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The Interactions Between Vitamin A, Vinblastine, and Cytochalasin B in Parathyroid Hormone Secretion[1] (38272)

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Microtubules and microfilaments are subcellular organelles which are involved in many cellular biological processes (1, 2). Of particular interest to us has been the role of these subcellular organelles in hormone secretion. Microtubules have been implicated in the secretion of hormone from the islets of Langerhans (3, 4), thyroid gland (5), adrenal medulla (6), and neurohypophysis (7), and the release of neurotransmitters from sympathetic nerve endings (8). Microfilaments may play a role in insulin secretion (9–11) and the release of neurotransmitters (8).

The role of microtubules and microfilaments in hormone secretion has been studied using Vinca alkaloids (3, 4, 6, 8) and the antibiotic cytochalasin B (CB) (8–11), agents which disrupt microtubules and microfilaments, respectively. Recently, we have shown that vitamin A (Vit A), an agent known to interact with the cell and lysosomal membrane (12–14), increases the release of parathyroid hormone (PTH) from parathyroid tissue *in vitro* (15).

Therefore, to determine (1) the role of microtubules and microfilaments in PTH secretion and (2) the interrelationship between these subcellular organelles and the effects of Vit A on the cell and/or secretion membrane, we studied the role of, and interactions between, Vit A, vinblastine sulfate (VB), and CB in PTH secretion.

Methods. Bovine parathyroid glands, which were obtained from an abattoir shortly after slaughter, were cut into 2 × 2 mm pieces. In each experiment over 100–150 pieces were cut from 10–15 glands. Six pieces were then randomly selected for each incubation flask and incubated in Eagle media with 1.5 m*M* calcium, 2 m*M* glutamine, and 10% calf serum. The parathyroid tissues were incubated for a total period of 5 hr in a Dubnoff metabolic shaker at 37° in 95% O_2 and 5% CO_2. The first 2 hr of incubation served as an equilibration period. "Zero time" or the basal secretion rate was determined by measuring PTH in media collected during the second hour of incubation. After the zero-time collection was made, the media composition was changed to contain the following additives alone or in combination: Vit A alcohol, 0.35 or 0.035 μg/ml; VB (compliments of Eli Lilly, Indianapolis, Indiana), 8.0 μg/ml; and CB (compliments of Imperial Chemical Industries Limited, Macclesfield, England), 5.0 μg/ml. Since Vit A is dissolved in ethanol and CB in dimethylsulfoxide (DMSO), ethanol and DMSO in final concentrations of 0.2% were added to control media in each experiment. Each flask to which test agent was added had a matched control. During the incubation period, media was changed every 30 or 60 min and the PTH concentration determined by the radioimmunoassay method of Arnaud *et al.* (16) with slight modification. PTH secreted into the incubation media immunologically cross-reacted identically with the purified bovine PTH, used as the standard in our assay. At the end of the incubation period, tissues were blotted dry and weighed on a Mettler H-16 microbalance. The hourly secretion rate of PTH was calculated for each 30- or 60-min interval throughout the incubation and is expressed in pg per mg wet wt of tissue per hour.

In our model, we consistently found a slight increase in PTH release in control tissues in the early phases of incubation. Therefore, each ex-

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TABLE I. The Effects of Vitamin A, Vinblastine, and Cytochalasin B Alone or in Combination on PTH Secretion Rates [a]

Experiment	Time (Min)				
	0	30	60	120	180
I. Vitamin A (vit A)					
A. Control	550 ± 72[b]	702 ± 69	727 ± 42	531 ± 32	530 ± 13
Vit A—0.35 μg/ml	540 ± 55	1350 ± 142	1252 ± 71	823 ± 45	772 ± 56
P_1[c]	NS	< .01	< .01	< .01	< .01
B. Control	205 ± 16	489 ± 54	421 ± 11	260 ± 14	243 ± 24
Vit A—0.035 μg/ml	257 ± 30	820 ± 68	943 ± 30	428 ± 23	373 ± 16
P_1	NS	< .01	< .001	< .001	< .01
II. Vinblastine (VB)					
Control	273 ± 23	463 ± 28	423 ± 48	292 ± 12	249 ± 18
VB—8 μg/ml	296 ± 18	314 ± 23	291 ± 36	185 ± 7	154 ± 41
P_1	NS	< .05	NS	< .01	< .05
VB + vit A—0.35 μg/ml	285 ± 22	369 ± 23	353 ± 36	217 ± 10	197 ± 35
P_2[d]	NS	< .001	< .02	< .05	< .05
III. Cytochalasin B (CB)					
Control	282 ± 27	497 ± 76	431 ± 77	303 ± 26	276 ± 35
CB—5.0 μg/ml	306 ± 16	498 ± 17	638 ± 56	476 ± 23	367 ± 36
P_1	NS	NS	< .05	< .01	< .05
CB + vit A—0.35 μg/ml	273 ± 22	778 ± 62	1010 ± 71	642 ± 53	579 ± 40
P_2	NS	< .01	< .001	< .05	< .001

[a] Secretion rates are for the 30 or 60 min period preceding the indicated time and are expressed in pg/mg wet wt/hr. Zero time represents a 60-min collection of media prior to the addition of test agents.

[b] Values are the means ± SEM for 6 separate incubations in Expts IA and III and 4 separate incubations in Expts IB and II.

[c] P_1 is the significance level for the difference between control and test agent alone.

[d] P_2 is the significance level for the difference between VB or CB alone and the combination of VB or CB plus vit, A respectively. NS: No significance.

perimental value was compared to the secretion rate of control tissues during the same time interval of incubation and expressed as percent of control during that time period.

Previous studies with this model show that the secretory function of our tissues is intact; the tissues respond physiologically as expected to low and high calcium concentrations (17). Lysosomal catheptic activity and LDH release, parameters of cell injury, are not increased by the test agents, and electron microscopy shows intact cells and suborganelles (including microtubules and microfilament bundles) in all phases of activity (unpublished observation).

The experiments were designed to permit statistical analyses by analysis of variance or the paired *t* test.

Results. The effects of Vit A, VB, and CB, alone or in combination, on PTH secretion rates over 30- or 60-min intervals are tabulated in Table I.

Vit A alone. Vit A alone (0.35 μg/ml) increased the mean secretion rate during incubation to 163% of control ($P < .001$) and increased hourly secretion rates throughout incubation, peaking to 192% of control at 30 min and decreasing to 140% of control by 180 min. When the concentrations of Vit A was reduced to 0.035 μg/ml, PTH secretion rates were similarly stimulated, maximally in the first hour of incubation, peaking to 224% of control at 60 min, and then falling to 150% of control at 180 min.

VB, VB plus Vit A. VB consistently significantly inhibited the secretion rate of PTH throughout the 3-hr incubation. The mean secretion rate decreased to 65% of control ($P < .01$). Vitamin A (0.35 μg/ml) increased the VB-inhibited secretion rates, but only by 10–17%, and the mean PTH secretion rate remained decreased at 79% of control ($P < .02$).

CB, CB plus Vit A. CB stimulated the PTH secretion rate to a maximum of 157% of control at 120 min. The effect of CB was delayed in that the secretion rate at 30 min was not different from controls. In contrast, CB plus Vit A (0.35 μg/ml) increased the secretion rate to 150% of control during the first 30 min and further

increased secretion to a peak of 234% of control at 60 min. At 180 min, the secretion rate remained 210% of control.

Discussion. Vitamin A is a twenty-carbon monocyclic isoprenalogue which interacts with membranes and alters membrane surface area and tension independently (12–14). Vitamin A alone (0.35 μg/ml) in our study significantly increased PTH secretion (Fig. 1). 0.035 μg/ml Vit A, a concentration approximately one-tenth of the reported normal plasma levels in man (18), similarly stimulated PTH secretion.

In our study, VB inhibited PTH secretion, indicating that microtubules may also play a role in the secretion of PTH. Habener *et al.* (19) recently reported that microtubules play a role in the conversion of proparathyroid hormone to PTH but not in the release of immunoreactive PTH.

VB caused a marked decrease in Vit A-stimulated secretion, suggesting that microtubules or microtubular-like proteins within the cell membrane may mediate a primary effect of Vit A. Consistent with this latter possibility are recent reports indicating that (a) VB eliminates the normal topographical separation of membrane functions (20) and (b) that VB protects erythrocytes from osmotic hemolysis (21), in contrast to the effect of Vit A which induces erythrocyte hemolysis (13).

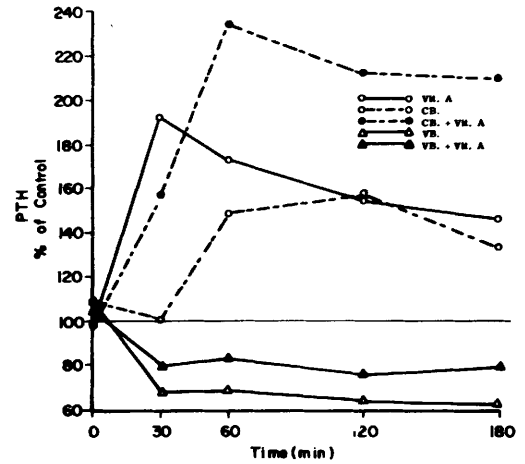

FIG. 1. A comparison of the effects of Vit A, 0.35 μg/ml; VB, 8.0 μg/ml; and CB, 5 μg/ml, alone and/or in combination on PTH secretion. Secretion rates are expressed as the percent of control values obtained during the same 30- or 60-min incubation interval in each experiment. Zero time represents a 1-hr collection of media prior to the addition of test agents.

Therefore, VB may be inhibiting Vit A-stimulated PTH secretion by an effect on the cell and/or secretion granule membrane.

The effects of CB, a microfilament disrupter, on hormone secretion have been variable depending on the tissue under study. CB inhibits release of neurotransmitter from nerve endings (8). On the other hand, CB stimulated insulin secretion by disrupting a zone of microfilaments at the periphery of the beta cell (9–11). In our study, CB stimulated PTH secretion, suggesting that microfilaments in the parathyroid cell, as in the pancreatic beta cell, have a retarding effect on PTH secretion.

Our data suggest that Vit A and CB act through different mechanisms. CB did not increase the PTH secretion rate until after 30 min of incubation, whereas Vit A increased secretion to 192% of control at 30 min (Fig. 1). Furthermore, the secretion rates in the presence of Vit A and CB together suggest that the combination of CB and Vit A has an additive effect at 120 min of incubation and synergistic effect at 180 min when compared to the release of PTH in the presence of each agent alone. If contractile microfilaments act by retarding the release of the secretion granule upon merger with the cell membrane, then Vit A may alter the membrane or microfilaments in such a way as to synergize the effect of CB on the secretion of PTH.

The effects of CB have been attributed to its effect on microfilaments. However, it is difficult to attribute the diverse effects of CB on hormone secretion (e.g., stimulation of PTH and insulin secretion and inhibition of secretion from sympathetic nerve endings) to a disrupting effect on microfilaments. Recent reports suggest that CB also affects membrane functions, such as transport of glucose and deoxyglucose (22, 23) and adhesiveness of cells in culture (24). Also, CB does not affect the structure or contractile function of skeletal muscle actin filaments *in vitro* (25). Therefore, although we have attributed the action of CB to its effect on microfilaments, CB may increase parathyroid hormone secretion through other cellular mechanisms, and possibly through an effect on the cell membrane.

Summary. The role of, and interrelationships between, microtubules, microfilaments, and membrane active agents in the secretion of parathyroid hormone were determined utilizing

VB, a microtubule disrupter, CB, a microfilament disrupter, and Vit A, an agent known to interact with membranes.

Parathyroid tissue pieces were incubated for 180 min in the presence of VB, CB, and Vit A, alone or in combination. VB alone consistently inhibited parathyroid hormone secretion. While Vit A alone stimulated the mean parathyroid hormone secretion rate, VB markedly inhibited this effect. CB alone did not change secretion in the first 30 min of incubation but thereafter stimulated secretion. In contrast to the delayed effect of CB alone, Vit A plus CB together increased the PTH secretion rate during the first 30 min, and their effects were at least additive at 120 and 180 min of incubation.

Assuming that the actions of the agents tested are reasonably specific and that immunoreactive PTH in media appropriately reflects secretion of intact hormone, our results suggest that: (a) Vit A increases PTH secretion perhaps through an interaction with the cell or secretion granule membrane; (b) microtubules or microtubular-like proteins facilitate PTH secretion; (c) microfilaments may retard the release of PTH; (d) there is a close interrelationship between membrane, microtubular protein, and microfilament function.

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