertussis toxin for 18 h completely abolished the CCK and gastrin inhibition of adenylate cyclase and revealed instead a small (+15%) but significant stimulation of the activity by CCK (fig.3) while basal activity and VIP stimulation of adenylate cyclase were not altered (not shown).

4. DISCUSSION

The present data represent the first direct evidence that CCK and gastrin can inhibit adenylate cyclase activity through a *Bordetella* pertussis toxin-sensitive mechanism, i.e. a guanine nucleotide-binding regulatory protein of the G_i type. This inhibitory effect of CCK and gastrin was observed in membranes from a transformed rat pancreatic acinar cell line but not in membranes from normal rat pancreas where, in contrast, the enzymatic activity was stimulated by CCK. This absence of CCK inhibition in normal pancreas could not be attributed to a lack of G_i since this organ contains a 41 kDa protein that can be ADP-ribosylated in the presence of *Bordetella* pertussis toxin [4]. Our results are, therefore, best explained by considering distinct populations of CCK receptors in AR 4-2J cells and in normal pancreas.

In AR 4-2J membranes, two types of CCK receptors coexist: CCK-preferring receptors of the CCK-A type that distinguish CCK and gastrin while an equal proportion (50%) of non-selective CCK-B receptors does not discriminate CCK and gastrin [3]. This second class of receptors, when characterized with radiolabelled gastrin [3], shows a relative affinity for CCK, gastrin, pentagastrin and tetragastrin similar to that observed in the present study for adenylate cyclase inhibition. However, the concentrations required in binding studies for receptor occupancy are lower than those provoking adenylate cyclase inhibition suggesting that CCK-B receptors, like selective CCK-A receptors, can exist in either a low- or a high-affinity state for agonists (see section 1).

In normal rat pancreas only selective CCK-A receptors have been described, their high-affinity state being coupled to calcium movements, cyclic GMP production, and amylase secretion [1], whereas the low-affinity state is coupled to cyclic AMP production, inhibition of amylase secretion, and increased amino acid transport [7,8].

In dog pancreas, the coexistence of CCK-A and CCK-B receptors has been reported with CCK-B receptors representing 15% of the total [9]. In guinea pig pancreas, 4% of CCK receptors can be considered to be 'non-selective or gastrin receptors' [10]. These receptors are not linked to enzyme secretion but might be involved in Na^+/H^+ exchange [11] (a phenomenon often linked to adenylate cyclase inhibition [12]) and in trophic effects [13].

To conclude, our data can be tentatively interpreted as follows: AR 4-2J cell membranes are characterized by an unusual balance of CCK receptors favoring non-selective CCK-B receptors. Due to their relatively high number and efficient coupling to a G_i type of regulatory protein, the overall effect exerted by CCK-9 on adenylate cyclase was inhibitory. After ADP-ribosylation of G_i, the inhibitory effect of CCK-9 disappeared revealing a potential for CCK-mediated adenylate cyclase stimulation, probably through the selective CCK-A receptors and G_s. In this respect, it is of interest to note that a dual effect of CCK on adenylate cyclase has already been observed in rat brain

where CCK inhibits dopamine-stimulated adenylate cyclase in the anterior part of the nucleus accumbens but potentiates the dopamine effect in the posterior part of the area [2].

Acknowledgements: This work was supported by grants no. ST2J-001-1-B and STJ-001-3-F from the EEC.

REFERENCES

[1] Von Shrenck, T., Jensen, R.T. and Gardner, J.D. (1987) in: Gastrin and Cholecystokinin, Chemistry, Physiology and Pharmacology (Bali, J.P. and Martinez, J. eds) pp.49–56, Excerpta Medica, Amsterdam.
[2] Studler, J.M., Reibaud, M., Herve, D., Blanc, G., Glowinski, J. and Tassin, J.P. (1986) Eur. J. Pharmacol. 126, 125–128.
[3] Scemama, J.L., Fourmy, D., Zahidi, A., Pradayrol, L., Susini, C. and Ribet, A. (1987) Gut 28, 233–236.
[4] Svoboda, M., Hannecart-Pokorni, E., Borremans, M. and Christophe, J. (1986) Anal. Biochem. 159, 402–411.
[5] Salomon, Y., Londos, C. and Rodbell, M. (1974) Anal. Biochem. 58, 541–548.
[6] Robberecht, P., Waelbroeck, M., Camus, J.C., De Neef, P. and Christophe, J. (1984) Biochim. Biophys. Acta 773, 271–278.
[7] Robberecht, P., Deschodt-Lanckman, M., Morgat, J.L. and Christophe, J. (1978) Eur. J. Biochem. 91, 39–48.
[8] Iwamoto, Y. and Williams, J.A. (1980) Am. J. Physiol. 238, G440–G444.
[9] Fourmy, D., Zahidi, A., Fabre, R., Guidet, M., Pradayrol, L. and Ribet, A. (1987) Eur. J. Biochem. 165, 683–692.
[10] Yu, D.-H., Noguchi, M., Zhou, Z.-C., Villanueva, M.L., Gardner, J.D. and Jensen, R.T. (1987) Am. J. Physiol. 253, G793–G801.
[11] Bastie, M.J., Delvaux, M., Dufresne, M., Saunier-Blache, J.S., Vaysse, N. and Ribet, A. (1988) Am. J. Physiol. 254, G25–G32.
[12] Limbird, L.E. (1988) FASEB J. 2, 2686–2695.
[13] Haarstad, H., Winnberg, A. and Petersen, H. (1985) Scand. J. Gastroenterol. 20, 530–538.