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EFFECTS OF VERAPAMIL AND DEXAMETHASONE ON THE 1,25-DIHYDROXYVITAMIN D_3-MEDIATED CALCIUM ABSORPTIVE MECHANISM IN THE ORGAN-CULTURED EMBRYONIC CHICK DUODENUM

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Abstract—1,25-Dihydroxyvitamin D_3 ($1,25(OH)_2D_3$) is known to induce the biosynthesis of a specific, calcium-binding protein (CaBP) and to stimulate calcium transport in the organ-cultured embryonic chick duodenum. The biosynthesis of CaBP has been shown previously to exhibit an absolute dependence on the ambient calcium concentration of the culture medium. Verapamil, a calcium-channel blocker, decreased calcium influx into the organ-cultured duodenum and inhibited the induction of CaBP by $1,25(OH)_2D_3$. Raising ambient calcium concentrations to as high as 10 mM did not prevent or reverse the inhibitory actions of verapamil. Dexamethasone, known to augment CaBP biosynthesis and calcium uptake in the organ-cultured duodenum in response to $1,25(OH)_2D_3$, largely prevented inhibition of CaBP by verapamil. The actions of verapamil and dexamethasone were correlated with altered steady-state calcium concentrations of the organ-culture duodenum, strongly supporting a regulatory role of calcium in the $1,25(OH)_2D_3$-mediated, intestinal calcium absorptive mechanism.

Any of a large number of vitamin D seco-steroids induces the *de novo* biosynthesis of a specific, intestinal calcium-binding protein (CaBP) in the embryonic chick duodenum maintained in organ culture [1]. The naturally-occurring, vitamin D metabolite, 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$), the hormonal form of vitamin D, stimulates both the unidirectional, passive influx of calcium at the mucosal surface [2] and the transmucosal transport [3] of calcium in this system. CaBP appears to be directly involved in these transport processes [3]. Using this organ culture system, it was established that $1,25(OH)_2D_3$ induction of CaBP was absolutely dependent on the ambient calcium concentration of the culture medium [4], suggesting a local, regulatory role for calcium at physiologic levels.

Verapamil, a calcium-channel blocker, widely employed in the treatment of cardiac arrhythmias [5], has been shown to inhibit influx of calcium into many cell types in addition to cardiac muscle. Calcium-dependent cell functions, including hormone (insulin) secretion [6] and protein (collagen) synthesis [7], are inhibitable by verapamil and other calcium antagonists. It seemed reasonable to assess the effects of verapamil on $1,25(OH)_2D_3$/calcium-dependent CaBP biosynthesis and calcium influx in the organ-cultured duodenum. Since glucocorticoids have been shown to augment $1,25(OH)_2D_3$-mediated responses in this system [8, 9], any possible interaction with verapamil was also assessed.

METHODS

The technique for maintenance of embryonic chick duodenum in organ culture in serum-free medium has been described [1]. Measurement of the $1,25(OH)_2D_3$-induced CaBP was by a specific and sensitive radial immunodiffusion assay [10]. $^{45}Ca^{2+}$ uptake by the tissue, essentially a measure of unidirectional influx of calcium at the duodenal mucosal surface [2], was determined by a published method [10]. Estimation of the steady-state tissue calcium concentration was accomplished by inclusion of $^{45}Ca^{2+}$ (0.5 μCi/ml) in the culture medium from initiation of culture until such time as the rate of influx approximated the rate of efflux (≥24 hr).

RESULTS

Initially, a series of concentration–response experiments were done to establish whether verapamil had any effect on $1,25(OH)_2D_3$ induction of CaBP at sub-cytotoxic levels. The results shown in Table 1 illustrate a concentration-dependent inhibition of CaBP by verapamil over the range 10^{-6}–10^{-4} M verapamil; maximum inhibition was 63% of control. Whereas tissue weight, total protein content and histologic appearance were unaltered over this range, greater than 10^{-4} M verapamil concentrations were cytotoxic by these criteria.

The time course of the inhibitory action of verapamil (10^{-4} M) on CaBP and $^{45}Ca^{2+}$ uptake, the latter measured during a short-term (30 min) post-culture incubation in a $^{45}Ca^{2+}$-labeled buffer solution [10], is shown in Table 2. Verapamil inhibited CaBP biosynthesis as early as 12 hr after the start of culture. This inhibition (approximately 50%) persisted through 48 hr of incubation. $^{45}Ca^{2+}$ uptake was also inhibited by verapamil as early as 12 hr after the start of culture, but remained at the same low level, relative to the $1,25(OH)_2D_3$-stimulated $^{45}Ca^{2+}$

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Table 1. 1,25-Dihydroxyvitamin D_3 induction of CaBP in organ-cultured duodenum: Effect of verapamil concentration

Verapamil (M)	CaBP* (μg/100 mg duodenum)
0	15.5 ± 1.8
10^{-7}	12.5 ± 1.2
10^{-6}	12.6 ± 1.1
10^{-5}	10.5 ± 0.7†
10^{-4}	5.7 ± 0.3†,‡

* Values: mean ± S.E.; six duodena/group. Culture period: 48 hr. $1,25(OH)_2D_3$ concentration: 5 nM. Verapamil was present for the entire culture period.

† Significantly lower than *minus verapamil* at 1% level.

‡ Significantly lower than *10^{-5} M verapamil* at 1% level.

uptake, through 48 hr. Thus, in the presence of verapamil, CaBP concentration in the duodena continued to increase (although only 50% of maximal), while $^{45}Ca^{2+}$ uptake remained constant. It was interesting to note that verapamil promoted the fairly rapid "relaxation" (within 12 hr) of the serosal coats (smooth muscle) of the duodenal strips, as expected from its well-known action on smooth muscle tissue, presumably as a result of calcium-channel blocking [11].

In subsequent experiments, it was demonstrated that verapamil had to be present for at least the first 36 hr of a 48-hr culture period to observe maximal inhibition of CaBP and $^{45}Ca^{2+}$ uptake (Table 3). When present for only the first 12–24 hr or the last 12–24 hr of a 48-hr culture period, the inhibitory actions of verapamil were either less marked or absent (Table 3).

A series of experiments were undertaken to determine if the calcium-channel blocking action of verapamil could be prevented, or overcome, by raising ambient calcium concentration. Calcium concentrations as high as 10 mM (the basal culture medium contains 2.5 mM calcium) could not prevent the inhibitory actions of verapamil (data not shown). For example, an experiment such as that depicted in Table 3, Protocol II, in which the only change was increasing the calcium concentration to 10 mM, produced similar results.

When dexamethasone was included in the culture medium, the inhibitory effect of verapamil on CaBP induction was almost completely abolished (Table 4). However, dexamethasone did not prevent verapamil inhibition of $^{45}Ca^{2+}$ uptake (Table 4). Similar experiments in which the duodena were incubated in the presence of $^{45}Ca^{2+}$ in the culture medium for the entire period revealed that dexamethasone increased the steady-state tissue calcium concentration (steady-state was reached between 24 and 48 hr), while verapamil decreased it (Table 4). The combination of dexamethasone and verapamil resulted in a steady-state tissue calcium concentration statistically equivalent to that seen with dexamethasone alone (Table 4).

DISCUSSION

The involvement of calcium in the $1,25(OH)_2D_3$-dependent biosynthesis of CaBP at the intestinal level has been suggested from several *in vivo* studies [12–14] and, more directly indicated, from *in vitro* studies with the organ-cultured duodenum [4]. The present studies of $^{45}Ca^{2+}$ uptake by the organ-cultured duodenum (essentially a reflection of the unidirectional influx of calcium at the mucosal surface [2]), revealed that verapamil inhibited calcium influx and, consequently, CaBP biosynthesis. The inhibition of CaBP was not absolute, nor irreversible (see Tables 2 and 3); this and several other evaluated characteristics proved that the actions of verapamil were not the result of generalized cytotoxicity.

The augmentation of $1,25(OH)_2D_3$ induction of CaBP by glucocorticoids, including dexamethasone, appears to be related to prolongation of the

Table 2. 1,25-Dihydroxyvitamin D_3 activity in organ-cultured duodenum: Time course of verapamil inhibition*

Time of culture (hr)	Verapamil (10^{-4} M)	CaBP (μg/100 mg duodenum)	$^{45}Ca^{2+}$ uptake (% of minus $1,25(OH)_2D_3$ controls)
12	–	4.6 ± 0.5	139 (2.73 ± 0.05 vs 3.80 ± 0.14)
12	+	3.0 ± 0.1†	123† (3.08 ± 0.12 vs 3.79 ± 0.14)
24	–	22.4 ± 1.7	156 (2.58 ± 0.09 vs 4.02 ± 0.09)
24	+	11.5 ± 1.2†	127† (2.90 ± 0.06 vs 3.66 ± 0.05)
48	–	49.1 ± 3.2	228 (2.38 ± 0.08 vs 5.42 ± 0.18)
48	+	21.7 ± 1.9†	124† (2.88 ± 0.09 vs 3.55 ± 0.13)

* Values: CaBP, mean ± S.E.; $^{45}Ca^{2+}$ uptake, mean; six duodena/group. $1,25(OH)_2D_3$ concentration: 50 nM. Minus $1,25(OH)_2D_3$ controls had no detectable CaBP. $^{45}Ca^{2+}$ uptake by the tissue was measured during a 30-min post-culture incubation in a $^{45}Ca^{2+}$-labeled buffer solution [10]. Values are expressed as a "% of minus $1,25(OH)_2D_3$ controls" for ease of presentation. The raw data, "% of available $^{45}Ca^{2+}$ taken up/100 mg duodenum" (mean ± S.E.), is shown for the minus $1,25(OH)_2D_3$ control group versus the plus $1,25(OH)_2D_3$-treated group, in parentheses. These data were subjected to Students' *t*-test. The coefficient of variation of the $^{45}Ca^{2+}$ uptake in this and Tables 3 and 4 was always ≤10%.

† Significantly lower than *minus verapamil* control at 1% level at each time.

Table 3. $1,25(OH)_2D_3$ activity in organ-cultured duodenum: Effect of preincubation time with verapamil*

First 12 hr Verapamil (10^{-4} M)	*Next 36 hr* Verapamil (10^{-4} M)	Protocol I CaBP (μg/100 mg duodenum)	$^{45}Ca^{2+}$ uptake (% of minus $1,25(OH)_2D_3$ controls)
−	−	45.7 ± 4.2	161
+	−	34.9 ± 3.9†,‡	147†,‡
−	+	18.3 ± 2.7†	122†
+	+	16.8 ± 2.3†	117†
First 24 hr Verapamil (10^{-4} M)	*Next 24 hr* Verapamil (10^{-4} M)	Protocol II CaBP (μg/100 mg duodenum)	$^{45}Ca^{2+}$ uptake (% of minus $1,25(OH)_2D_3$ controls)
−	−	43.6 ± 1.0	169
+	−	30.8 ± 1.7†,‡	147†,‡
−	+	31.8 ± 1.7†,‡	155†,‡
+	+	22.5 ± 1.6†	129†
First 36 hr Verapamil (10^{-4} M)	*Next 12 hr* Verapamil (10^{-4} M)	Protocol III CaBP (μg/100 mg duodenum	$^{45}Ca^{2+}$ uptake (% of minus $1,25(OH)_2D_3$ controls)
−	−	43.5 ± 1.7	152
+	−	16.0 ± 1.1†	120†
−	+	36.0 ± 1.3†,‡	146‡
+	+	17.1 ± 1.4†	125†

* Values: CaBP, mean ± S.E., eight duodena/group; $^{45}Ca^{2+}$ uptake, mean, six duodena/group. $1,25(OH)_2D_3$ concentration: 50 nM. For further details see legend of Table 2.

† Significantly lower than *minus verapamil* control at 1% level.

‡ Significantly higher than *continuous verapamil* control at 1% level.

$1,25(OH)_2D_3$ receptor's residence time in the nucleus of the intestinal cell [9]. This phenomenon has not been shown to be calcium sensitive. Regardless, dexamethasone largely prevented the inhibition by verapamil of CaBP synthesis (Table 4). Interestingly, dexamethasone did not prevent the action of verapamil on short-term influx of calcium. Thus, the restoration of CaBP biosynthesis to nearly control levels apparently had little or nothing to do with re-opening verapamil-blocked calcium channels. Alternatively, dexamethasone seemed to affect tissue steady-state calcium levels, which were much more highly correlated with CaBP concentration than with short-term calcium influx.

It is not known to what extent the measured tissue calcium concentrations reflect alterations in ionic calcium concentrations nor what component of the total intracellular calcium may be involved in the regulation of CaBP synthesis. However, a recent report attests to the essentiality of ionic calcium in

Table 4. $1,25(OH)_2D_3$ activity in organ-cultured duodenum: Dexamethasone and the inhibitory action of verapamil*

Dex (2.75 μM)	Verapamil (10^{-4} M)	CaBP (μg/100 mg duodenum)	$^{45}Ca^{2+}$ uptake (% of minus $1,25(OH)_2D_3$ controls)	Calcium concentration (nmoles/g tissue)
−	−	34.6 ± 3.0	161	20.8 ± 0.8
+	−	51.6 ± 2.0†	185†	23.1 ± 1.4†
−	+	22.1 ± 1.8‡	131‡	18.0 ± 0.4‡
+	+	41.3 ± 1.8†,§	131‡	21.7 ± 0.6§

* Values: mean ± S.E.; six to eight duodena/treatment. Culture period: 48 hr. $1,25(OH)_2D_3$ concentration: 50 nM. For further details see legend of Table 2.

† Significantly higher than *minus dexamethasone minus verapamil* control at 1% level (CaBP); 5% level (calcium concentration).

‡ Significantly lower than *minus verapamil* control at 1% level.

§ Significantly higher than *minus dexamethasone plus verapamil* control at 1% level.

the biosynthesis of casein in response to prolactin in organ-cultured mouse mammary gland [15]. Interestingly, in neither the organ-cultured chick duodenum (unpublished) nor the mouse mammary gland [15] are calcium-dependent hormonal responses mediated by the intracellular calcium receptor, calmodulin.

In conclusion, the present results support the hypothesis that calcium regulates the biosynthesis of CaBP induced by $1,25(OH)_2D_3$. Compounds such as verapamil and dexamethasone, which alter the biosynthesis and function of CaBP, may do so via alteration of steady-state calcium concentrations. The clinical significance of these findings is unclear, but there is a suggestion that verapamil and/or glucocorticoids may be having some unexpected pharmacologic consequences on intestinal calcium absorption.

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