VINBLASTINE POTENTIATES THE SECRETAGOGUE ACTION OF DIBUTYRYL CYCLIC AMP ON THE EXOCRINE PANCREAS[1]

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SUMMARY. The effects of VB[4] on both structure and function of the pancreatic acinar cell have been examined in vitro. VB potentiates the secretagogue effect of DbcAMP[5] but fails to affect the spontaneous release of enzymes. This potentiation is parallel to the disappearance of cellular microtubules and depends on the dose and the time of exposure to VB. The potentiation is energy dependent; it is more obvious in calcium containing medium than in absence of calcium. A damaging effect of VB on acinar cells is ruled out. These findings suggest the participation of microtubules in the secretory cycle of the pancreatic acinar cell.

INTRODUCTION

It has been shown that vinca alcaloids interfere with a variety of secretory processes (1-4). In the exocrine pancreas, these drugs inhibit the secretion of enzymes induced by cholinergics (5,6) or digestive hormones (6). Such effects have been attributed to the ability of periwinkle alcaloids to disrupt the microtubules (7). The dynamic equilibrium between microtubules and the subunit protein (tubulin), which occurs in the cell, is modulated by calcium (8,9) and cyclic AMP (8, 10) or DbcAMP (10, 11, 12, 13). Since the secretagogue properties of DbcAMP on the pancreatic acinar cell are no more disputed (14, 15, 16, 17), it was of great interest to determine the participation of microtubules in the secretory action of the nucleotide derivative.

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[4]VB = Vinblastine sulphate. It was a generous gift from Eli Lilly (St-Cloud, France).

[5]DbcAMP = N6-2' - O - Dibutyryl - adenosine 3', 5' - monophosphate, cyclic.

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	CONTROL	VINBLASTINE 5×10^{-5} M	Dbc AMP 2 mM	VINBLASTINE 5×10^{-5}M + Dbc AMP 2 mM
L-Leucine incorporated into "proteins" (% of control)	100 (6) (79 ± 3 n mole / 100mg/h)	70.9 ± 6.4 (3)	84.3 ± 6.1 (12)	69.0 ± 5.0 (7)
LDH release (% of tissue content)	2.36 ± 0.26 (14)	2.05 ± 0.10 (3)	2.21 ± 0.44 (16)	2.95 ± 0.95 (6)
ATP (µmole / 100mg tissue)	0.36 ± 0.06 (8)	0.34 ± 0.08 (6)	0.39 ± 0.05 (8)	0.42 ± 0.06 (6)

Table 1. Influence of VB on metabolic parameters in the pancreas in vitro. Incorporation of L-leucine was calculated after the specific activity of L-[4,5-^{3}H] leucine in the medium and the activity incorporated into 5 % TCA precipitable proteins. VB and DbcAMP were added to the medium respectively 30 and 60 min. after the pulse. Time of incubation with DbcAMP and/or VB : 90 min. The activity of lactic dehydrogenase (LDH) in the tissue was: 4.1 ± 0.4 U/100 mg (n = 25). Each value is given with its SEM and the number of experiments in brackets.

In the present investigation, the effects of vinblastine on both structure and function of the acinar cell are examined.

METHODS

Pieces of rat pancreas were obtained from 2.5 month old Wistar rats after 12 hours fast. They were incubated as previously described (18). The incubation medium, enriched by the L-amino-acid mixture of CAMPAGNE and GRUBER (18) and D-glucose 10 mM, was buffered with bicarbonate at pH 7.4. The gas phase was 0_2 95 %, $C0_2$ 5 %. DbcAMP was generally added to the medium after a 30 minutes preincubation with or without VB. Secretion of enzymes was determined by the activity of amylase (19), lipase (20) or chymotrypsinogen (21) in aliquots from the incubation medium.

Incorporation of labeled amino acid into the proteins (5 %TCA precipitable material) was measured by the method of MANS and NOVELLI (22). Pancreatic fragments were pulse labeled with L-[4,5 - ^{3}H] leucine 5 µCi/ml (53 Ci/mmol) for 5 minutes, then incubated in a cold leucine medium (800 nmol/ml) for 150 minutes.

ATP content of incubated glands was estimated by the luciferine luciferase method of STANLEY and WILLIAMS (23).

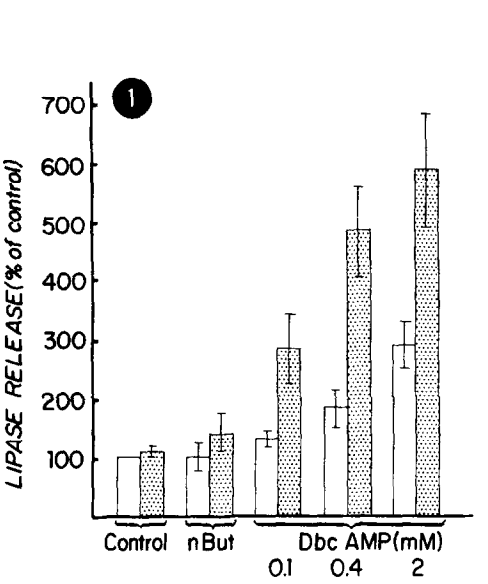
The lactic dehydrogenase (LDH) activity was estimated by following the reduction of pyruvate by NADH (24).

Electron microscope examination was performed on pancreatic fragments, fixed in 2 % buffered glutaraldehyde, postfixed in 1 % buffered osmium tetroxide, dehydrated in alcohols and embedded in araldite. Thin sections, doubly stained with uranyl acetate and lead citrate, were examined with a Philips EM 300 electron microscope.

DbcAMP was purchased from Boehringer, Pharma (Mannheim, Germany). Antimycin A was from Calbiochem (San Diego, USA). All other chemicals were Merck products, analytical grade.

RESULTS

VB 5.10^{-5}M induces the disappearance of microtubules while the paracrystalline deposits develope in the cytoplasm of the acinar cells. This structural alteration requires a lag time of 20 - 30 minutes and a minimal concentration of 5.10^{-7}M.



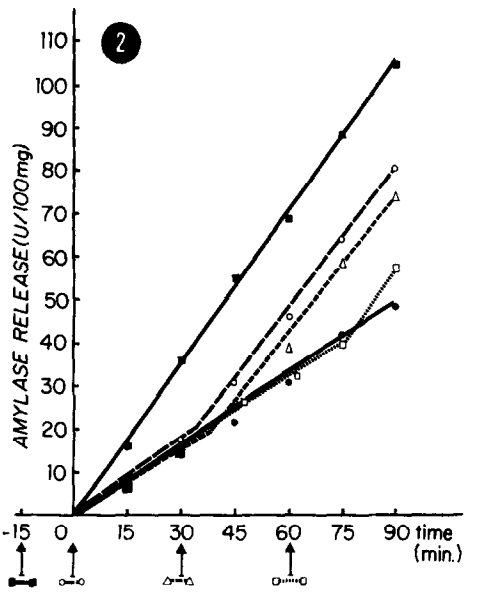


Figure 1. Influence of VB on lipase release induced by DbcAMP at different concentrations or n-butyric acid. The pancreatic fragments were incubated for 90 min. after a preincubation of 30 min. in presence or in absence of VB. Results expressed in % of control are means ± SEM of 10 experiments. Control : 100 % = 19.5 ± 3.2 µEq/100 mg/90 min. Open columns : without VB. Stippled columns : with VB 5.10^{-5}M. Concentration of n butyric acid in the medium was 2 mM. Concentrations ofDbcAMP were 0.1, 0.4 and 2 mM.

Figure 2. Influence of the time of addition of VB on the release of amylase induced by DbcAMP. Pancreatic fragments from one rat were incubated for 90 min. after a 15 min. preincubation. Each curve is representative of one observation. Time of addition of VB 5.10^{-5}M is indicated by the arrow above the corresponding symbol. DbcAMP was added at time zero. The rate of amylase release, when the effect of VB had fully developed, was 1.2 U per 100 mg per min. ; the control rate (DbcAMP induced secretion) was 0.55U per mg per min.

While VB 5.10^{-5}M inhibits the incorporation of L-[4,5 - ^{3}H] leucine into TCA precipitable proteins (Table 1), it potentiates the secretory response to DbcAMP (0.1 - 2mM) (Fig. 1).

Alike the alteration of microtubules, this potentiation depends 1. on the time of exposure to VB : a lag time of 20 - 30 min. is necessary at the given concentration of 5.10^{-5}M (Fig. 2) ; 2. on the concentration of VB : the threshold dose is 5.10^{-7}M (Fig. 3). VB 5.10^{-9}M to 5.10^{-5}M do not alter significantly the basal release of enzymes (Fig. 3).

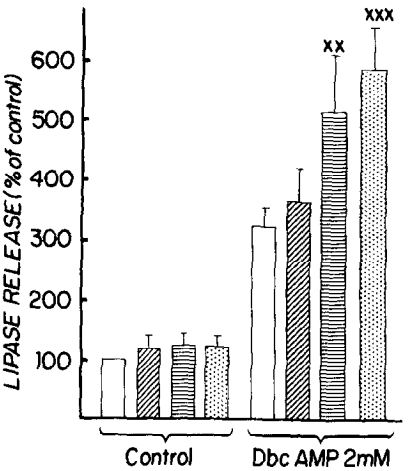


Figure 3. Influence of the concentration of VB on lipase release induced by DbcAMP. Pancreatic fragments were incubated for 90 min. after a preincubation of 30 min. with or without VB at the given concentration. Open columns : control ; Hatched columns : VB 5.10^{-9}M ; Barred columns : VB 5.10^{-7}M ; Stippled columns : VB 5.10^{-5}M. Results expressed in % of control are means $\pm$ SEM of at least 7 experiments. Control 100 % = 17.7 ± 2.3 µEq/100 mg/90 min. The statistical significance was calculated (paired tests) between VB treated tissue and corresponding control -P > 0.05 ; xx P < 0.01. xxx P < 0.001.

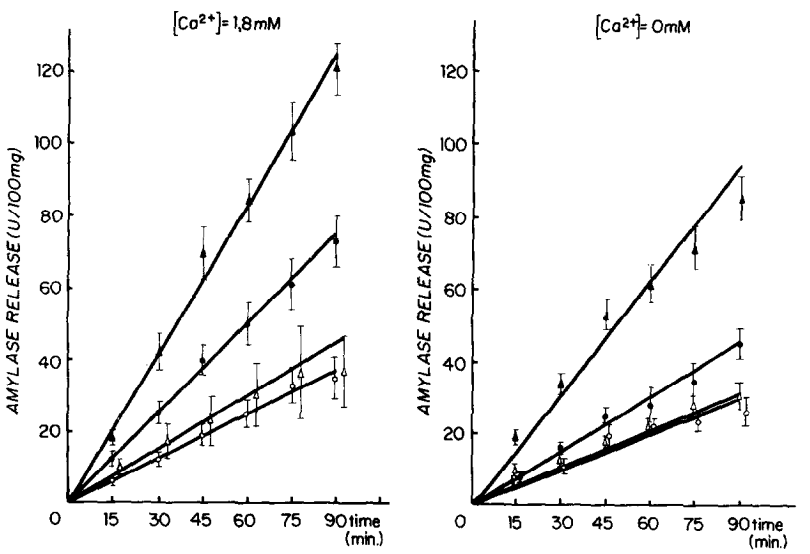


Figure 4. Influence of VB on DbcAMP induced amylase release ; effect of calcium depletion. After two washes of 15 min. each in a calcium free medium containing EGTA 1 mM with or without VB the pancreatic fragments were incubated for 90 min. in presence of calcium (1.8 mM) or in the absence of calcium (0 mM). Each value is the mean $\pm$ SEM of 8 experiments. O Control ; Δ VB 5.10^{-5}M ; ● DbcAMP 2 mM ; ▲ DbcAMP 2 mM + VB 5.10^{-5}M.

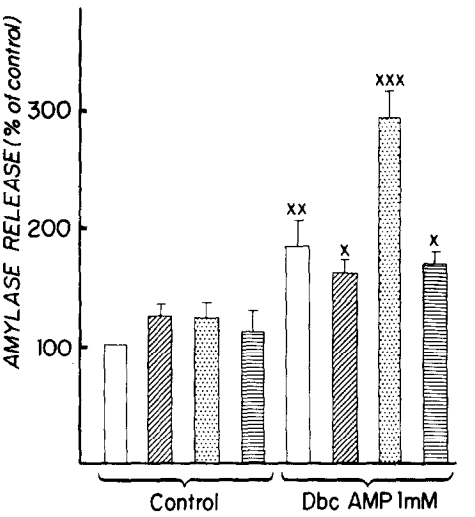


Figure 5. Influence of antimycin A on the interaction of VB with the amylase release induced by DbcAMP. Pancreatic fragments were incubated for 90 min. after a preincubation of 30 min. with or without VB 5.10^{-5}M. Open columns : control ; Hatched columns : antimycin A 10^{-5}M ; Stippled columns ; VB 5.10^{-5}M ; Barred columns : antimycin A 10^{-5}M. Results expressed in % of control are means ± SEM of 9 experiments. Control : 100 % = 102 ± 17 U/100 mg/90 min. Statistical significance was calculated (paired tests) vis the non stimulated respective controls x $P < 0.05$; xx $P < 0.01$; xxx $P < 0.001$.

The VB potentiation of the secretory response to DbcAMP is effective for at least three enzymes : lipase (Fig. 1, 3) amylase (Fig. 2) and chymotrypsinogen (not shown). It is more obvious in presence of calcium 1.8 mM than in the absence of calcium ($[Ca^{2+}] < 10^{-8}$M, obtained after two 15 min. washes of the gland in EGTA 1 mM containing medium) (Fig.4).

While the ATP content of the pancreatic tissue do not vary after VB and/or DbcAMP treatment (Table 1), the potentiation by VB of DbcAMP induced enzyme release is abolished by antimycin A (Fig. 5), an inhibitor of the oxidative phosphorylation. However, the later do not affect the spontaneous enzyme release nor the secretory response to DbcAMP.

At last, VB and DbcAMP did not cause any leakage of lactic dehydrogenase (Table 1), a cytoplasmic marker, in the extracellular medium. This evidence and the morphological integrity of acinar cells after 2 hours exposure to VB 5.10^{-5}M, ruled out the possibility of a damaging effect on the pancreatic tissue by the association of the vinca alcaloid with DbcAMP.

DISCUSSION

In a previous work (25), it was suggested that DbcAMP stimulates enzyme release from the pancreas through a direct interaction with the acinar cell. The present findings support this hypothesis and imply the participation of microtubules in the secretory cycle of the acinar cell. Indeed, the VB potentiation of enzyme secretion induced by DbcAMP is parallel to the disappearance of microtubules in the acinar cell ; both features show a similar dependence on the concentration and time of exposure to the vinca alcaloid.

A cytotoxic action of VB on pancreatic acinar cells, as reported by NEVALAINEN (26) was ruled out in our experimental conditions on histological grounds as well biochemical evidences.

Although the unspecificity of the periwinkle alcaloid is well known(27), none of its effects on DNA, RNA (27) or protein synthesis (table 1, 5,27) could explain an increase of the discharge of zymogen induced by DbcAMP. The interference of VB with the intermediary metabolism (alteration of glycolysis (27), decrease of palmitate oxidation (5)) does not alter the energy balance in the gland (table 1, 6) ; this makes the side effects of the drug unrelated to its secretory interaction.

The blockage by antimycin A of the potentiation by VB of the secretory response to DbcAMP shows that the process requires a high energy supply that only oxidative phosphorylation can afford. Indeed, glycolysis which provides less than 30 % of the energy requirements in the non stimulated gland (17) can sustain the secretion induced by DbcAMP alone but not the potentiation effect of the vinca alcaloid (Fig. 5). Three steps of the secretory cycle : protein synthesis, transport of secretory material through the Golgi area and exocytosis, are endergonic processes (28). The inhibition of protein synthesis by VB, already reported by SEYBOLD et al. (5) and confirmed in this study, cannot be correlated with the secretion of digestive enzymes (28). Thus, the action of VB is located between the formation of zymogen granules and their extrusion in the acinar lumen. The microtubular system is the most likely site of action of VB in the acinar cell as discussed above. How this system plays a role in the secretory cycle remains unclear. The microtubules may guide the secretory granules to the membrane (29), organize the distribution of cell membrane components (30) or interact with microfilaments (3). Since the potentiation by VB of DbcAMP induced enzyme release is 1. energy dependent, 2. influenced by calcium and 3. inhibited by cytochalasin B, an agent know to disrupt microfilaments (31), the involvement of a contractile process in this potentiation, at the level of the microfilamentous-microtubular system, seems to be a very likely hypothesis. Further work is in progress to clarify this assumption.

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