IMPLICATION OF MICROTUBULES AND MICROFILAMENTS IN THE CYCLIC GMP RESPONSE OF RAT EXOCRINE PANCREAS TO SECRETAGOGUES

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1. Introduction

Microtubules and microfilaments have been shown to be involved in the secretion processes of many glands: thyroid gland [1], pancreatic islets [2], adrenal medulla [3], anterior pituitary [4], parotid gland [5] and exocrine pancreas [6,7]. Most of these studies used drugs known to alter the structure of these cellular organelles. On the other hand, microtubules as well as microfilaments have been implicated in the control of the redistribution and motility of membrane surface components [8–12]. Furthermore, recent studies suggested that microtubules and/or microfilaments are probably involved in the process of adenylate cyclase stimulation by hormones [13,14].

In pancreatic acinar cells the initial step in the sequence of events mediating the action of secretagogues is an increase in intracellular guanosine 3′:5′ cyclic monophosphate (cyclic GMP) [15,16]. Further, microtubules and microfilaments were shown to be widely distributed in these cells [7]. Thus the present study was undertaken in order to investigate the possible role of microtubules and microfilaments in the secretagogue-induced cyclic GMP accumulation by rat exocrine pancreas. We report that both vinblastine which causes the disappearance of microtubules [7,17], and cytochalasin B which impairs the function of microfilaments [7,18] inhibit the cyclic GMP response of rat exocrine pancreas to peptide hormones (caerulein), cholinergic agents (carbachol) or the calcium ionophore A 23187.

2. Materials and methods

Pancreata were taken from 2.5-month-old male Wistar rats as previously described [19]. The incubations were performed at 37°C in a Krebs-Ringer bicarbonate medium supplemented with 10 mM D-glucose and the amino acid mixture of Campagne and Gruber [19] under a 95% O_2, 5% CO_2 atmosphere. The pancreatic fragments (about 50 mg wet weight) were treated for 1, 2, 5, 10 or 30 min with 3×10^{-10} M caerulein, 10^{-6} M carbachol or 10^{-5} M ionophore A 23187, in the presence or absence of vinblastine or cytochalasin B. Timed incubations were terminated by quickly removing the fragments, instant freezing in liquid N_2 and homogenization in 2 ml of ice-cold 6% trichloroacetic acid. After centrifugation at 0°C of the acidified homogenate, the acid-soluble supernatant was collected, extracted twice with 10 vol. of water-saturated ether and assayed for cyclic GMP content using a commercially available protein-binding assay (Boehringer Test Kits, Mannheim, Germany). Recovery (80–90%) was monitored with 1 nCi of [^{3}H]cyclic GMP and the specificity of the assay was checked by the cyclic phosphodiesterase test [20]. The acid-precipitable material was dissolved in 2.0 ml of 1 N NaOH and assayed for protein content by the method of Lowry et al. [21]. In parallel experiments, the pancreatic fragments were preincubated for 15, 30, 60 or 120 min in the presence of vinblastine or cytochalasin B before the cyclic GMP response to secretagogues was measured.

Results are expressed as the mean of 4 to 6 replicates in pmol/mg of protein. Similar results were obtained when samples were purified on a column of Dowex AG1X8. However, in the majority of experiments, the cyclic GMP concentration was determined without the prior column step since this procedure was found to give the most consistent results.

Caerulein was a generous gift from Farmitalia (Milan, Italy). Carbachol was obtained from Sigma Chemical Co. (St. Louis, MO, USA). Ionophore

A 23187 and vinblastine sulphate (Velbe) were a generous gift from Eli Lilly (St. Cloud, France). Cytochalasin B was purchased from EGA Chemie (Steinheim Albuch, W. Germany) and 3-isobutyl-1-methyl xanthine (MIX) from Aldrich Chemical Co. (Milwaukee, WI, USA).

3. Results

As shown in fig.1, 3×10^{-10} M caerulein, 10^{-6} M carbachol and 10^{-5} M ionophore A 23187, induced a rapid and transient accumulation of cellular cyclic GMP in pancreatic fragments. This increase was maximal (8- to 10-fold over the basal value) after 2 min and then decreased steadily toward control values by 30 min.

In contrast, pancreatic fragments pretreated with 5×10^{-5} M vinblastine for 60 min were no longer responsive to either caerulein, carbachol or ionophore A 23187, whereas the basal cyclic GMP levels were not significantly affected. This vinblastine-mediated inhibitory effect depended on dose and duration of the treatment with the drug. Fig.2 shows the relative effects of both parameters on the cyclic GMP accumulation in response to caerulein. At 5×10^{-5} M vinblastine had no significant effect when introduced at the beginning of the incubation along with the secretagogues (fig.2A and fig.1) and a 15–30 min preincubation with the drug was necessary for decreasing significantly the response to caerulein (fig.2A). The vinblastine-induced refractoriness was maximal at 5×10^{-5} M for a 60 min preincubation, but a dose as low as 5×10^{-8} M was sufficient for eliciting the loss of responsiveness to caerulein (fig.2B).

Cytochalasin B was similarly studied. At 2×10^{-5} M cytochalasin B also abolished the cyclic GMP response to either caerulein, carbachol or ionophore, without altering the basal levels of this cyclic nucleotide (fig.1). In contrast to vinblastine, cytochalasin B was immediatly effective without preincubation (fig.3A). This refractoriness was maintained even after a 2-h preincubation with the drug. Fig.3B depicts the effect of different concentrations of cytochalasin B on the caerulein-induced cyclic GMP accumulation without preincubation with the drug: A dose as low as 2×10^{-8} M was sufficient for eliciting a 50% inhibition of the response to caerulein.

The suppression of the cyclic GMP response to caerulein, carbachol and ionophore A 23187 by either

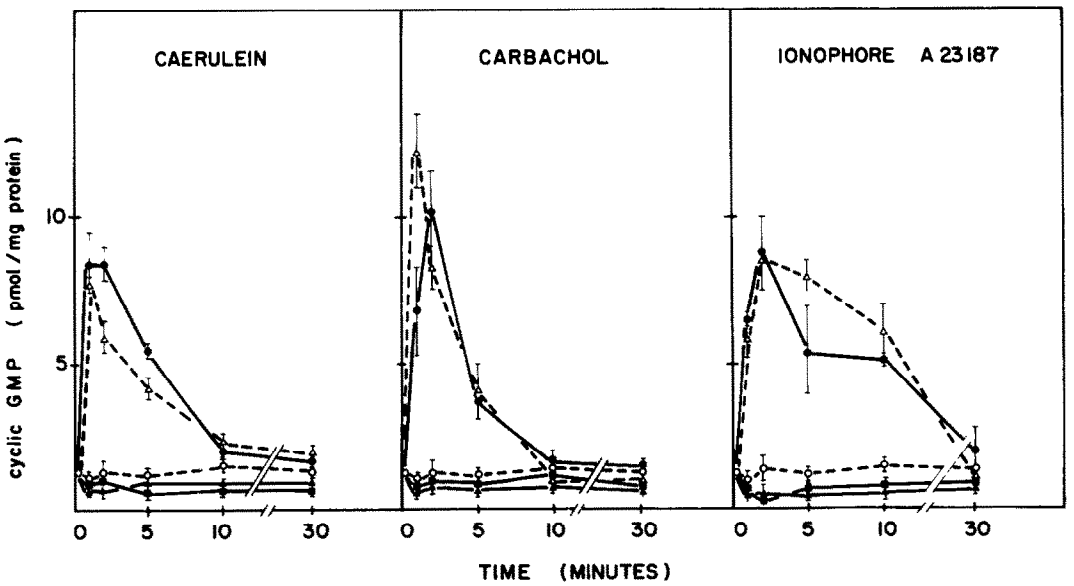

Fig.1. Time course of cyclic GMP accumulation in rat exocrine pancreatic fragments exposed to 3×10^{-10} M caerulein, 10^{-6} M carbachol or 10^{-5} M ionophore A 23187. Secretagogues were added without (●——●), or with 2×10^{-5} M cytochalasin B (■——■) or 5×10^{-5} M vinblastine (△– – –△). The cyclic GMP response of pancreatic fragments pretreated for 60 min with 5×10^{-5} M vinblastine (▲——▲), was studied in parallel. Unstimulated controls (○– – –○) were run with hormone or ionophore solvents, 5×10^{-5} M vinblastine, or 2×10^{-5} M cytochalasin B.

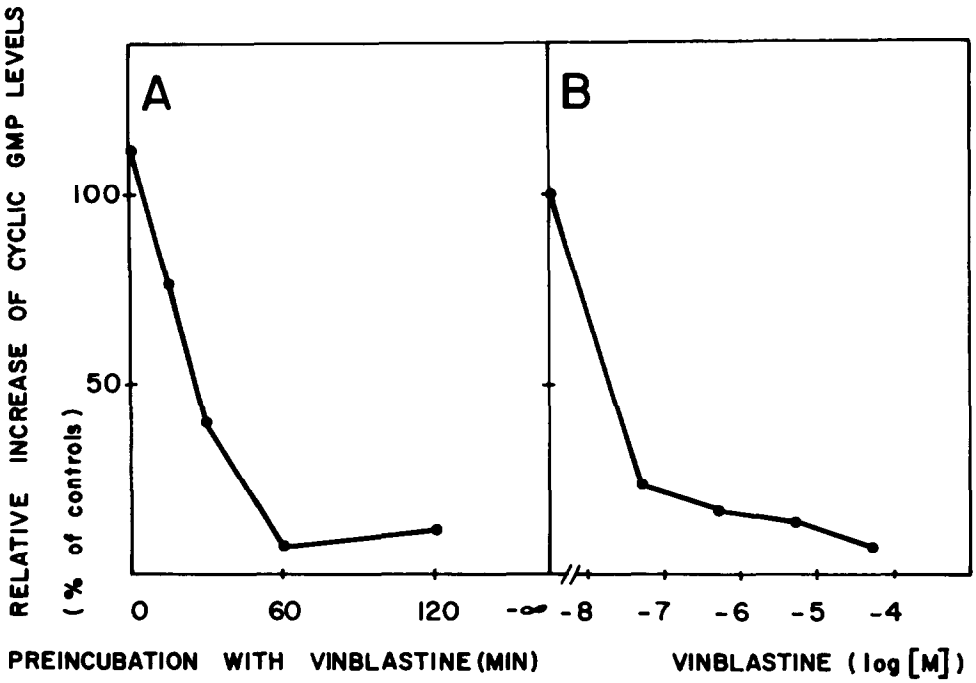

Fig.2. (A) Cyclic GMP response to 3×10^{-10} M caerulein of pancreatic fragments pretreated for various periods of time with 5×10^{-5} M vinblastine. The cyclic GMP levels were measured 2 min after the addition of 3×10^{-10} M caerulein. Control values represent the increase in the cyclic GMP content of untreated cells which were exposed for 2 min to caerulein. (B) Response of pancreatic fragments to 3×10^{-10} M caerulein following a 60 min pretreatment with graded doses of vinblastine. The intracellular cyclic GMP content was measured 2 min after the exposure to 3×10^{-10} M caerulein.

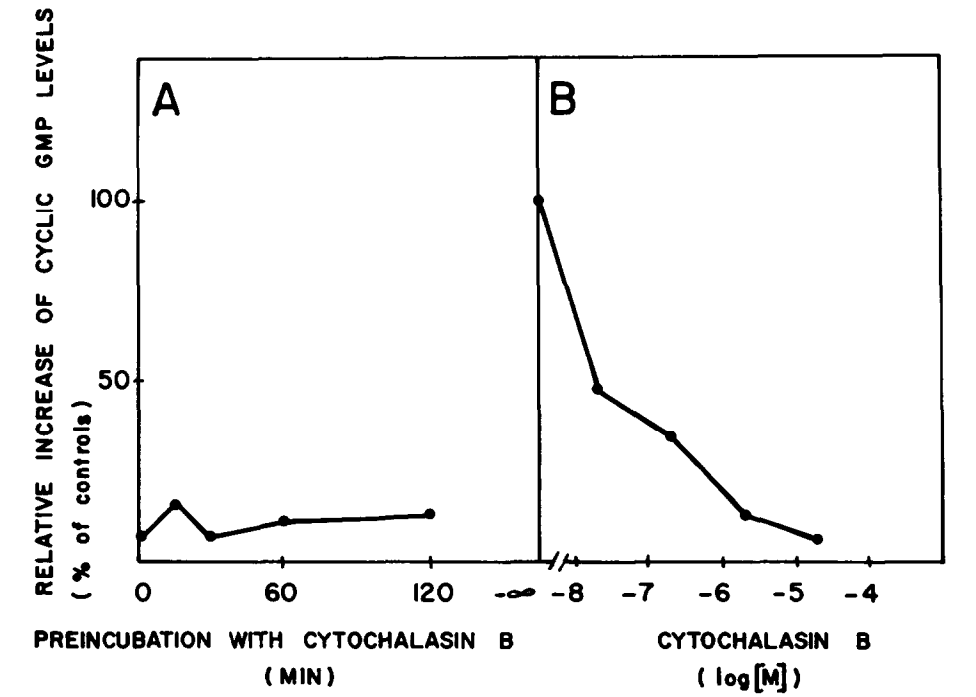

Fig.3. Cyclic GMP response to 3×10^{-10} M caerulein of pancreatic fragments pretreated for various periods of time with 2×10^{-5} M cytochalasin B (A), or treated with graded doses of cytochalasin B without preincubation (B). The intracellular cyclic GMP concentration was determined 2 min after the addition of 3×10^{-10} M caerulein.

Table 1
Effect of 1-methyl-3-isobutylxanthine (MIX), on the vinblastine and cytochalasin B-mediated suppression of cyclic GMP accumulation

	Cyclic GMP (pmol/mg protein)	
	No MIX	0.5 mM MIX
Controls	1.16 ± 0.10	1.54 ± 0.39
VB	1.18 ± 0.48	1.40 ± 0.50
CB	0.88 ± 0.26	1.43 ± 0.18
Caerulein	3.55 ± 0.61	4.66 ± 0.14
Caerulein + VB	1.29 ± 0.10	1.69 ± 0.21
Caerulein + CB	1.43 ± 0.25	1.82 ± 0.31
Carbachol	4.93 ± 0.13	5.53 ± 1.48
Carbachol + VB	1.51 ± 0.19	1.25 ± 0.14
Carbachol + CB	1.38 ± 0.04	1.08 ± 0.13
Ionophore A 23187	4.17 ± 0.24	5.08 ± 1.24
Ionophore A 23187 + VB	2.14 ± 0.39	1.31 ± 0.07
Ionophore A 23187 + CB	2.67 ± 0.16	1.26 ± 0.13

Rat exocrine pancreatic fragments were pretreated for 60 min with 5×10^{-5} M vinblastine (VB) or treated with 2×10^{-5} M cytochalasin B (CB) without preincubation; then the intracellular cyclic GMP concentration was determined 2 min after the addition of 3×10^{-10} M caerulein, 10^{-6} M carbachol or 10^{-6} M ionophore A 23187 with or without 0.5 mM MIX

vinblastine or cytochalasin B was not modified when both drugs were combined (data not shown).

It should be stressed that the addition of 0.5 mM MIX, a potent cyclic nucleotide phosphodiesterase inhibitor, did not overcome the vinblastine and cytochalasin B-mediated suppression of the cyclic GMP response in rat pancreatic fragments (table 1).

4. Discussion

Although the cellular mechanism of action of secretagogues such as caerulein, carbachol and calcium ionophore A 23187 is not yet clear, the first step of their action is well established: caerulein and carbachol primarily bind to a receptor present in the plasma membrane of pancreatic exocrine cells [22–24] whereas ionophore A 23187 would facilitate the movement of divalent cations across biological membranes including plasma membrane [25]. This first step is followed, through a mechanism which still has to be clarified, by a rapid and transient accumulation of cyclic GMP.

Our results show that vinblastine and cytochalasin B inhibit the cyclic GMP response to caerulein, carbachol and ionophore A 23187. Cytochalasin B was immediately effective, whereas vinblastine needed 15–30 min to exert a significant effect.

These drugs might alter the binding of caerulein and carbachol to their receptor sites and/or impair a coupling mechanism between the hormone–receptor complexes and guanylate cyclase which has been localized in the apical plasmalemma membrane of the pancreatic acinar cell [26]. However, these suggestions could not be ascertained since the calcium ionophore A 23187-induced cyclic GMP accumulation was similarly inhibited.

The inhibitory effect of vinblastine and cytochalasin B on the cyclic GMP response could be manifested through a decrease in the rate of production of cyclic GMP or an increase in its rate of hydrolysis. Although we did not measure guanylate cyclase activity in membrane preparations from vinblastine or cytochalasin B-treated pancreatic fragments, the inability of both drugs to affect the basal cyclic GMP levels even after a 2-h treatment argue against the possibility that guanylate cyclase was directly affected. On the other hand, since the addition of MIX failed to restore the normal response to caerulein, carbachol and ionophore A 23187 in vinblastine and cytochalasin B-treated pancreata, the possibility that an increased breakdown of cyclic GMP could account for the observed lack of responsiveness seems unlikely. Further, neither vinblastine, or cytochalasin B enhanced the efflux or hormone-elevated intracellular cyclic GMP (data not shown).

Since guanylate cyclase activation has been reported to be calcium-dependent [27], the vinblastine and cytochalasin B-mediated refractoriness could result from alterations in calcium fluxes. Although previous studies have shown that neither cytochalasin B [6] nor vinblastine (H. Bauduin, personal communication) altered the calcium exchanges between the extracellular medium and the pancreatic tissue, an effect of the drugs on the intracellular movements could not be ruled out.

Under our experimental conditions, a damaging effect of vinblastine and cytochalasin B on the pancreatic tissue seems unlikely. Neither drugs altered significantly the basal release of amylase and lipase [6,19,28], nor did they cause any leakage of lactic dehydrogenase (a cytoplasmic marker) in the extracellular medium [28]. Further the morphological integrity of acinar cells after exposure to vinblastine or cytochalasin B has been evidenced by electron microscopy [6,7].

An alternative candidate for the vinblastine and cytochalasin B-mediated refractoriness would be the microtubule–microfilament system. Such an argument is based on the observation that the inhibition of the cyclic GMP response to hormones, as the disappearance of microtubules and microfilaments in vinblastine and cytochalasin B-treated pancreas show similar time courses [7]. The time-dependence of the vinblastine effect could be due to limitation of the rate of entry of the drug into the cells, or to limitation of the rate of its binding to tubulin.

Guanylate cyclase has been localized in the plasmalemma membrane of the pancreatic cells [26], but the nature of the cytoskeleton links to the plasma membrane that influence guanylate cyclase remains unclear and intriguing. In many systems, cytochalasin B has been shown to bind to plasma membranes [29,30] and microfilaments have been demonstrated in association with plasma membranes [12]. On the other hand, although vinblastine-binding activity has been described for plasma membrane [31], microtubular structures have been generally observed in the cytoplasm [7] and their association with plasma membranes remains questionable [11,12]. Since both drugs have a common effect on the cyclic GMP response, despite two different sites of action, it could be postulated that microtubules are linked to microfilaments which are associated with the membrane [32]. Alteration of the latter either directly by cytochalasin B, or via the perturbation of the former by vinblastine, would cause a change in the membrane reflected in the behavior of guanylate cyclase.

From our results, it seems that microfilaments and microtubules (cytoplasmic microtubules or vinblastine sensitive structures) are involved in guanylate cyclase stimulation by caerulein, carbachol and ionophore A 23187. However, further work is necessary to learn the mechanism by which the microfilament–microtubule system is implicated in the process of guanylate cyclase stimulation.

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