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INTRACELLULAR PATHWAYS TRIGGERED BY GALANIN TO INDUCE CONTRACTION OF PIG ILEUM SMOOTH MUSCLE CELLS

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SUMMARY

1. In order to determine the intracellular mechanisms by which galanin induces contraction of isolated smooth muscle cells from pig ileum, we examined the effects of external Ca^{2+}, relaxing agents, pertussis toxin and forskolin on the galanin-induced contraction and compared these effects to those observed on the cholecystokinin derivative CCK8-induced contraction.

2. Galanin induced a concentration-dependent cell contraction. The maximal contraction ($24{\cdot}5 \pm 2{\cdot}1$% of the length of resting cells) was observed at 1 nM of galanin. When cells were incubated in the simultaneous presence of concentrations of galanin (10 fM) and CCK8 (1 pM) which were ineffective alone, or galanin (10 fM) and acetylcholine (100 pM), a synergistic action was observed corresponding to a submaximal contraction.

3. Incubation of cells in Ca^{2+}-free medium caused a significant decrease in galanin- but not in CCK-induced contraction. Nifedipine, a Ca^{2+} channel blocker, provoked a concentration-dependent inhibition of galanin-induced contraction while it had no effect on the contraction induced by CCK8.

4. Vasoactive intestinal polypeptide (VIP) and isoprenaline, known to induce cell relaxation through an increase in intracellular cAMP level, inhibited CCK-induced cell contraction at concentrations ranging from 1 pM to 1 μM but failed to inhibit cell contraction induced by galanin.

5. When cells were pre-incubated for 3 h in the presence of 200 ng/ml of pertussis toxin, the contraction induced by galanin was abolished while the CCK-induced contraction remained unchanged. On the contrary, 10 μM forskolin abolished the contraction induced by 10 nM CCK but had no effect on galanin-induced contraction.

6. These results indicate that galanin induces a concentration-dependent contraction of pig ileum smooth muscle by a direct myogenic effect. This effect of galanin involves the activation of a pertussis toxin-sensitive G protein, which results in an influx of Ca^{2+} into the cell. This intracellular pathway is insensitive to the relaxing effect of cAMP.

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INTRODUCTION

First described in the myenteric plexus of pig ileum (Tatemoto, Rökaeus, Jörnvall, McDonald & Mutt, 1983), the 29 amino acid peptide galanin has been found to be widely distributed, not only in the myenteric plexus of the rat, mouse, guinea-pig, pig and man (Rökaeus, Melander, Hökfelt, Lundberg, Tatemoto, Carlquist & Mutt, 1984; Ekblad, Rökaeus, Hakanson & Sundler, 1985*b*; Melander, Staines, Hökfelt, Rökaeus, Eckenstein, Salvaterra, Weiner, 1985; Bishop, Polack, Bauer, Christofides, Carlei & Bloom, 1986; Tamura, Palmer & Wood, 1987) but also in the brain (Rökaeus *et al.* 1984; Melander, Hökfelt, Nilsson & Brodin, 1986; Bonnefond, Palacios, Probst & Mengod, 1990) and in the pancreas (Dunning, Ahren, Veith, Böttcher, Sundler & Taborsky, 1986; Su, Bishop, Power, Hamada & Polak, 1987).

Galanin alters the activity of digestive smooth muscle in several mammalian species (Fox, 1989). It displays different effects resulting in either a contraction (Ekblad, Hakanson, Swindler & Wahlstedt, 1985*a*) or a relaxation (Fox, Brooks & McDonald, 1988), depending on the organ and the species studied. In humans, it was recently reported that galanin has a potent inhibitory effect on postprandial gastrointestinal motility (Bauer, Zintel, Kenny, Calder, Ghatei & Bloom, 1989).

In vitro studies have reported a direct myogenic effect of galanin on the longitudinal muscle of the rat jejunum (Rökaeus *et al.* 1984) and pig (Tatemoto *et al.* 1983) resulting in a contraction while an inhibition of neurally evoked contraction was observed in guinea-pig taenia coli (Ekblad *et al.* 1985*a*, *b*; Brown, Hildebrand, Parsons & Soldani, 1990), or *in vivo* on gastric, ileal and colonic motility in dog (Fox, McDonald, Kostolanska & Tatemoto, 1986; Fox *et al.* 1988; Gonda, Daniel, McDonald, Fox, Brooks & Oki, 1989). Moreover, galanin may act as a neurotransmitter and its participation in the non-adrenergic, non-cholinergic transmission has been suggested both *in vitro* and *in vivo* (Fox *et al.* 1986; Muramatsu & Yanaihara, 1988).

We previously described the direct myogenic effect of galanin on isolated smooth muscle cells from pig ileum (Delvaux, Botella, Fioramonti, Frexinos & Bueno, 1991). The present study was designed to investigate the intracellular pathways triggered by galanin to induce directly a contraction in pig ileum smooth muscle cells. In order to assess the specificity of the action of galanin on these cells, we compared its effects to those of widely evaluated contracting agents e.g. cholecystokinin (as CCK8) and acetylcholine (ACh). We also tested the influence of extracellular Ca^{2+} level on the galanin-induced contraction, its sensitivity to the relaxing agents, vasoactive intestinal polypeptide (VIP) and isoprenaline (ISO), which are known to increase intracellular cAMP levels, and we compared their effects to those of pertussis toxin and forskolin.

METHODS

Materials

Collagenase (Type V) and pronase were purchased from Boehringer Mannheim Ltd (Meylan, France). Penicillin G and streptomycin G were from Specia (Paris, France). L365,260 was obtained from Merck Sharp and Dohme Laboratories (West Point, PA, USA). All other reagents were obtained from Sigma (St Louis, MI, USA).

Cell dispersion

Cell dispersion was achieved according to previous reports in the literature (Bitar & Makhlouf, 1982*a*). Smooth muscle cells were isolated from the circular muscle layer of the ileum of 6-month-old male pigs of the Large White breed. Samples were obtained from pigs killed in an abattoir according to the legal procedure in France. The pigs were stunned by electric shock and were then bled. Intestinal samples were removed from a position 10 cm proximal to the ileo-caecal junction. After removal of serosa and mucosa–submucosa layers, circular and longitudinal muscle layers were separated by stripping. Small muscle strips were incubated for two successive periods of 30 min at 31 °C in a medium (132 mM NaCl, 5·4 mM KCl, 5 mM Na_2HPO_4, 1 mM NaH_2PO_4, 1·2 mM $MgSO_4$, 1 mM $CaCl_2$, 25 mM HEPES, 0·2% glucose (w/v), 0·2% bovine serum albumin (w/v); pH 7·4, bubbled with 95% O_2–5% CO_2 and supplemented with antibiotics, 100 i.u./ml penicillin G and 50 μg/ml streptomycin), containing 0·25 i.u./ml collagenase, 0·2 mg/ml pronase and 0·2 mg/ml soybean trypsin inhibitor. At the end of the second incubation, the medium was filtered and the partly digested muscle strips were washed four times with enzyme-free medium. These strips were then transferred into fresh and enzyme-free medium and left to stand for 20 min to allow the muscle cells to disperse spontaneously under very slow mechanical agitation. Cells were harvested through a 500 μm nylon filter. It is emphasized that only those cells that had dissociated spontaneously in enzyme-free medium were used for functional measurements.

Measurement of contractile response

Cell suspensions were usually studied within 30 min of dispersion. The cell density of the suspension was about 250000 cells/ml. Aliquots of 250 μl of cell suspension were added to 250 μl solution containing the agent to be tested, thereby ensuring rapid mixing, and incubated for 30 s at 31 °C. The reaction was interrupted by addition of glutaraldehyde to a final concentration of 2·5%.

In control experiments, 250 μl of the same medium were substituted for the tested agent. To measure cell length, an aliquot of cells fixed with glutaraldehyde was placed on a Malassez slide and the lengths of the first fifty cells randomly encountered in successive microscopic fields were measured. Only cells appearing entire at microscopic examination were measured.

Experiments in inhibition or relaxation. Procedure in Ca^{2+}-free medium

After dispersion, cells were pre-incubated for 1 min in the presence of various concentrations of the inhibitory or relaxing agents to be tested. Then the contracting agent was added and the reaction stopped after 30 s as described above. For experiments in Ca^{2+}-free medium, cells were first dispersed in the presence of proteolytic enzymes as described above. Thereafter, the muscle strips were washed four times and incubated for 30 min to allow spontaneous dispersion in an enzyme- and Ca^{2+}-free medium, with or without addition of 2 mM EGTA.

Expression of results

The contractile response was defined as the decrease in the mean cell length of a population of muscle cells exposed to a tested agent in comparison to controls. Cell contraction was expressed as the percentage decrease in cell length compared to the control. The decrease in cell length was calculated using the following formula: $[(L_0 - L_x)/L_0] \times 100$, where L_0 is the mean length of cells in resting state and L_x, the mean length of treated cells.

In relaxation experiments, the degree of inhibition was expressed as the percentage decrease in the contractile response from the maximal response observed in the absence of inhibitors, taken as 100%. Throughout this paper, n refers to the number of experiments, each performed on samples from different animals. Statistical evaluation was carried out using Student's t test and data were tested for significant difference from a normal distribution by the Kolmogoroff test.

RESULTS

Direct myogenic effect of galanin, CCK8 and ACh on isolated smooth muscle cells

Galanin, CCK8 and ACh induced a contraction of smooth muscle cells in a concentration-dependent manner ($n = 7$). Maximal contractions were observed at 1 nM galanin, 10 nM CCK8 and 1 μM ACh and were respectively 24·5 $\pm$ 2·1%,

25·2 ± 2·2 and 23·0 ± 2·0% of the resting cell length. The concentration inducing a half-maximal contraction (EC_{50}) was 3 pM for galanin, 100 pM for CCK8 and 10 nM for ACh (Figs 1 and 2*A*).

Contraction due to interaction between galanin and ACh

At 100 pM ACh, no significant contraction was observed (4·5 ± 1·1% decrease in cell length). In the same way, 10 fM galanin induced no significant contraction. When

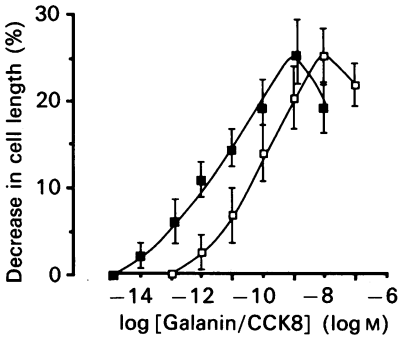

Fig. 1. Contraction induced by galanin and CCK8 in isolated smooth muscle cells from pig ileum. Cells were incubated for 30 s at 31 °C with increasing concentrations of galanin (■) or CCK8 (□), and then fixed by 2·5% glutaraldehyde. Results are expressed as the percentage decrease in cell length compared to control. Values are means ± S.E.M. of seven separate experiments.

cells were incubated in the presence of 10 fM galanin together with increasing concentrations of ACh ($n = 4$), contraction was observed for ACh concentrations ranging from 10 fM to 10 nM (Fig. 2*A*). At higher concentrations of ACh, a maximal response was observed (Fig. 2*A*). When the cells were incubated for 1 min in the presence of increasing concentrations of atropine before 10 nM ACh and 10 fM galanin were added, the synergistic effect was inhibited in a concentration-dependent manner. At 1 μM atropine, the effect of ACh was abolished and the residual contraction was the same size as the effect of 10 fM galanin (Fig. 2*B*).

Contraction due to interaction between galanin and CCK8

At 1 pM CCK8, no significant contraction was observed (2·9 ± 1·8% decrease in cell length; Table 1). When cells were simultaneously incubated with 1 pM CCK8 and 10 fM galanin, the cell contraction was 21·0 ± 2·1% of resting cell length and was significantly more intense than the theoretical sum of the responses to either 10 fM galanin or 1 pM CCK8 ($P < 0·01$; Table 1). When cells were incubated in the presence of 1 pM CCK8 and with larger concentrations of galanin ($n = 4$), a potentiation of the same order was observed when the concentrations of galanin were less than 1 pM. At galanin concentrations > 1 pM, the cell contraction was not different from that observed in the presence of galanin alone. When the cells were incubated for 1 min in the presence of L365,260 (10 μM) before CCK8 and galanin (10 fM) were added, the synergetic effect was abolished and the contraction observed corresponded to the effect of galanin alone (Table 1).

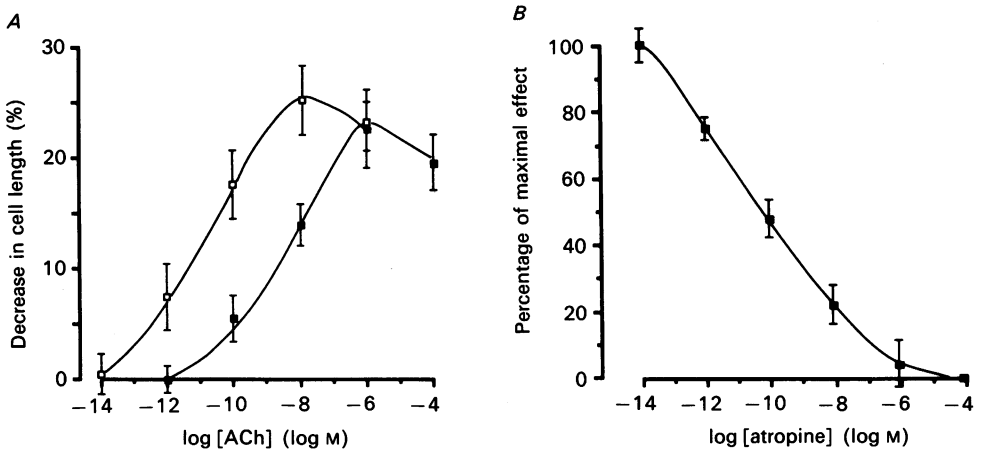

Fig. 2. *A*, synergism between galanin and ACh to induce cell contraction of isolated smooth muscle cells from pig ileum. Cells were incubated for 30 s at 31 °C in the presence of ACh alone (■) and of 10 fM galanin together with increasing concentrations of ACh (□), and then fixed by 2·5% glutaraldehyde. Results are expressed as the percentage decrease in cell length compared to control. Values are means ± S.E.M. of four separate experiments. *B*, effect of atropine, in increasing concentrations (abscissa) on the synergism between galanin and ACh to induce contraction of isolated smooth muscle cells. Cells were pre-incubated with increasing concentrations of atropine. Galanin (10 fM) and ACh (10 nM) were then simultaneously added for 30 s and cells were fixed with 2·5% glutaraldehyde. Results are expressed as the percentage of the contraction observed in the presence of contracting agents in the absence of atropine, taken as 100%. Values are means ± S.E.M. of four separate experiments.

TABLE 1. Synergism between galanin and CCK8 to induce contraction of isolated smooth muscle cells from pig ileum

	Contraction (%)
Galanin (1 nM)	24·5 ± 2·1
CCK8 (10 nM)	25·2 ± 2·2
Galanin (10 fM)	2·2 ± 0·8
CCK8 (1 pM)	2·0 ± 1·8
Galanin (10 fM) + CCK8 (1 pM)	21·0 ± 2·1
Galanin (10 fM) + CCK8 (1 pM) + L365,260 (1 μM)	2·2 ± 1·7

Values are means ± S.E.M. of four separate experiments. Cells were incubated at 31 °C for 30 s in the presence of galanin and/or CCK8, and/or L365,260 and then fixed by 2·5% glutaraldehyde. Contraction is expressed as the percentage decrease in cell length compared to control.

Effect of extracellular calcium on the contractile response induced by galanin and CCK8

The incubation of cells in Ca^{2+}-free medium caused a significant decrease in the galanin-induced contraction down to 32·5 ± 2·1% of the maximal effect observed in a 1 mM Ca^{2+} medium. By contrast, removing extracellular Ca^{2+} did not impair contraction caused by CCK8 (Fig. 3). When cells were incubated in Ca^{2+}-free medium to which 2 mM EGTA was added, the contraction induced by galanin was abolished

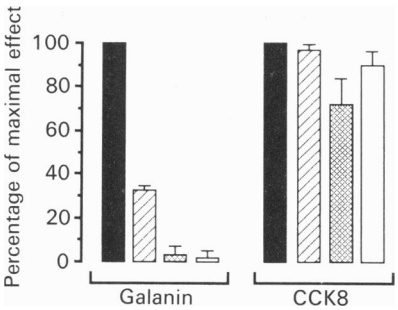

Fig. 3. Influence of extracellular calcium on the contraction induced by galanin and CCK8 on isolated smooth muscle cells. Cells were incubated in the presence of galanin (1 nM) or CCK8 (10 nM) in standard medium (■), in Ca^{2+}-free medium (□), in Ca^{2+}-free medium + 2 mM EGTA (▨) and standard medium + 1 μM nifedipine (□). Results are expressed as the percentage of the contraction induced by a maximal concentration of each contracting agent in the standard medium, taken as 100%. Values are means ± S.E.M. of four separate experiments.

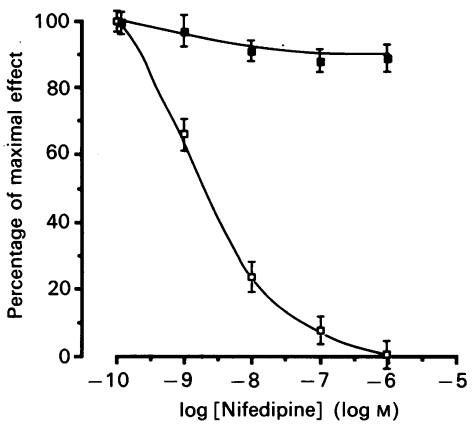

Fig. 4. Effect of the Ca^{2+} channel blocker nifedipine on the contraction induced by galanin and CCK8 on isolated smooth muscle cells. Cells were pre-incubated with increasing concentrations of nifedipine ranging from 1 pM to 1 μM. Galanin (1 nM, □) or CCK8 (10 nM, ■) were then added for 30 s and cells were fixed with 2·5% glutaraldehyde. Results are expressed as the percentage of the contraction induced by a maximal concentration of each contracting agent in the absence of nifedipine, taken as 100%. Values are means ± S.E.M. of four separate experiments.

(3·3 ± 3·0% of maximal effect, Fig. 3) while the CCK8-induced contraction was unchanged. Nifedipine, an organic Ca^{2+} channel blocker (Godfraind, Miller & Wibo, 1986) provoked a concentration-dependent inhibition of galanin-induced contraction. The effect of galanin was abolished at 1 μM nifedipine. The concentration of nifedipine leading to half-maximal (IC_{50}) was 7 nM. In the same experiments, the Ca^{2+} channel blocker nifedipine had no effect on the contraction induced by CCK8 (Fig. 4).

Effect of vasoactive intestinal polypeptide (VIP) and isoprenaline (ISO) on galanin- and CCK8-induced contraction

Cells were incubated for 1 min in the presence of various concentrations of VIP (Fig. 5*A*) or ISO (Fig. 5*B*) ranging from 10 pM to 1 μM, before 1 nM galanin or 10 nM

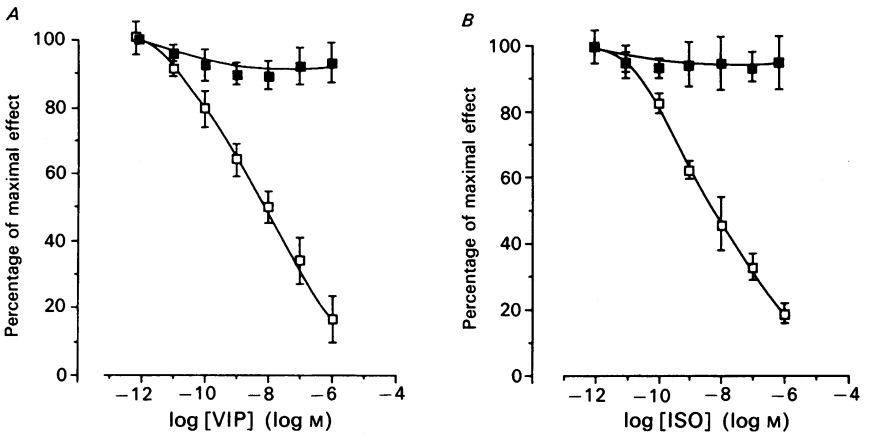

Fig. 5. Effects of relaxing agents VIP and isoprenaline (ISO) on galanin- and CCK8-induced contraction. Cells were pre-incubated with various concentrations of VIP (*A*) or ISO (*B*) for 60 s at 31 °C, before 1 nM galanin (■) or 10 nM CCK8 (□) was added for 30 s and fixed by 2·5% glutaraldehyde. Results are expressed as the percentage of the contraction induced by a maximal concentration of each contracting agent in the absence of VIP and ISO, taken as 100%. Values are means ± S.E.M. of four separate experiments.

CCK8 were added. Neither 1 μM VIP nor 1 μM ISO altered *per se* the mean cell length of isolated smooth muscle cells. Both VIP and ISO failed to inhibit the galanin-induced contraction at concentrations ranging from 1 pM to 1 μM. On the contrary, VIP and ISO induced a concentration-dependent relaxation of cells contracted by 10 nM CCK8. The maximal inhibition was obtained at 1 μM VIP and ISO and corresponded to 16·5 ± 4·5% of the contraction induced by 10 nM CCK8 in the absence of inhibitors. IC_{50} was 0·1 nM for both VIP and ISO.

Effect of pertussis toxin and forskolin on galanin- and CCK8-induced contraction

Prolonged incubation (3 h) in the presence of 200 ng/ml of pertussis toxin did not alter the mean cell length of resting smooth muscle cells (72 ± 17 *versus* 70 ± 15 μm). When cells were first incubated with pertussis toxin 200 ng/ml for 3 h and then challenged with contracting agents, the contraction induced by 1 nM galanin was abolished (Fig. 6). By contrast, pertussis toxin failed to inhibit the contraction induced by 10 nM CCK8 (Fig. 6).

Forskolin is known to directly stimulate adenylate cyclase, thereby increasing the intracellular level of cAMP (Laurenza, Sutkowski & Seamon, 1989). Incubation (10 min) in the presence of 10 μM forskolin did not alter the mean cell length of resting smooth muscle cells (data not shown). In our experiments, a pre-incubation of cells in the presence of forskolin (10 μM) inhibited the contraction induced by

CCK8 (10 nM), in the same way as did ISO and VIP. On the contrary, 10 μM forskolin was unable to inhibit the contraction induced by 1 nM galanin and this was 89·2 ± 12·5% of the maximal response observed when the cells were incubated in the presence of galanin alone (Fig. 6).

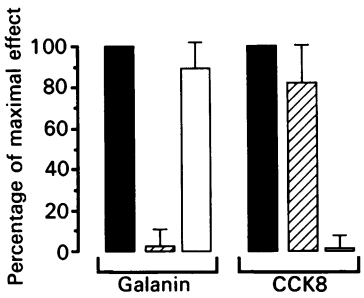

Fig. 6. Effect of pertussis toxin and forskolin on galanin- and CCK8-induced contraction in isolated smooth muscle cells. Cells were pre-incubated in the presence of pertussis toxin (200 ng/ml, ▨) for 3 h or forskolin (10 μM, □) for 10 min. Then, galanin or CCK8 were added for 30 s and fixed with 2·5% glutaraldehyde. Results are expressed as the percentage of the contraction induced by a maximal concentration of each contracting agent in the absence of inhibitors (■), taken as 100%. Values are means ± S.E.M. of four separate experiments.

DISCUSSION

These results confirm previous reports from our laboratory (Delvaux *et al.* 1991) and from others (Ekblad *et al.* 1985*a*; Brown *et al.* 1990) demonstrating a direct myogenic effect of galanin on intestinal smooth muscle in pig, this effect resulting in a cell contraction. In the present study, we observed a synergism between concentrations of ACh and galanin, which were ineffective alone, as well as between CCK8 and galanin. Galanin (10 fM) potentiated the effect of increasing concentrations of CCK8 or ACh. A potentiation was also observed in the experiments carried out in a reverse manner, except that incubating the cells in the presence of ineffective concentrations of CCK or ACh and increasing concentrations of galanin did not alter the maximal response to galanin. A similar potentiation has already been described between gastrin and ACh on isolated smooth muscle cells from guinea-pig stomach (Bitar & Makhlouf, 1982*a*) as well as between CCK and ACh in pig ileum (Berry, Delvaux, Botella, More, Frexinos & Bueno, 1991). The potentiation of the action of one drug by another may be explained by various mechanisms. It may result from interactions between the receptors for these drugs on the cell membrane, by the addition of two separate biological effects or by the fact that these drugs trigger different intracellular pathways. In the present study, we observed only one biological effect: the cell contraction. This contracting effect of CCK, ACh and galanin seems to be receptor-mediated as shown previously in many studies (Bitar & Makhlouf, 1982*a*; Delvaux *et al.* 1991; Botella, Delvaux, Berry, Frexinos & Bueno, 1992). An interaction between receptors at the level of the cell membrane seems not to be involved since the potentiation we observed occurred between ACh and galanin

as well as between CCK and galanin. A link between at least three different receptors is extremely improbable. Thus the potentiation observed could result from the fact that CCK8 and galanin trigger distinct intracellular pathways to induce cell contraction.

Galanin and CCK are known to trigger different second messengers in other cell types. The binding of CCK to its receptor results in activation of phospholipase C and subsequent release of diacylglycerol and inositol trisphosphate, leading to internal Ca^{2+} release (Florholmen, Malm, Vonen & Burhol, 1989). Galanin is known to interact with the adenylate cyclase complex in pancreatic islet cells (Amiranoff, Lorinet, Lagny-Pourmir & Laburthe, 1988). To test the assumption that galanin and CCK8 could act through different intracellular pahways in smooth muscle cells, we studied the effects of extracellular Ca^{2+}, relaxing agents (VIP and ISO) and the effects of pertussis toxin and forskolin on the galanin- and CCK-induced cell contraction. Mobilization of internal Ca^{2+} and Ca^{2+} influx into the cell may both occur in smooth muscle cells and is specific for the action of stimulating agents (Moummi, Magous & Bali, 1989). Our results indicate that the galanin-induced contraction is dependent on the influx of extracellular Ca^{2+}. Galanin-induced contraction was impaired in the absence of extracellular Ca^{2+} and blocked by 1 μM nifedipine, a blocker of Ca^{2+} channels. The dependence of the action of galanin on extracellular calcium was previously reported on isolated mouse distal colon (Fontaine & Lebrun, 1989). On the contrary, the CCK-induced contraction was insensitive to the removal of extracellular Ca^{2+} in our experiments. It was previously shown that CCK and ACh trigger internal Ca^{2+} mobilization to induce cell contraction in gastric (Makhlouf, 1987; Moummi *et al.* 1989) and intestinal muscle (Bitar & Makhlouf, 1982*a*). Opposite effects of galanin and CCK8 on Ca^{2+} fluxes could account for the synergism we observed between these agents to induce cell contraction and thus strongly suggest that different intracellular pathways may be triggered by galanin, CCK8 and ACh.

VIP and ISO inhibit, in a concentration-dependent manner, the CCK-induced cell contraction of smooth muscle cells brought about in various ways (Bitar & Makhlouf, 1982*b*; Makhlouf, 1987). This relaxing effect is known to be mediated through an increase of intracellular cAMP level and thus due to stimulation of adenylate cyclase (Honeyman, Merriam & Fay, 1977; Scheid, Honeyman & Fay, 1979; Gonda, Daniel, McDonald, Fox, Brooks & Oki, 1989; Moummi *et al.* 1989). We observed a similar effect of VIP and ISO on the CCK-induced smooth muscle cell contraction in pig ileum. On the contrary, in our experiments, both VIP and ISO failed to inhibit the galanin-induced contraction. Several possibilities can be raised in view of these results: (i) the intracellular pathway triggered by galanin could be insensitive to an increase of intracellular cAMP level; (ii) galanin could inhibit the activity of adenylate cyclase at the same time as it induces cell contraction; (iii) VIP, ISO and galanin receptors could be interdependent, the occupation of the galanin receptor impairing the effect of VIP or ISO on their specific receptor. This last assumption seems not to be true since linkage between receptor sites would involve at least three types of receptor. In other cell types, the galanin receptor is negatively coupled to the adenylate cyclase complex (Amiranoff *et al.* 1988; Lagny-Pourmir, Amiranoff, Lorinet, Tatemoto & Laburthe, 1989). However, it seems that the inability of VIP

and ISO to inhibit galanin-induced contraction may not be explained by an inhibition of adenylate cyclase by galanin. Indeed, forskolin which increases cAMP by interacting directly with adenylate cyclase (Laurenza *et al.* 1989) was unable to inhibit contraction induced by galanin in our experiments.

Most of the membrane receptors are linked to G proteins. Pertussis toxin is known to inhibit several different G proteins (Casey & Gilman, 1988) including the G_i protein that is negatively coupled to the adenylate cyclase complex (Fleming, Hodges & Watanabe, 1988). In further experiments, we observed that pertussis toxin inhibited the contraction induced by galanin but not that induced by CCK8. Thus, galanin could activate a pertussis toxin-sensitive G protein to induce contraction of pig ileum smooth muscle cells, while a G protein is obviously not involved in CCK8-induced contraction.

Finally, the synergistic action of galanin, CCK and ACh to induce contraction of pig ileum smooth muscle cells could result from their ability to trigger specific, different intracellular pathways and thereby different Ca^{2+} movements. Galanin seems to activate a pertussis toxin-sensitive G protein which triggers a specific intracellular pathway insensitive to common relaxing agents and insensitive also to the increase in cAMP level, which results in an influx of Ca^{2+} into the cell. This specificity of the pathway triggered by galanin, suggests that this peptide has a modulatory role in the control of intestinal motility by acting directly at a cellular level. However, *in vivo*, the regulatory effect of galanin on digestive motility could result from a more complex combination of this cellular action, with effects on enteric nerves (Yau, Dorsett & Youther, 1986) and at the level of the central nervous system (Rokaeus *et al.* 1984; Maiter, Hooi, Koenig & Martin, 1990).

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