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INHIBITORY INTERACTIONS BETWEEN STIMULUS–SECRETION PATHWAYS IN THE EXOCRINE RAT PANCREAS

P. J. CAMELLO* and G. M. SALIDO

Department of Physiology, Faculty of Veterinary Science, University of Extremadura, Apdo. 618–10080 Cáceres, Spain

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Abstract—In many tissues the cellular responses mediated through different intracellular messenger systems are mutually interactive. In the exocrine pancreas the secretagogues acting via adenosine cyclic monophosphate (cAMP) and those acting via calcium-phosphoinositides can potentiate one another. On the other hand, protein kinase C (PK-C) modulates receptor-induced responses in exocrine pancreatic cells and other cell types. Recording total protein output, monitored on-line at 280 nm, from superfused rat pancreatic segments, we demonstrate that secretin (a cAMP-acting hormone) reduces the efficacy of the calcium-mediated secretagogue cholecystokinin-octapeptide (CCK-8). Likewise, the PK-C activator 12,*O*, tetradecanoyl phorbol 13 acetate (TPA) reduces both the efficacy of secretin and the potency of cholecystokinin. Thus, the hypothesis of potentiation between different stimulus–secretion coupling mechanisms must be revised, and receptor-activated responses in the exocrine pancreas must be considered a complex model with multiple inhibitory and stimulatory interactions.

Exocrine pancreatic secretion is the final result of multiple extracellular signals acting simultaneously on acinar and ductular pancreatic cells. Some of these signals [e.g. secretin or vasoactive intestinal peptide (VIP†)] evoke the cellular response through the cAMP pathway, whereas secretagogues such as cholecystokinin (CCK) or acetylcholine (ACh) activate the phosphoinositide pathway, increasing intracellular levels of Ca^{2+} and diacylglycerol, the activator of protein kinase C (PK-C) [1, 2].

For more than a decade it has been accepted that when two secretagogues acting via different stimulus–secretion coupling mechanisms are administered together, the overall secretion is potentiated, that is, it is greater than the addition of the individual responses [3]. Association of cAMP-mobilizing hormones with calcium-mediated secretagogues evokes a potentiated response [4, 5]. Similar findings have been obtained with a combination of compounds inducing (or imitating) an increase in the intracellular levels of second messengers [4–6]. Besides this type of interaction, in the exocrine pancreas as well as other tissues, phorbol esters, known activators of PK-C, modulate some cellular responses elicited either by cAMP- [7–10] or by phosphoinositide-linked mechanisms [10–14].

Our object was to investigate, using an on-line *in vitro* method, the potentiation hypothesis between secretin and cholecystokinin-octapeptide (CCK-8) over a wide range of concentrations, and to establish if the phorbol ester 12, *O*, tetradecanoyl phorbol 13 acetate (TPA) modulates the secretion induced by these two peptides.

MATERIALS AND METHODS

Perfusion technique. All the experiments were performed on isolated segments of rat pancreas. Adult animals (200–300 g) were killed by cervical dislocation, and the pancreas was quickly removed and placed in modified Krebs–Henseleit (KH) solution of the following composition (mM): NaCl 103, KCl 4.7, $CaCl_2$ 2.56, $MgCl_2$ 1.13, $NaHCO_3$ 25.0, NaH_2PO_4 1.15, D-glucose 5.5, sodium pyruvate 4.9 and sodium glutamate 4.9. The solution, gassed with 5% CO_2 in O_2, was maintained at 37° and pH 7.3–7.4.

Following the procedure described previously [15], the pancreas was cut into small segments (5–10 mg), and a total weight of about 200 mg was placed in a Perspex flow chamber (volume 1 mL) and superfused with the normal KH solution (1.5 mL/min) for 30–40 min to allow the tissue to equilibrate. Then, the compounds were dissolved in the KH solution immediately prior to applying the stimuli for 10 min. Total protein concentration in the effluent was assayed by UV absorbance at 280 nm (using an on-line UV detector for liquid chromatography—EM1, Biorad, Richmond, CA, U.S.A.), monitoring the optical density with a pen recorder. The method was validated by performing an amylase assay on the effluent. The values referred to 100 mg tissue, using bovine serum albumin (fraction V, Sigma Chemical Co., St Louis, MO, U.S.A.) as standard. The responses were expressed as net increases of total protein output (μg protein/mL/min/100 mg) by measuring the area under the curve (i.e., test minus basal values during the 10 min of stimulation). At the end of the experiment the segments were exposed to 10^{-5} M ACh to check the viability of the tissue.

* Corresponding author. FAX (34) 927-25 71 10.

† Abbreviations: ACh, acetylcholine; CCK-8, cholecystokinin octapeptide; EC_{50}, effective concentration; $Ins(1,4,5)P_3$, inositol 1,4,5 trisphosphate; KH, Krebs–Henseleit solution; MR, maximal response; PK-C, protein kinase C; TPA, 12,*O*,tetradecanoyl phorbol 13 acetate; VIP, vasoactive intestinal peptide.

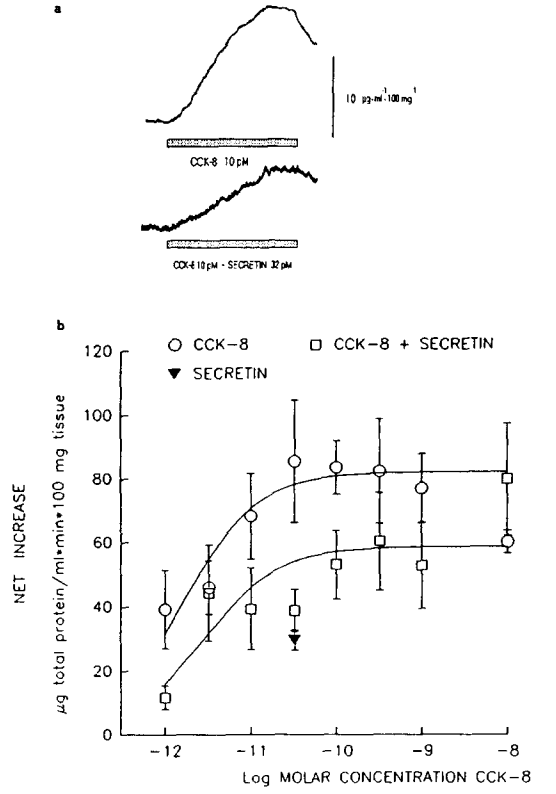

Fig. 1. (a) Original records showing the effects of 10 pM CCK-8 alone or in the presence of 32 pM secretin on the total protein output from superfused pancreatic segments. The horizontal bar is equivalent to 10 min, and a vertical calibration bar is displayed. The responses were recorded from independent experiments, and are representative of six more. (b) Effects of CCK-8 alone or in the presence of 32 pM secretin on the protein output from rat pancreatic segments. Each point is mean ± SE from five to nine separate experiments. The sigmoid lines represent the best fitted models.

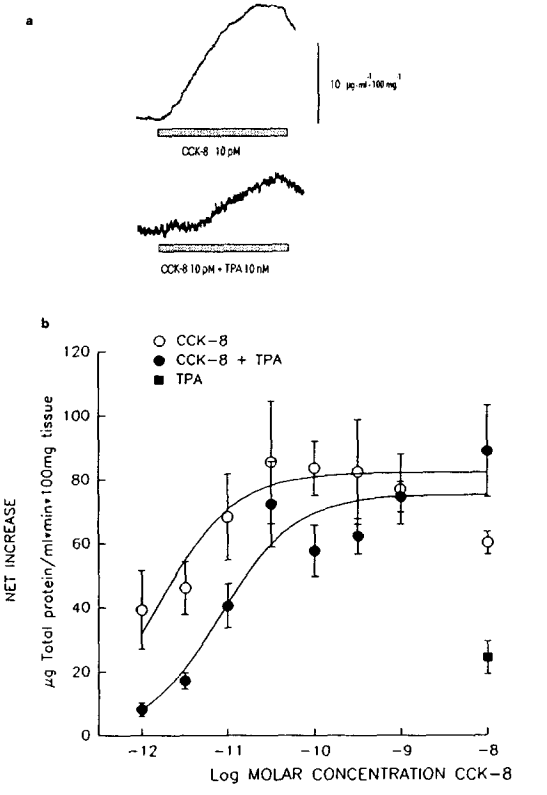

Fig. 2. (a) Protein output induced by 10 pM CCK-8 alone or in the presence of 10 nM TPA. The horizontal bar is equivalent to 10 min, and a vertical calibration bar is displayed. The traces were obtained from separate experiments representative of five to eight more. (b) Dose–response curves for CCK-8- and for CCK-8 + 10 nM TPA-induced protein output from superfused rat pancreatic segments. Data are means ± SEM of five to eight experiments. The sigmoid lines represent the best fitted models.

To study the interaction between secretin and CCK-8 and between TPA and secretin or CCK-8, we carried out dose–response curves (10^{-12}–10^{-8} M) for CCK-8 and secretin (Sigma), alone or in combination with 10 nM TPA (Sigma), and for CCK-8 in the presence of 32 pM secretin.

Statistical analyses. For each dose the mean and the standard error (SE) were calculated and plotted. To study the dose–response parameters median effective concentration (EC_{50}) and maximal response (MR), a nonlinear fitting method was used, following the procedure developed by Meddings *et al.* [16], that allows a statistical comparison of these parameters between different dose–response curves.

RESULTS

Total protein basal output from superfused pancreatic segments was 37.29 ± 0.58 μg/mL/100 mg (mean ± SE, N = 127). Figures 1–3 show original chart records obtained with the indicated stimuli. The pattern of the response was similar to that described by several authors using an on-line amylase detecting technique [15].

The protein output evoked by CCK-8 at concentrations between 10^{-12} and 10^{-8} M is shown in Fig. 4. As can be seen, the pattern was biphasic, reaching a maximum at a concentration of 32 pM. Greater concentrations produced smaller or equal responses. This biphasic contour is a well known characteristic of CCK- and cholinergic-induced enzymatic secretion *in vitro* [3]. As shown also in Fig. 4, the effect of secretin on protein output was dose-dependent. As was expected, secretion was smaller than that from similar doses of CCK-8, reaching a maximum at 320 pM. In this case the biphasic contour was not observed. Both potency (EC_{50}) and efficiency (MR) were greater for CCK-8 (Table 1); so, EC_{50} was significantly smaller for CCK-8 alone, and MR was smaller for secretin ($P < 0.01$).

When the dose–response curve for CCK-8 was repeated in the presence of 32 pM secretin, the protein output was impaired for all concentrations,

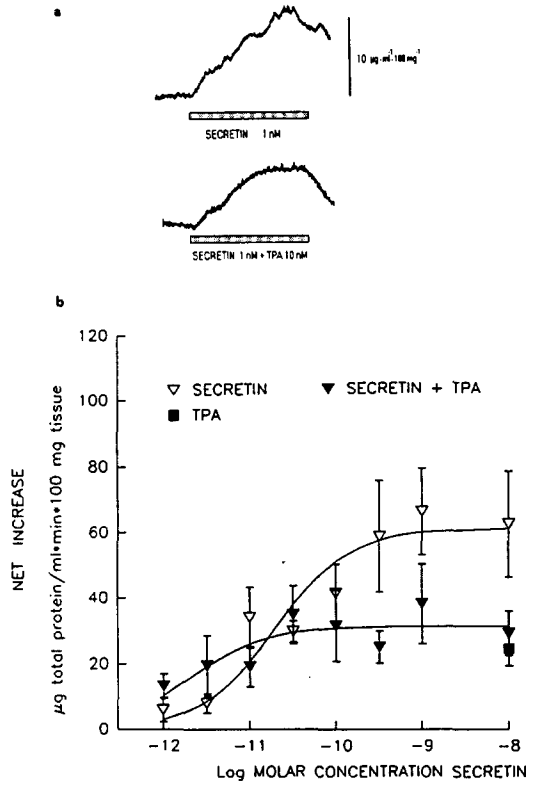

Fig. 3. (a) Effect of 1 nM secretin and 1 nM secretin + 10 nM TPA on the protein output from pancreatic superfused segments. A horizontal 10-min bar and a vertical calibration line are displayed. The traces were obtained from separate experiments representative of five to nine more. (b) Effect of 10 nM TPA on the secretin-evoked response. Data are means ± SE of five to nine experiments. The solid lines are the best fitted models.

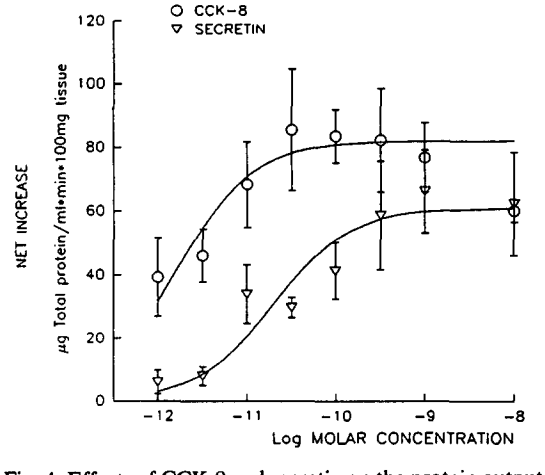

Fig. 4. Effects of CCK-8 and secretin on the protein output from perfused segments of rat pancreas. The tissue was stimulated for 10 min with the indicated concentrations of peptide, and the responses are expressed as net increases above pre-stimulus values. Each point represents mean ± SE of five to nine separate experiments. The solid lines are the best fitted models obtained using the nonlinear procedure described in Materials and Methods.

except for 3.2 pM and 10 nM (Fig. 1). The MR of CCK-8 (but not its EC_{50}) was statistically decreased with secretin ($P < 0.01$) (Table 1). Only for 10 nM of CCK-8 was the response additive. These results do not suggest either potentiation or addition between 32 pM secretin and a wide range of CCK-8 concentrations.

In order to check the effect of PK-C activation on the responses evoked by secretin and CCK-8, we selected a submaximal concentration of 10 nM of TPA (protein output was 15.33 ± 4.1 µg/mL/min/100 mg for 3.2 nM TPA, 24.51 ± 5.14 µg/mL/min/100 mg for 10 nM and 24.93 ± 5.9 µg/mL/min/100 mg for 1 µM TPA, N = 5). This concentration activates PK-C in acinar cells in just a few minutes [17].

As shown in Fig. 2, in the presence of TPA the dose–response curve for CCK-8 was shifted to the right. The EC_{50} was significantly increased (1.61 ± 0.44 pM for CCK-8, and 8.27 ± 3.47 pM for CCK-8 + TPA, $P < 0.01$), while the efficacy was not changed (Table 1). As for the response to secretin, the phorbol ester decreased the secretion induced by the highest concentrations tested of the hormone (Fig. 3). Thus, the MR decreased to 50% (61.85 ± 4.84 µg/mL/min/100 mg in the presence of secretin, and 31.30 ± 2.50 in the presence of secretin and TPA, $P < 0.01$). Surprisingly, the potency of the hormone was improved, since a marked decrease in EC_{50} was observed (Table 1), although the responses to 1 and 3.2 pM secretin were only slightly higher in the presence of TPA (Fig. 3) and nevertheless smaller than the addition of the individual responses to secretin and TPA.

DISCUSSION

In many experimental systems, including the exocrine pancreas, cAMP and Ca^{2+}/PK-C transduction systems have shown to be subject to reciprocal interaction [3, 18]. It has been postulated that a calcium-mediated and a cAMP-mediated secretagogue can potentiate one another [3]. However, in agreement with previous studies showing lack of potentiation between pancreatic secretagogues [19, 20] our results do not support this concept. In addition, other authors found potentiation only for some secretory parameters [21, 22] or certain secretagogue concentrations, even some of the studies that gave rise to the theory [3, 4, 6]. Recent data show that this process depends on a cholinergic tone [23]. In light of all these findings the hypothesis of potentiation between pancreatic secretagogues must be revised, and probably is only valid under particular circumstances of dose, secretory parameter and type of secretagogue.

Moreover the present results indicate that secretin can inhibit CCK-8-induced enzymatic secretion, since their combined effect is smaller than the addition of the separate responses (this can not be explained as an incapacity to secrete more proteins, because it also occurs at low concentrations of CCK-8). We showed previously a similar response in rat pancreas (both *in vivo* and *in vitro*) associated to a decrease in the CCK-8-evoked calcium signal when it is combined with secretin [19]. In that work we

Table 1. EC_{50} and MR of the dose–response curves for CCK-8 (alone or plus 10^{-8} M TPA or plus 32 pM secretin) and for secretin (alone or plus 10^{-8} M TPA)

	EC (pM)	MR (μg/mL min/100 mg tissue)
CCK-8	1.61 ± 0.44	82.25 ± 3.44
Secretin	19.65 ± 8.28*	61.18 ± 4.84*
CCK-8 + 32 pM secretin	2.72 ± 1.83	58.75 ± 6.01*
CCK-8 + 10^{-8} M TPA	8.27 ± 3.47*	75.37 ± 5.45
Secretin + 10^{-8} M TPA	2.03 ± 1.11†	31.30 ± 2.50†

* $P < 0.01$ with respect to CCK-8 alone.
† $P < 0.001$ with respect to secretin alone.
Values are means ± SEM.

ruled out the possibility that cAMP is responsible for this inhibition reducing the amount of calcium released by inositol trisphosphate [Ins(1,4,5)P_3] [24], since neither dibutyryl cAMP nor forskolin inhibited the response to CCK-8 [19]. The inhibition could be mediated by Mg^{2+} because it inhibits the ACh-evoked secretion [25], and CCK-8 stimulates MGg^{2+} efflux from pancreatic cells while secretin induces a net uptake alone or with CCK-8 [19]. In any case, it is possible that secretin inhibits CCK-8 via Gs proteins (the link between secretin receptors and adenylate cyclase [1]) functionally coupled to CCK receptors in rat acinar cells [26].

Concerning the effects of TPA on the CCK-8-induced secretion, our results are similar to previous reports showing a decrease in potency (but not the efficacy) of carbachol [14] and CKK [11, 12] in pancreatic acini, probably due to the effect of 10 nM TPA on the affinity of the high-affinity CCK receptors in rat acini [12]. Another possible target for TPA is the mobilization of calcium because it impairs the calcium signal evoked by CCK-8 [11] and carbachol [27]: PK-C stimulates the Ca-ATPase membrane pump (finalizing the calcium signal) [28], and reduces the Ins(1,4,5)P_3 receptor affinity and the total amount of Ca^{2+} released by this messenger [29]. Similar effects are also present in other tissues [10].

In the case of secretin, although the main effect of TPA was a marked decrease in the efficacy of the hormone (in line with previous studies *in vivo* [9] and *in vitro* [8]), TPA can increase the VIP-evoked amylase secretion by potentiating the rise in cAMP levels [7]. The effects of phorbol esters on cAMP pathways range from potentiation (salivary glands [30]) to reduction (MDCK cells [31]); both effects can even be observed depending on the stimulus [10, 32, 33]. Available evidence indicates receptors and G proteins (or coupling between them) as the point of interaction [33, 34]. The only explanation we can suggest is a difference in the receptors and/or G proteins mediating the response to secretin and VIP [2], thus allowing PK-C to modulate selectively the response to these hormones.

In conclusion, our data show that the hypothesis of potentiation between cAMP- and phosphoinositide-linked secretagogues in the exocrine pancreas must be revised. We also demonstrate an inhibitory role for PK-C in the responses mediated by cAMP or Ca^{2+}/PK-C.

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