Effects of stimulation of muscarinic and of β -catecholamine receptors on the intracellular distribution of protein kinase C in guinea pig exocrine glands

Estela MACHADO-DE DOMENECH and Hans-Dieter SÖLING*

Abteilung Klinische Biochemie, Zentrum Innere Medizin, Universität Göttingen, Robert Koch-Strasse 40, D-3400 Göttingen, Federal Republic of Germany

Stimulation of exocrine cells via muscarinic receptors is associated with an activation of protein kinase C [Padel & Söling (1985) *Eur. J. Biochem.* **151**, 1–10]. We show here that stimulation of isolated parotid gland lobules with 8×10^{-6} M-carbamoylcholine leads to a translocation of protein kinase C from the cytosolic to the particulate compartment within 30 s (25% and 45% of total activity recovered in the particulate fraction of controls and stimulated samples respectively). The specific enzyme activity in the particulate fraction increased to 169% of the corresponding control value. After 10 min the changes started to reverse and, after 30 min, cytosolic protein kinase C was higher in stimulated than in unstimulated lobules. Isoproterenol (2×10^{-5} M) stimulated the release of amylase more than did carbamoylcholine, but did not significantly affect intracellular distribution of protein kinase C during the observation time of 30 min. In isolated pancreatic lobules a significant carbamoylcholine-mediated translocation of protein kinase C into the particulate fraction could be observed after 5 and 20 min, but not after 1 min. After 5 min the specific enzyme activity in the particulate fraction had increased to 153% of the corresponding controls. The corresponding decrease (–38%) in the specific enzymic activity of cytosolic protein kinase C stayed constant up to 30 min. In isolated parotid gland lobules α -amylase secretion proceeded at a linear rate already during the first 1 min of stimulation, whereas in pancreatic lobules a measurable rate of α -amylase secretion did not occur before 5 min. These differences in time course paralleled the differences in the onset of translocation of protein kinase C. The results support a direct involvement of protein kinase C in carbamoylcholine-mediated but not in isoproterenol-mediated stimulation of exocytosis in exocrine cells.

INTRODUCTION

Protein kinase C represents one of the intracellular protein kinases that can be regulated by second messengers (Takai *et al.*, 1979a,b; Kishimoto *et al.*, 1980). Among them, diacylglycerol is of special importance as it increases the affinity of the enzyme for calcium. Phorbol esters seem to activate protein kinase C by the same mechanism as do diacylglycerols (Castagna *et al.*, 1982). They induce in addition a shift of the intracellular distribution of the enzyme from the cytosolic to the membrane compartment(s) of the cell (Kraft *et al.*, 1982; Kraft & Anderson, 1983; McCaffrey *et al.*, 1984). Wooten & Wrenn (1984) have observed such an effect of phorbol esters also in isolated rat pancreatic acini. The question is whether more physiological stimuli also lead to a redistribution of protein kinase C. In experiments with permanent cultured cell lines various groups have shown that receptor-mediated stimulation involving the polyphosphoinositide cycle indeed leads to a shift of protein kinase C from the cytosol to the membrane fraction (Fearon & Tashjian, 1985; Farrar & Anderson, 1985; Farrar *et al.*, 1985; Drust & Martin, 1985). Only Hirota *et al.* (1985) studied the effect of a stimulus on protein kinase C distribution in a non-permanent cell culture system (rat pituitary gonadotrophs) and found a shift of protein kinase into the membrane fraction within 10–20 min following stimu-

lation with gonadotropin-releasing hormone. Data concerning the distribution behaviour of protein kinase C in exocrine glands in the presence of physiological stimuli are missing. We show here for the first time that stimulation with the acetylcholine analogue carbamoylcholine leads to a translocation of protein kinase C from the soluble to the particulate compartment in isolated lobules from the parotid gland and the pancreas of the guinea pig. The different time course of onset of exocytosis in the two glands is paralleled by corresponding differences in the time course of translocation of protein kinase C. Isoproterenol, which stimulates exocytosis in the guinea pig parotid gland even more than carbamoylcholine, does not have a measurable effect on the distribution of protein kinase C.

EXPERIMENTAL

Materials

Male guinea pigs of the Pirbright white strain (150–200 g body wt.; fasted overnight) were obtained from Winkelmann, Borcheln bei Paderborn, Germany. They received a standard chow (Altromin R; Altrogge, Lage/Lippe, Germany) and vitamin C in the drinking water. Histone III S, phosphatidylserine, diolein, isoproterenol, and carbamoylcholine chloride were purchased from Sigma, leupeptin was from the Peptide Institute

* To whom correspondence and reprint requests should be addressed.

Inc., Osaka, Japan, and aprotinin was a kind gift from Dr. Puls, Bayer Werke AG, Aprath, Germany. DEAE-cellulose (DE-52) and glass microfibre filters (type GF/C, diam. 2.4 cm) came from Whatman. All other chemicals were from Merck. [γ - 32 P]ATP was obtained from Amersham-Buchler, Braunschweig, Germany.

Preparation and incubation of isolated lobules from parotid glands and pancreas

This was performed as described previously (Scheele & Palade, 1975; Padel *et al.*, 1982). After preincubation for 15 min in Krebs–Ringer/Hepes supplemented with 5 mM-glucose and 0.02% bovine serum albumin the medium was replaced by fresh medium and 15 min later, carbamoylcholine (final concn. 8×10^{-6} M) was added. At the time points indicated, aliquots (100 μ l) of the incubation medium were removed for the determination of α -amylase activity according to Bernfeld (1955). In experiments where the distribution of protein kinase C was determined earlier than 15 min after application of the stimulus, incubations for the determination of α -amylase secretion were run in parallel. At the time points indicated after addition of carbamoylcholine the incubated lobules were rapidly removed and washed with ice-cold 'stop solution' (0.3 M-sucrose, 20 mM-Tris/HCl, pH 7.4, 2 mM-EDTA, 50 mM-2-mercaptoethanol, 4 μ g of leupeptin/ml, 0.1 mM-phenylmethanesulphonyl fluoride and 10 μ g of aprotinin/ml). Subsequently the lobules were homogenized in a glass/Teflon Potter homogenizer with 5 vol. (v/w) of the 'stop solution' and centrifuged at 600 *g* for 5 min to remove unbroken cells. The pellet was resuspended in 1 ml of 'stop solution' and centrifuged again at 600 *g* for 5 min. The two resulting supernatants were combined and centrifuged for 60 min at 100 000 *g*_{av.}. The resulting pellet was resuspended in buffer A (20 mM-Tris/HCl, pH 7.4, 2 mM-EDTA, 50 mM-2-mercaptoethanol, 4 μ g of leupeptin/ml and 100 μ g of aprotinin/ml) containing in addition 2.5 mM-EGTA and 0.3% Triton X-100 and left on ice for 1 h. The suspension was centrifuged for 60 min at 100 000 *g*_{av.} and the

supernatant used for the determination of particulate protein kinase C activity.

DEAE-cellulose chromatography

The supernatants from the first 100 000 *g* centrifugation ('cytosolic fraction') as well as from the second 100 000 *g* centrifugation ('particulate fraction') were loaded onto small DEAE-cellulose columns (0.5 cm $\times$ 4 cm) which had been equilibrated with buffer A. After loading, the columns were washed with buffer A and protein kinase C activity was eluted with 75 mM-KCl. Protein kinase C was usually eluted completely in 2–2.5 ml.

Assay of protein kinase C activity

The enzyme activity was assayed in a final volume of 0.1 ml containing (final concentrations): 20 mM-Hepes, pH 7.4, 10 mM-MgCl₂, 0.5 mM-CaCl₂, 0.25 mg of histone IIIS/ml, 50 μ g of phosphatidylserine/ml, 5 μ g of diolein/ml and 20 μ l of the protein-kinase-C-containing DEAE-cellulose eluate. Assays in the absence of phosphatidylserine and diolein served as controls. After thermoequilibration (30 °C for 5 min), the reaction was started by addition of [γ - 32 P]ATP (final concn. 50 μ M; 200 d.p.m./pmol). After 10 min the reaction was stopped by addition of 20 μ l of 50 mM-potassium phosphate, pH 7, containing in addition 10 mM-EGTA, 2 mM-EDTA, 5 mM-unlabelled ATP and 5 mg of bovine serum albumin/ml. The stopped reaction mixture was applied to Whatman glass fibre filters (diam. 2.4 cm) and the protein precipitated onto the filter with 25% (w/v) trichloroacetic acid. The filter plates, which had been placed on a multi-well suction funnel, were washed under vacuum with 25% trichloroacetic acid (about 15 ml/filter) and placed into glass counting vials. The radioactivity was counted as Cerenkov radiation. The sum of protein kinase C activity in the soluble and the particulate fraction amounted to 83% of the activity measured in total homogenates treated with 0.3% Triton X-100. In the following, total protein kinase C activity is

Table 1. Effect of stimulation with carbamoylcholine on the intracellular distribution of protein kinase C in isolated guinea pig parotid gland lobules

Guinea pig parotid gland lobules were prepared and incubated as given in the Experimental section. Immediately before and 30 s, 10 min and 30 min after addition of carbamoylcholine (8×10^{-6} M), lobules were removed and analysed for soluble and particulate protein kinase C activity. The distribution of protein kinase C is expressed as a percentage of total protein kinase C measured in the total particulate and the total soluble fractions. Total protein kinase C activity at time zero (particulate + soluble) was 2479 ± 142 pmol/min per 300 mg wet wt. (means $\pm$ S.E.M., $n = 6$). The numbers in parentheses give the distribution of total protein in the protein-kinase-C-containing soluble and particulate fractions as a percentage of the sum of total protein in both fractions. The values in the table represent means $\pm$ S.E.M. from three to five experiments for each time point. (P) and (S), particulate and soluble fractions respectively. * $P < 0.001$ versus controls, ** $P < 0.01$ versus controls.

Experimental conditions		Distribution of protein kinase C (% of total activity) at:			
		Time zero	30 s	10 min	30 min
Controls	(P)	25 $\pm$ 1.4	25 $\pm$ 1.6	34 $\pm$ 1.7	46 $\pm$ 2.9
		(35 $\pm$ 1.4)	(40 $\pm$ 1.0)	(28 $\pm$ 1.0)	(24 $\pm$ 3.5)
	(S)	75 $\pm$ 1.6	75 $\pm$ 1.4	66 $\pm$ 1.8	54 $\pm$ 3.1
		(65 $\pm$ 1.3)	(60 $\pm$ 0.7)	(72 $\pm$ 0.5)	(76 $\pm$ 3.5)
Carbamoylcholine	(P)	–	45 $\pm$ 1.5*	43 $\pm$ 2.4**	33 $\pm$ 1.8*
		–	(36 $\pm$ 1.5)	(28 $\pm$ 2.0)	(26 $\pm$ 3.4)
	(S)	–	55 $\pm$ 1.4*	57 $\pm$ 2.7**	67 $\pm$ 1.7*
		–	(64 $\pm$ 1.2)	(72 $\pm$ 2.0)	(74 $\pm$ 3.4)

Fig. 1. Protein kinase C specific activity and amylase secretion following stimulation of isolated guinea pig parotid gland lobules with carbamoylcholine

Parotid gland lobules were prepared and incubated as given in the Experimental section. Lobules and medium were analysed immediately before, and at the indicated time points after, addition of 8×10^{-6} M-carbamoylcholine for protein kinase C activities and α -amylase release respectively. (a) Specific activities of protein kinase C. Lobules were analysed for protein kinase C specific activities in the soluble and particulate fractions as described in the Experimental section. The specific activities are given for the eluates from the DEAE-cellulose chromatography. The values are expressed as percentages of specific activity measured in unstimulated lobules at the corresponding time points (means $\pm$ S.E.M.; numbers of separate experiments are given in parentheses). The specific activities of protein kinase C taken at time zero were 3380 ± 138 pmol/min per mg of protein and 1632 ± 101 pmol/min per mg of protein in DEAE-cellulose eluates from the soluble and the particulate compartment respectively (means $\pm$ S.E.M., $n = 6$). (b) Cumulative release of α -amylase, from the experiments given in (a).

defined as the sum of soluble and particulate protein kinase C activity.

Other methods

Protein was determined by the dye-binding assay of Bradford (1976).

RESULTS

Experiments with guinea pig parotid lobules

Control state. Total protein kinase C activity amounted to 2479 ± 142 pmol/min per 300 mg wet wt. of tissue (mean $\pm$ S.E.M., $n = 6$). It was not significantly affected by stimulation with 8×10^{-6} M-carbamoylcholine. It was $88 \pm 5\%$, $90 \pm 5\%$, and $99 \pm 9\%$ of the corresponding control values at 30 s, 10 min and 30 min respectively.

In the unstimulated state only 25% of the total activity of protein kinase C was recovered in the particulate fraction (Table 1). During the 30 min of incubation a shift of protein kinase C from the cytosol to the particulate fraction could be observed under control conditions: after 10 min 34% and after 30 min 46% of total protein kinase C activity was detected in the particulate fraction (Table 1).

Stimulation with carbamoylcholine. Following stimulation with 8×10^{-6} M-carbamoylcholine a significant shift of protein kinase C activity from the cytosol to the particulate fraction occurred which was most pronounced 30 s after the addition of the secretagogue (Table 1). The specific enzymic protein kinase C activities cannot be given per mg of particulate or soluble protein as protein kinase C activity could only be measured reliably after DEAE-cellulose chromatography. However, since tissue preparation and DEAE-cellulose chromatography were performed in a standardized way, the specific enzyme activities in the eluates from the DEAE-cellulose columns can be compared. Moreover, the total amounts of protein in the protein-kinase-C-containing fractions remained unchanged (Table 1). After 30 s of stimulation the specific protein kinase C activity in the particulate fraction had increased to about 170% of that of unstimulated controls, whereas the specific activity in the eluate from the soluble fractions had decreased to about 55% of that of unstimulated controls (Fig. 1). After 10 min of stimulation the specific activities in the stimulated samples had already started to revert to the control state (Fig. 1).

In accordance with the rapid effect of carbamoylcholine on the intracellular distribution of protein kinase C, the stimulation of exocytosis seems also to take place almost immediately after application of the stimulus, as indicated by the release of amylase (Fig. 1).

Stimulation with isoproterenol. Under our experimental conditions, isoproterenol stimulated exocytosis as least as well as did carbamoylcholine (compare Fig. 1 and Table 2), but had no significant effect on the distribution of protein kinase C or its specific activity at any of the time points measured (Table 2).

Stimulation of pancreatic lobules

Total protein kinase C activity of the guinea pig pancreas amounted to 4560 ± 743 pmol/min per 300 mg wet wt. (mean $\pm$ S.E.M., $n = 6$). Stimulation with 8×10^{-6} M-carbamoylcholine did not significantly affect total (= particulate + soluble) protein kinase C activity. Total activity of the stimulated samples amounted to $99 \pm 3\%$, $93 \pm 6\%$ and $101 \pm 6\%$ of the corresponding unstimulated samples at 1, 5 and 20 min after stimulation, respectively.

Upon stimulation with carbamoylcholine (8×10^{-6} M)

Table 2. Effects of isoproterenol on the intracellular distribution of protein kinase C and on the release of α -amylase in experiments with isolated guinea pig parotid gland lobules

Parotid gland lobules were isolated and incubated as given in the Experimental section. At the time points indicated after addition of isoproterenol (final concn. 2×10^{-5} M) the lobules were removed and analysed for the intracellular distribution of protein kinase C as described in the legends to Fig. 1 and Table 1. At time zero and after 30 s and 15 and 30 min of stimulation aliquots of the incubation medium were removed and analysed for α -amylase activity. The values given represent amylase secreted during 15 and 30 min respectively. Values in parentheses give the distribution of protein kinase C as percentage of total protein kinase C, which was set as 100%. The values in brackets give the distribution of total protein in the protein-kinase-C-containing soluble and particulate fractions (as a percentage of total protein in these fractions). Values are means $\pm$ s.e.m. from three separate experiments for each time point. (S), soluble fraction; (P), particulate fraction. * $P > 0.5$ versus controls. Abbreviation: PK-C, protein kinase C.

Experimental conditions	30 s				15 min				30 min			
	PK-C activity				PK-C activity				PK-C activity			
	Total (pmol/min)	(S)	(P)	Specific (pmol/min per mg)	Total (pmol/min)	(S)	(P)	Specific (pmol/min per mg)	Total (pmol/min)	(S)	(P)	Specific (pmol/min per mg)
Controls	2355 $\pm$ 65 (78 $\pm$ 1) [64 $\pm$ 2]	3723 $\pm$ 219 (22 $\pm$ 1) [36 $\pm$ 2]	1934 $\pm$ 179 (36 $\pm$ 2) [64 $\pm$ 2]	678 $\pm$ 52 (22 $\pm$ 1) [36 $\pm$ 2]	2253 $\pm$ 77 (73 $\pm$ 4) [69 $\pm$ 5]	847 $\pm$ 63 (27 $\pm$ 2) [31 $\pm$ 4]	3084 $\pm$ 112 (27 $\pm$ 2) [31 $\pm$ 4]	3792 $\pm$ 89 (27 $\pm$ 2) [31 $\pm$ 4]	645 $\pm$ 260 (23 $\pm$ 6) [31 $\pm$ 1]	3223 $\pm$ 151 (23 $\pm$ 6) [31 $\pm$ 1]	1983 $\pm$ 88 (23 $\pm$ 6) [31 $\pm$ 1]	15 $\pm$ 4
Isoproterenol	1946 $\pm$ 95 (74 $\pm$ 2)* [65 $\pm$ 1]	3107 $\pm$ 216 (26 $\pm$ 3)* [35 $\pm$ 1]	2072 $\pm$ 220 (26 $\pm$ 3)* [35 $\pm$ 1]	715 $\pm$ 67 (26 $\pm$ 3)* [35 $\pm$ 1]	2991 $\pm$ 106 (72 $\pm$ 1)* [65 $\pm$ 2]	972 $\pm$ 82 (28 $\pm$ 1)* [35 $\pm$ 3]	3043 $\pm$ 44 (28 $\pm$ 1)* [35 $\pm$ 3]	4278 $\pm$ 203 (72 $\pm$ 1)* [35 $\pm$ 3]	780 $\pm$ 257 (28 $\pm$ 7)* [35 $\pm$ 5]	3197 $\pm$ 341 (28 $\pm$ 7)* [35 $\pm$ 5]	2220 $\pm$ 141 (28 $\pm$ 7)* [35 $\pm$ 5]	106 $\pm$ 22
				Amylase released (units/100 mg)				Amylase released (units/100 mg)				Amylase released (units/100 mg)
				4.3 $\pm$ 1.0				4.3 $\pm$ 1.0				4.3 $\pm$ 1.0
				34 $\pm$ 6				34 $\pm$ 6				34 $\pm$ 6

Fig. 2. Specific enzymic activities of protein kinase C and amylase secretion following stimulation of isolated guinea pig pancreatic lobules

Pancreatic lobules were prepared and incubated as described in the Experimental section. Following stimulation with 8×10^{-6} M-carbamoylcholine, lobules and medium were removed for the determination of specific protein kinase C activities and α -amylase release respectively. (a) Specific activities of protein kinase C. Lobules were analysed for protein kinase C specific activities in the soluble and particulate fractions as described in the Experimental section. The specific activities are given for the eluates from the DEAE-cellulose chromatography. The results designated by squares are from initial experiments in which only the effect of stimulation on cytosolic protein kinase C had been studied. All values are expressed as percentages of specific activity measured in unstimulated lobules at the corresponding time points (means $\pm$ s.e.m.; number of separate experiments given in parentheses). The protein kinase C specific activities taken at time zero were 1595 ± 335 pmol/min per mg of protein and 845 ± 113 pmol/min per mg of protein in DEAE-cellulose eluates from the soluble and the particulate compartment respectively (means $\pm$ s.e.m., $n = 6$). (b) Cumulative release of α -amylase, from the experiments given in (a).

a maximal shift of protein kinase activity to the membrane fraction could be observed no earlier than 5 min after stimulation, but membrane protein kinase C remained at this level over the following 15 min (Table 3). The changes in distribution of total protein kinase C following carbamoylcholine treatment are paralleled by

Table 3. Effect of stimulation with carbamoylcholine on the intracellular distribution of protein kinase C in isolated guinea pig pancreatic lobules

Preparation and incubation of isolated pancreatic lobules was performed as given in the Experimental section. Pancreatic lobules were removed immediately before and at the indicated time-points after stimulation with 8×10^{-6} M-carbamoylcholine and analysed for soluble and particulate protein kinase C activity. The distribution is expressed as a percentage of total protein kinase C activity. Total protein kinase C activity at time zero was 4502 ± 743 pmol/min per 300 mg wet wt. (means $\pm$ S.E.M.; $n = 6$). The values in parentheses give the distribution of total protein in the protein-kinase-C-containing soluble and particulate fractions as a percentage of the sum of total protein in both fractions. The values in the Table are mean values $\pm$ S.E.M. from three to five separate experiments for each time point. (P) and (S), particulate and soluble fractions respectively. * $P < 0.001$, ** $P < 0.01$, *** $P > 0.20$ versus controls.

Experimental conditions		Distribution of protein kinase C (% of total activity) at:			
		Time zero	1 min	5 min	20 min
Controls	(P)	22 $\pm$ 1.9 (35 $\pm$ 1.2)	22 $\pm$ 1.8 (34 $\pm$ 1.9)	20 $\pm$ 2.0 (34 $\pm$ 0.5)	28 $\pm$ 2.6 (38 $\pm$ 6.2)
	(S)	78 $\pm$ 1.8 (65 $\pm$ 1.7)	78 $\pm$ 2.1 (66 $\pm$ 1.9)	80 $\pm$ 2.1 (66 $\pm$ 0.5)	72 $\pm$ 2.8 (72 $\pm$ 6.0)
	(P)	—	25 $\pm$ 2.7*** (36 $\pm$ 3.0)	38 $\pm$ 4.0** (34 $\pm$ 0.1)	45 $\pm$ 2.4* (35 $\pm$ 3.1)
Carbamoylcholine	(S)	—	75 $\pm$ 2.5*** (64 $\pm$ 3.1)	62 $\pm$ 3.9** (66 $\pm$ 1.0)	55 $\pm$ 2.3* (65 $\pm$ 3.4)

corresponding changes in the specific enzyme activities (Fig. 2).

The slower onset of intracellular translocation of protein kinase C in pancreatic lobules following stimulation in comparison with parotid gland lobules corresponds with a slower onset of exocytosis (Fig. 2).

DISCUSSION

The results reported here show for the first time that stimulation of exocytosis in exocrine glands by the acetylcholine analogue carbamoylcholine leads to a shift in the intracellular distribution of protein kinase C. It is unknown at present to what extent the measured distribution gives a quantitatively exact picture of the real distribution in the intact cell. It cannot be excluded that homogenization of the cells and the subsequent procedures lead to some redistribution of the enzyme. On the other hand, the time course of the distribution changes corresponds well with that of onset of amylase secretion. This holds especially for a comparison of the time course between the parotid gland and the exocrine pancreas (Figs. 1 and 2). We tend to believe, therefore, that the observed shifts, although not necessarily reflecting the true quantitative conditions in intact cells, do represent qualitatively the receptor-mediated shifts of protein kinase C distribution.

It has recently been proposed by Ballester & Rosen (1985) that translocation of protein kinase C is mainly involved with its degradation. However, our results obtained in parotid gland lobules clearly show that the process of translocation is reversible to a large extent, indicating that protein kinase C translocated to the membranes is not, or only to a low extent, degraded during the duration of the experiments.

It seems likely that the receptor-mediated formation of diacylglycerol resulting from the breakdown of polyphosphoinositides is responsible for the increased membrane binding of protein kinase C. Our results support a

direct involvement of protein kinase C in exocytosis in exocrine cells, especially when combined with the following additional findings. (1) We have previously shown that stimulation of guinea pig parotid and pancreatic lobules or acini by carbamoylcholine is indeed associated with an activation of protein kinase C as judged from the phosphopeptide pattern of the ribosomal protein S6 (Padel & Söling, 1985). (2) Phorbol esters can stimulate exocytosis from isolated guinea pig pancreatic acini without the need for further agonists (Gunther, 1981; Wrenn, 1984) and from guinea pig parotid glands in the presence of low concentrations of a calcium ionophore (Padel & Söling, 1985).

Stimulation of exocytosis with isoproterenol is associated with the same phosphopeptide pattern in the ribosomal protein S6 as observed during stimulation with carbamoylcholine (Padel *et al.*, 1983; Padel & Söling, 1985) and also with a similar rapid increase in diacylglycerol (Söling *et al.*, 1985). We had therefore discussed the possibility that isoproterenol might in addition to stimulating cyclic AMP-dependent protein kinase also enhance protein kinase C activity. This has to be questioned now in the light of the results reported here and on the basis of more recent experiments which indicate that the cellular diacylglycerol pools seem to be compartmentalized (H.-D. Söling & W. Fest, unpublished work). Protein-kinase-C-catalysed phosphorylations that could be directly linked to exocytotic processes have not yet been identified. Wrenn (1984) as well as Burnham *et al.* (1985) have recently described an association of protein kinase C with the secretory granule membrane from rat and mouse pancreas respectively. These authors have also described a number of proteins associated with the secretory granule membrane which could be phosphorylated by protein kinase C. However, one has to await further research in order to see whether these proteins also become phosphorylated by protein kinase C in intact stimulated exocrine glands.

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REFERENCES

- Ballester, R. & Rosen, O. M. (1985) *J. Biol. Chem.* **260**, 15194–15199
- Bernfeld, P. (1955) *Methods Enzymol.* **1**, 149–158
- Bradford, M. M. (1976) *Anal. Biochem.* **72**, 248–254
- Burnham, D., Munowitz, P., Thorn, N. & Williams, J. A. (1985) *Biochem. J.* **227**, 743–751
- Castagna, M., Takai, Y., Kaikuchi, K., Sano, K., Kikkawa, U. & Nishizuka, Y. (1982) *J. Biol. Chem.* **257**, 7847–7851
- Drust, D. S. & Martin, T. F. (1985) *Biochem. Biophys. Res. Commun.* **128**, 531–537
- Farrar, W. L. & Anderson, W. B. (1985) *Nature (London)* **315**, 233–235
- Farrar, W. L., Thomas, T. P. & Anderson, W. B. (1985) *Nature (London)* **315**, 235–237
- Fearon, C. W. & Tashjian, A. H., Jr. (1985) *J. Biol. Chem.* **260**, 8366–8371
- Gunther, G. R. (1981) *J. Biol. Chem.* **256**, 12040–12045
- Hirota, K., Hirota, T., Aquilera, G. & Catt, K. J. (1985) *J. Biol. Chem.* **260**, 3243–3246
- Kishimoto, A., Takai, Y., Mori, T., Kikkawa, U. & Nishizuka, Y. (1980) *J. Biol. Chem.* **255**, 2273–2276
- Kraft, A. S. & Anderson, W. B. (1983) *Nature (London)* **301**, 621–623
- Kraft, A. S., Anderson, W. B., Cooper, H. L. & Sando, J. J. (1982) *J. Biol. Chem.* **257**, 13193–13196
- McCaffrey, P. G., Friedman, B. & Rich Rosner, M. (1984) *J. Biol. Chem.* **259**, 12502–12507
- Padel, U., Unger, C. & Söling, H.-D. (1982) *Biochem. J.* **208**, 205–210
- Padel, U., Kruppa, J., Jahn, R. & Söling, H.-D. (1983) *FEBS Lett.* **159**, 112–118
- Padel, U. & Söling, H.-D. (1985) *Eur. J. Biochem.* **151**, 1–10
- Scheele, G. A. & Palade, G. E. (1975) *J. Biol. Chem.* **250**, 2867–2875
- Söling, H.-D., Padel, U., Jahn, R., Thiel, G., Kricke, P. & Fest, W. (1985) *Adv. Enzyme Regul.* **23**, 141–156
- Takai, Y., Kishimoto, A., Iwasa, Y., Kawahara, Y., Mori, T. & Nishizuka, Y. (1979a) *J. Biol. Chem.* **254**, 3692–3695
- Takai, Y., Kishimoto, A., Kikkawa, U., Mori, T. & Nishizuka, Y. (1979b) *Biochem. Biophys. Res. Commun.* **91**, 1218–1224
- Wooten, M. W. & Wrenn, R. W. (1984) *FEBS Lett.* **171**, 183–186
- Wrenn, R. W. (1984) *Biochim. Biophys. Acta* **775**, 1–6

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