Vol. 147, No. 1, 1987 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
August 31, 1987 Pages 140-144

MECHANISMS FOR THE STIMULATORY AND INHIBITORY EFFECTS OF CARBAMOYLCHOLINE ON CANINE GASTRIC D-CELLS

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Received July 15, 1987

We have previously reported that carbamoylcholine (carbachol), a recognized inhibitor of somatostatin release from D-cells, can act as stimulant following pretreatment of cells with pertussis toxin. In the present studies we have observed that pertussis toxin reverses the inhibitory effects of carbachol on D-cell stimulated with either cAMP or 12-O-tetradecanoyl-phorbol 13-acetate. Furthermore, the stimulatory effects of carbachol on D-cells pretreated with pertussis toxin potentiated the actions of pentagastrin without further enhancing the release of ^{3}H-inositol trisphosphate from prelabeled cells. These studies suggest that carbachol exerts its inhibitory effects on D-cells via pertussis toxin sensitive guaninine nucleotide binding proteins at a point distal to the activation of different signal transduction mechanisms and that the stimulatory effects of carbachol are mediated by mechanisms that are independent of membrane phosphoinositide turnover.

In previous studies we have demonstrated that carbachol, an inhibitor of somatostatin release from gastric D-cells under normal circumstances (1), can stimulate D-cells after pertussis toxin pretreatment (2). The decreased cellular production of adenosine 3':5'-cyclic monophosphate (cAMP) and enhanced turnover of membrane inositol phospholipids that accompany carbachol action on D-cells suggest possible mechanisms for, respectively, the inhibitory and stimulatory effects that were observed. We undertook these studies to further explore the potential mechanisms for muscarinic regulation of D-cells.

MATERIALS AND METHODS

Materials: Carbachol, dibutyryl cAMP (dbcAMP), 12-O-tetradecanoyl-phorbol 13-acetate (TPA), collagenase I (type I) and ethylenediaminetetra-acetic acid (EDTA) were purchased from Sigma Chemical (St. Louis, MO). Pentagastrin was a product of Peninsula Laboratories (Belmont, CA) and pertussis toxin was obtained from List Biological Laboratories (Campbell, CA). Earle's balanced salt solution and Ham's F12-Dulbecco's modified Eagle's (50:50, vol/vol) culture media were obtained from Irvine Scientific (Santa Ana, CA). (2-^{3}H)myo-inositol (15.8 Ci/mmol) was obtained from New England Nuclear (Boston, MA), and Dowex-1 resin (100-200 mesh; x 8 in the formate form) was purchased from Bio Rad (Richmond, CA).

Cell Isolation and Culture: Mucosal cells were dispersed from stripped canine fundus by sequential exposure to collagenase (0.35 mg/ml) and EDTA (1 mM), as

0006-291X/87 $1.50

described previously (1,3). Somatostatin containing D-cells were further enriched by centrifugal elutriation and then cultured at a concentration 4 x 10^6 cells/well on a bed of collagen in Ham's F12-Dulbecco's modified Eagle's medium (50:50, vol/vol) containing 10% dog serum, insulin (8 ug/ml) and hydrocortisone (1 ug/ml) in the presence of 95% air - 5% CO_2 at 37° C. After 40h of stabilization, the cells were incubated in Earle's balanced salt solution containing various test substances for a subsequent 2h experiment and somatostatin released into the media was measured by radioimmunoassay as described previously (4). Some experiments were performed after the cells had been preincubated with pertussis toxin (200 ng/ml) for 4h.

Production of ^3H-IP_3: Turnover of membrane inositol phospholipids was assessed by measuring the release of ^3H-IP_3 from D-cells after first prelabeling for 24h at 37° in culture medium (see above) containing ^3H-inositol (20 uCi/well). During the last 15 min of incubation, lithium chloride was added to achieve a final concentration of 10 mM. Following the prelabeling period, the cells were further incubated for 5 min in 1 ml of Earle's balanced salt solution containing various test substances. The incubations were terminated by the addition of 3 ml of chloroform:methanol (1:2) and ^3H-IP_3 was separated by Dowex-resin chromatography using the method of Fisher and Bartus (5). The fractions denoted as containing IP_3 may consist of a mixture of the 1,4,5 and 1,3,4 isomers.

RESULTS

As depicted in Fig. 1, carbachol inhibited the increase in somatostatin release induced by dbcAMP and TPA. Increases in cytosolic ^3H-IP_3 content were always noted in the presence of carbachol although dbcAMP and TPA by themselves had no effect. These changes in cytosolic ^3H-IP_3 content induced

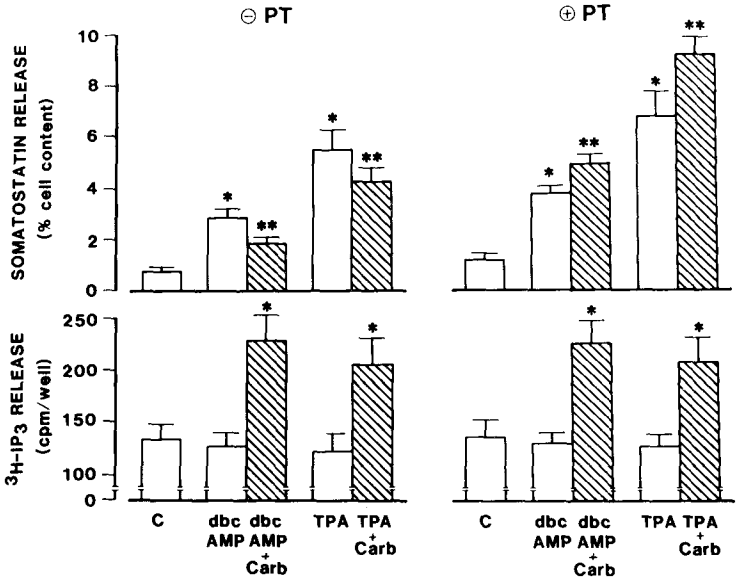

Fig. 1. Effects of pertussis toxin (200 ng/ml) pretreatment on inhibition of dbcAMP (10^{-3}M)- and TPA (10^{-7}M)-induced somatostatin release by carbachol (Carb, 10^{-4}M) and on carbachol-induced release of ^3H-IP_3 from D-cells. Data are presented as means ± SE from 4 separate dog preparations and were analyzed using Student's t-tests for paired samples. *P < 0.01 vs control (c), **P < 0.01 vs dbcAMP or TPA alone.

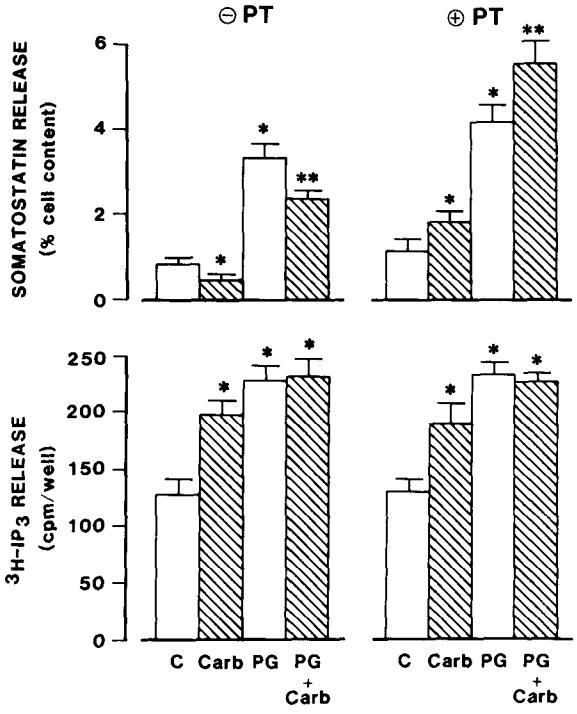

Fig. 2. Effects of pertussis toxin (200 ng/ml) pretreatment on inhibition of pentagastrin (PG, 10^{-7}M)-induced somatostatin release by carbachol (Carb, 10^{-4}M) and on carbachol and pentagastrin-induced release of ^{3}H-IP$_3$ from D-cells. Data are presented as means ± SE from 4 separate dog preparations and were analyzed using Student's t-tests for paired samples. *P < 0.01 vs control (C), **P < 0.01 vs PG alone.

by carbachol were unaltered after pre-incubation of D-cells in pertussis toxin, however, such treatment converted the inhibitory effect of the muscarinic agonist to one in which the stimulatory actions of TPA and dbcAMP were potentiated. In contrast to TPA and dbcAMP, pentagastrin, like carbachol, enhanced ^{3}H-IP$_3$ production in D-cells (Fig. 2). Carbachol inhibited the stimulatory effect of pentagastrin on somatostatin release without altering ^{3}H-IP$_3$ production. However, after pretreatment of D-cells with pertussis toxin, carbachol potentiated the stimulatory effect of pentagastrin on somatostatin release, again without changing the level of ^{3}H-IP$_3$ production.

DISCUSSION

In previous studies we have observed that inhibition of basal as well as forskolin-stimulated somatostatin release from gastric D-cells is paralleled by decreases in cellular cAMP content, and that these changes could be prevented by pretreatment with pertussis toxin (2). Since pertussis toxin is

known to inactivate the inhibitory guanine nucleotide binding proteins regulating adenylate cyclase activity by promoting their ADP-ribosylation (6), our data suggest that muscarinic agonists exert their inhibitory effects on D-cells, at least in part, by attenuation of cellular cAMP generation. The present studies indicate, however, that the inhibitory action of muscarinic agonists may occur at a point distal to the production of cAMP since the stimulation of somatostatin release by dbcAMP itself could be inhibited by carbachol in a fashion preventable with pertussis toxin. Moreover, we observed that stimulation of somatostatin release by both TPA and pentagastrin, processes which we have previously demonstrated to be mediated via protein kinase C activation (7), could also be inhibited by carbachol via a pertussis toxin sensitive pathway. Taken together, these data suggest that muscarinic inhibitory actions on D-cells may be mediated by inhibitory guanine nucleotide regulatory proteins that exert their effects at multiple sites or at some single site at which various signal transduction pathways converge to stimulate D-cells. The mechanisms for such an activity is unknown but may possibly involve such basic processes as phosphoprotein dephosphorylation or ion channel gating, both of which are known guanine nucleotide binding protein dependent actions of cholinergic agonists in other systems (8,9).

We have confirmed our original observations that after pretreatment with pertussis toxin D-cell somatostatin release can be stimulated by carbachol (2). We had speculated previously that this effect might be mediated by the various intracellular signal transduction events triggered by the pertussis toxin insensitive turnover of membrane phosphoinositides induced by carbachol in D-cells. Furthermore, in the presence of pertussis toxin carbachol potentiated D-cell stimulation by forskolin, an agent known to activate adenylate cyclase directly. However, we noted in the present studies that carbachol was also able to potentiate the stimulation of somatostatin secretion induced by pentagastrin which, as noted above, acts via protein kinase C. Furthermore, the potentiating effect of carbachol was not accompanied by a further increment in cytosolic $^{3}H\text{-}IP_3$ content. These data lead us to conclude that the stimulatory effects of carbachol on D-cells may involve additional mechanisms beyond those dependent on membrane phosphoinositide turnover.

ACKNOWLEDGEMENTS

We gratefully acknowledge the technical support of Jung Park and the secretarial assistance of Lori Ennis. This study was supported by NIH grant RO1-DK34306 and funds from the Michigan Gastrointestinal Peptide Research Center (NIH grant P30-DK34933).

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