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GALANIN INHIBITS CHOLECYSTOKININ SECRETION IN STC-1 CELLS[1]

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Summary-Neuropeptides such as gastrin releasing peptide and pituitary adenylate cyclase activating polypeptide (PACAP) stimulate CCK secretion from CCK producing cells. We hypothesized that in addition to somatostatin, galanin may also play an inhibitory role on CCK secretion. The effect of galanin on CCK secretion was studied in a CCK-producing murine neuroendocrine tumor cell line, STC-1. Galanin below 10 nM did not affect basal CCK secretion but dose- and time-dependently inhibited KCl-stimulated CCK secretion. Galanin also inhibited forskolin-, bombesin- and PACAP- but not dibutyryl cAMP- or β-TPA-stimulated CCK secretion. The inhibitory effect of galanin was reduced partially by a blocker of ATP-sensitive K^+ channel (K^+_{ATP}), glibenclamide, and prevented by pretreatment of the cells with PTX. The results indicated galanin regulates CCK secretion by modulation of K^+_{ATP} and cAMP production through receptors coupled to a PTX-sensitive G protein

Secretion of cholecystokinin (CCK) from special endocrine cells (I cells) of upper small intestine is regulated by food derived lumenal stimulants and may be subjected to neuromodulation (1). The release of CCK is also stimulated by monitor peptide which was isolated from rat pancreatic juice (2) and by a putative CCK-releasing peptide (CCK-RP), which has been proposed to be released in the intestinal lumen to mediate feedback regulation of pancreatic enzyme secretion by pancreatic proteases (3, 4). Recently, neuropeptides such as bombesin/GRP (5) and PACAP (6, 7) have been shown to stimulate the release of CCK. On the other hand, except for somatostatin, which has been shown to inhibit release and action of CCK-RP (8) and may act via circulation or release locally from the intestinal D cell (9), the possibility that other neuropeptides may also participate in the inhibitory regulation of CCK secretion from CCK producing cells of the small intestine has not been studied.

Galanin is a 29 amino acid neuropeptide, originally isolated from porcine small intestine by detection of the C-terminal amidated structure of peptides (10). Galanin-like immnoactivity is widely

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Abbreviations used are: CCK, cholecystokinin; GRP, gastrin releasing polypeptide; IBMX, 3-isobutyl-1-methylxanthine, K^+_{ATP}, ATP-sensitive potassium channel; NPY, neuropeptide Y; PACAP, pituitary adenylate cyclase activating polypeptide; β-TPA, 4β-12-tetradecanoylphorbol-13-acetate; PTX, pertussis toxin; VIP, vasoactive intestinal polypeptide.

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distributed in both the central and peripheral nervous system of various species (11 - 13). In the periphery, Gal-LI is found abundantly and co-localize with other neuropeptides (VIP, NPY) in nerve cell bodies and fibers of the myenteric plexus and submucosal plexus close to the mucosa of the small intestine or other organs of the gastrointestinal tract (11 - 13). It is also present in the nerve fibers of the pancreas, adrenal medulla, genitourinary tract and respiratory tract (11 - 13). This ubiquitous neuropeptide participates regulation of many biological functions such as stimulation of intestinal motility, inhibition of pancreatic exocrine secretion, and insulin release from β-cells, and stimulation of growth hormone release and food intake (12, 13). Studies in pancreatic β-cell and other tissues suggested that actions of galanin are mediated via high affinity G_i/G_o-coupled receptors involving multiple effector systems such as ATP sensitive K^+ and L-type Ca^{2+} channels as well as adenylate cyclase (12, 13). However, a possible regulatory role of galanin and its mechanism of action on the secretion of gut hormones such as CCK have not been investigated.

In the present study, we hypothesized that galanin may participate in the regulation of CCK secretion by acting directly on CCK producing cells. STC-1 cell line, a murine intestinal neuroendocrine cell line that has been established as an alternative model for study of CCK secretion (14), was chosen for the present study. The data indicated that galanin alone had little effect on basal CCK secretion. However, it exhibited an inhibitory effect on various secretagogues-stimulated CCK release from STC-1 cells in a time- and dose-dependent manner. The inhibitory effect of galanin was abolished by PTX, suggesting the involvement of a PTX-sensitive G protein coupled receptor.

Materials and Methods

Rat galanin and PACAP-27 were synthesized by Dr. David Coy of Tulane University, New Orleans, LA. Bombesin was purchased from Peninsula Laboratories, Inc., Belmont, CA. Glibenclamide was purchased from Research Biochemicals International, Natick, MA. Streptomycin, penicillin and gentamycin sulfate were obtained from Flow Laboratories, Mclean, VA. Forsklin, β-TPA, IBMX, PTX and other chemicals were purchased from Sigma Chemical Co., St Louis, MO. STC-1 cells were obtained form Dr. Seth Grant, Columbia University, NY thought Dr. Andrew Leiter, Tuft University, Boston, MA. All tissue culture wares and media were either purchased from Grand Island Biological Co., Grand Isand, NY. or Costar, Cambridge, MA.

Cell culture: Monolayer cultures of STC-1 cells were maintained in 6 well tissue culture plates as described previously (14).

Studies of the release of CCK from STC-1 cells: Monolayer culture of STC-1 cells were incubated in the presence or absence of galanin (in various doses) and/or a CCK secretagogue at 37° C for 60 min or an indicated time period as described previously (14). The content of CCK-LI in the cells and the incubation medium was then determined by a specific RIA as described previously (14).

Statistical analysis: Comparison of more than one experimental means with that of a control was performed by one way analysis of variance followed by post hoc analysis of Dunnett using a Systat software (Systat Inc., Evanston, IL) as described previously (14). In cases that only two means were compared, Student's paired t test was used. A difference at $p < 0.05$ or lower was regarded as statistically significant.

Results and Discussion

Dose depentent inhibitory effect of galanin on secretagouge-stimulated CCK secretion: Since actions of galanin has been shown to involve multiple signal transduction pathways, we investigated its effect on CCK secretion from STC-1 cells stimulated by different secretagogues known to involve different effector systems. We first studied dose-dependent effect of galanin on CCK release from

STC-1 cells. As show in Fig 1, galanin alone at concentrations below 10^{-8} M had no effect on basal CCK-LI release; wereas at 10^{-7} M it exhibited a significant stimulatory effect. However, galanin dose-dependently inhibited KCl-, bombesin-, PACAP-27- and forskolin-stimulated CCK secretion , reaching a maximal inhibition of 57.8%, 44.2%, 34.5% and 66.7%, respectively at 10^{-8} M. On the other hand, galanin had no effect on β-TPA-stimulated CCK secretion. The results of previous studies (6, 15, 16) have indicated that both bombesin and KCl stimulate CCK secretion in STC-1 cells via elevation of intracellular Ca^{2+} concentration and activation of protein kinase C. The failure of galanin to inhibit CCK secretion stimulated by the protein kinase C activator, β-TPA, thus suggested that galanin inhibited KCl- or bombesin-stimulated CCK release via modulation of Ca^{2+}-dependent event(s) activated by these stimulants. The inhibition by galanin of CCK release stimulated by the two activators of cAMP production, forskolin and PACAP-27 (6), further suggested that galanin can also modulate cAMP-dependent release of CCK in STC-1 cells. This effect appeared to involve production of cAMP rather than its action as galanin did not inhibit dibutyryl cAMP-stimulated CCK secretion (data not shown). Although galanin at 10^{-7} M stimulated basal CCK secretion in STC-1 cells through a yet unknown mechanism, it remained inhibitory to forskolin- and KCl-stimulated CCK release. In addition, the inhibition of secretagogue-stimulated CCK secretion by galanin at low concentrations and stimulation of basal CCK secretion at a high concentration might involve separate subtypes of galanin receptors that merit further investigation.

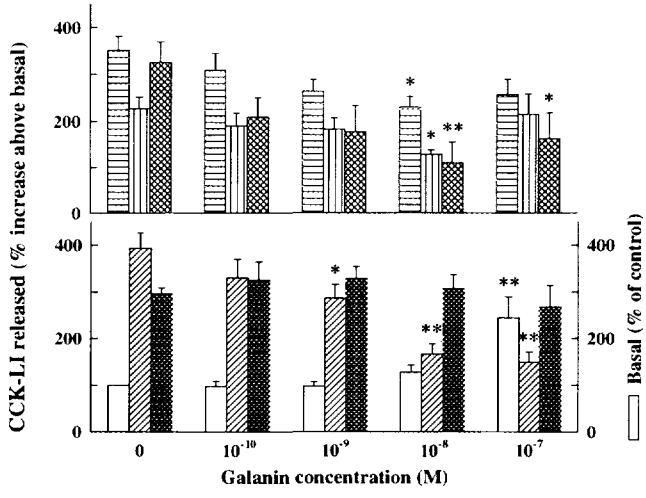

Figure 1. Effect of galanin on basal and secretagogue stimulated CCK secretion in STC-1 cells. Dose-dependent effect of galanin on basal CCK secretion (open bars, lower panel, n=10) or those stimulated by 0.1 μM β-TPA (filled bars, lower panel, n=7), 25 mM KCl (diagonally striped bars, lower panel, n=8), 50 nM PACAP-27 (horizontally striped bars, upper panel, n=6), 30 μM forskolin (diagonally crossed bars, upper panel, n=5), or 5 nM bombesin (vertically striped bars, upper panel, n=7). The stimulatory effect of a secretagogue in the presence of galanin was calculated by substracting the effect of galanin alone from that of secretagogue + galanin and then divided by the basal secretion determined in the same experiment. The data represent mean ± S.E. of n experiments as indicated. * and ** indicate significant inhibition by galanin with $p < 0.05$ and $p < 0.01$, respectively.

Time dependent inhibitory effect of galanin on KCl stimulated CCK release: Since galanin exhibited a profounded inhibitory effect on KCl-stimulated CCK secretion (Fig. 1), we studied the time course of inhibition of KCl-stimulated CCK release by galanin. STC-1 cells were incubated with 25 mM KCl in the absence or presence of 10^{-8} M galanin for various periods of time. As shown in Fig 2, galanin exhibited a significant inhibition of KCl-stimulated CCK release only after 30 min of incubation. A maximum inhibition appeared to be attained at 60 min of incubation.

Partial inhibition of KCl-stimulated CCK secretion by galanin: Plasma membrane depolarization resulted in activation of voltage-dependent ion channels in STC-1 cells (15, 16). Galanin appeared to inhibit only a portion of these activities. As shown in Fig. 3, incubation of STC-1 cells with increasing concentration of KCl resulted in a biphasic dose-dependent stimulation of CCK release. It appeared that one phase of stimulation by KCl reached a maximal effect at 25 mM KCl. In the presence of 10^{-8} M galanin, KCl exhibited a smaller but linear dose-dependent stimulation of CCK release that was parallel to that of KCl at concentrations above 25 mM, suggesting the presence of a galanin-insensitive pathway. This might involve activation of protein kinase C which was not sensitive to galanin.

Involvement of ATP-sensitive K^+ channel in the inhibitory effect of galanin: Previous studies have indicated that galanin can act via activation of ATP-sensitive K^+ channels (K^+_{ATP}) in other cell types (12, 13). Plasma membrane depolarization by KCl or glucose also appeared to activate K^+_{ATP} (16, 17) in STC-1 cells. Therefore, we studied the effect of a blocker of K^+_{ATP}, glibenclamide (18), on the inhibitory effect of galanin on KCl-stimulated CCK release. As shown in Fig. 4, glibenclamide at concentrations above 5 μM significantly antagonized (about 50%) the inhibitory effect of galanin on KCl (25mM)-stimulated CCK release. It should be noted that glibenclamide alone had no effect on

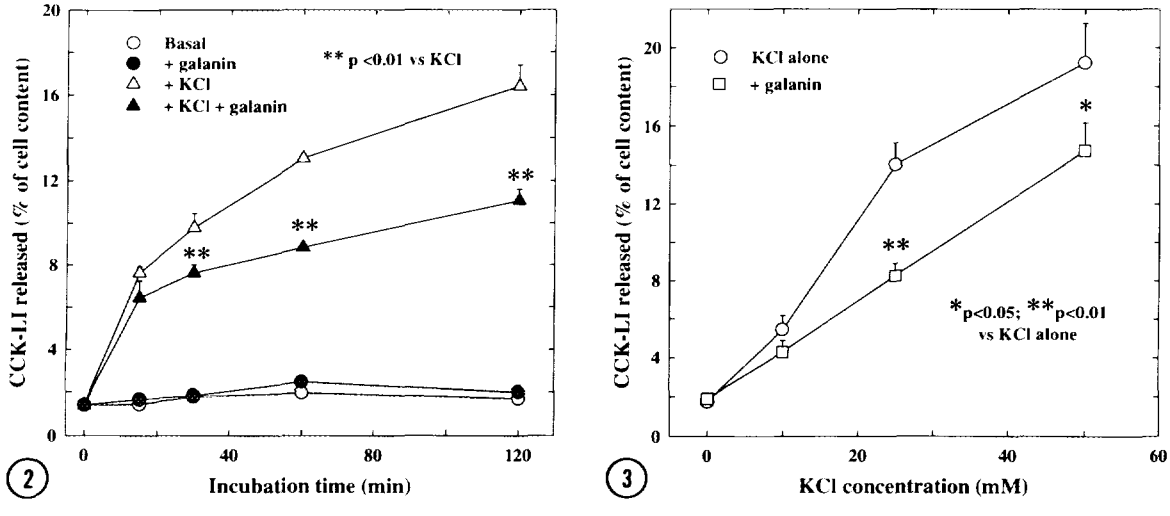

Figure 2. Time-dependent effect of galanin on basal and KCl-stimulated CCK secretion. The concentration of galanin and KCl were 10 nM and 25 mM, respectively. The data represent mean ± S.E. of 4 experiments.

Figure 3. Effect of galanin on CCK secretion stimulated by various doses of KCl. The concentration of galanin was 10 nM. The data represent mean ± S.E. of 4 experiments.

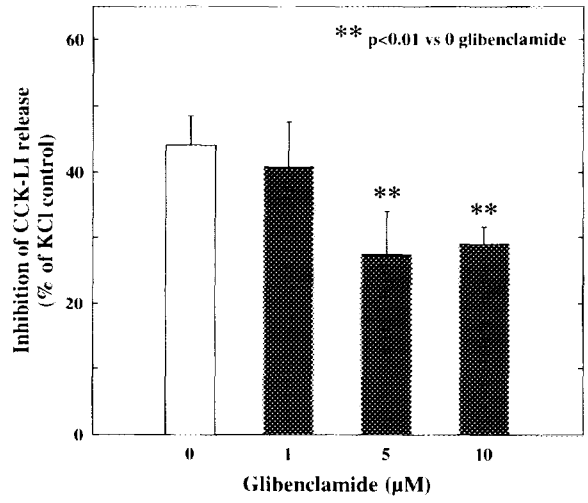

Figure 4. Effect of glibenclamide on inhibition of KCl-stimulated CCK secretion by galanin. STC-1 cells were incubated with 25 mM KCl in the absence or presence of 10 nM galanin and/or various doses of glibenclamide for 60 min and CCK secretion was then determined. The data represent mean ± S.E. of 6 experiments.

basal or secretagogues-stimulated CCK secretion (data not shown). Therefore, this observation suggested that inhibition by galanin was in part mediated through activation of K^+_{ATP} in STC-1 cells. In addition, this observation also supports the previous report that secretion of CCK is modulated by K^+_{ATP} (16, 17).

Involvement of a pertussis toxin-sensitive G protein in the inhibitory effect of galanin: The inhibitory effect of galanin appeared to involve a PTX-sensitive GTP binding protein-coupled receptor.

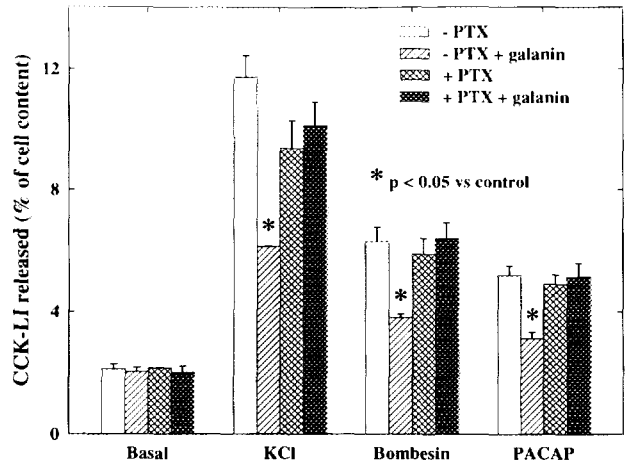

Figure 5. Effect of pretreatment of STC-1 cell with pertussis toxin on galanin produced inhibition of secretagogue-stimulated CCK secretion. STC-1 cells were preincubated with or without 100 ng/ml of PTX for 10 h before incubated in the absence or presence of KCl (25 mM), bombesin (5 nM), or PACAP (50 nM) either alone or together with 10 nM galanin. The extent of CCK secretion was then determined and compared. The data represent mean ± S.E. of 4 experiments.

As shown in Fig. 5, pretreatment of STC-1 cells with 100 ng/ml PTX for 10 h resulted in a complete prevention of inhibition by galanin of CCK release that was stimulated by KCl, bombesin or PACAP. It should be noted that pretreatment with PTX alone did not affect basal or secretagogue-stimulated CCK release. This observation strongly suggested that the inhibitory action of galanin is mediated through a PTX-sensitive G protein-coupled receptor.

In summary, the results of the present study have indicated that galanin regulates CCK secretion in STC-1 cells through PTX-sensitive G protein-coupled receptors. Its action involves in part activation of K^+_{ATP} and of a cellular factor or factors that modulate the cAMP-dependent signal cascade. Given the similar characteristics of CCK secretion in STC-1 cells and mucosal endocrine cell-enriched preparations isolated from the small intestine (14), galanin and other neuropeptides such as bombesin/GRP and PACAP may well participate in regulation of CCK secretion in vivo. Therefore, future studies on its mechanism of action in vivo and in vitro should be interesting and important subjects.

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