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# $Ca^{2+}$-CHANNEL AGONIST BAY K8644 MIMICS 1,25$(OH)_2$-VITAMIN $D_3$ RAPID ENHANCEMENT OF $Ca^{2+}$ TRANSPORT IN CHICK PERFUSED DUODENUM

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To further understand the molecular mechanism by which 1,25$(OH)_2$-vitamin $D_3$ [1,25$(OH)_2D_3$] rapidly stimulates intestinal calcium transport (termed "transcaltachia"), the effect of the calcium channel agonist BAY K8644 was studied in vascularly perfused duodenal loops from normal, vitamin D-replete chicks. BAY K8644, 2 uM, was found to stimulate $^{45}Ca^{2+}$ transport from the lumen to the vascular effluent to the same extent as physiological levels of 1,25$(OH)_2D_3$. The sterol and the $Ca^{2+}$ channel agonist both increased $^{45}Ca^{2+}$ transport 70% above control values within 2 min and 200% after 30 min of vascular perfusion. The effect of the $Ca^{2+}$ channel agonist was dose dependent. Also, 1,25$(OH)_2D_3$-enhanced transcaltachia was abolished by the calcium channel blocker nifedipine. Collectively, these results suggest the involvement of 1,25$(OH)_2D_3$ in the activation of basal lateral membrane $Ca^{2+}$ channels as an early effect in the transcaltachic response. 

Vitamin $D_3$ acting through its daughter metabolite 1,25-dihydroxy-vitamin $D_3$ [1,25$(OH)_2D_3$] is the major regulator of $Ca^{2+}$ homeostasis and it is now considered to be a true steroid hormone acting via intranuclear receptor binding and regulation of gene transcription (1). However, several studies have demonstrated a 1,25$(OH)_2D_3$-mediated rapid acute uptake of $Ca^{2+}$ in both classical and non classical target tissues (2-4). The perfused duodena of normal (vitamin D replete) chicks was also shown to respond very rapidly to physiological levels of the seco-sterol, increasing $Ca^{2+}$ transport from the brush border to the basal lateral membrane of the epithelial cells (5,6). This rapid response, termed "transcaltachia", is evidenced only when 1,25$(OH)_2D_3$ is introduced in the vascular perfusate or basal lateral membrane surface but not when the seco-sterol is introduced to the lumen or brush border membrane surface (5). This sidedness may reflect the existence of a specific receptor for 1,25$(OH)_2D_3$ at the basal lateral membrane as has been reported for other steroid hormones (7). The acute effect of 1,25$(OH)_2D_3$ on intestinal $Ca^{2+}$ transport is not affected by a wide variety of agents including actinomycin D, but can be abolished by colchicine (an anti-microtubule drug) and leupeptin (a lysosomal cathepsin B-inhibitor) (8,9). It has been proposed that 1,25$(OH)_2D_3$ increases intestinal $Ca^{2+}$ transport through a vesicular pathway: internalization of $Ca^{2+}$ in endocytic vesicles, fusion of the vesicles with lysosomes and movement of the lysosomes (along microtubules) to the basal lateral membrane where exocytosis of the contents completes the transport process (10). The purpose of the present study was to further investigate the possible

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mechanism(s) by which $1,25(OH)_2D_3$ mediates transcaltachia. We have evaluated the possibility that activation of intestinal membrane $Ca^{2+}$-channels is involved in the early response to the sterol.

## MATERIALS AND METHODS

### Materials

$^{45}CaCl_2$ (1 Ci/m mol) was obtained from New England Nuclear, Boston, MA. BAY K8644 was from Calbiochem, La Jolla, CA. Nifedipine was from Sigma Chemical Co., St. Louis, MO. All other chemicals used were reagent grade.

### Animals

White Leghorn cockerels (Lakeview, CA) were obtained on the day of hatch and maintained on a vitamin D-supplemented diet (O.H. Kruse Groting and Milling, Ontario, CA) for 4-5 weeks (300-500 g). When vitamin D-deficient chicks were employed, they were raised for 4 weeks on a rachitogenic diet (11).

### Intestinal $Ca^{2+}$ Transport Measurements

Measurements of $Ca^{2+}$ transport were carried out in perfused chick duodena essentially as previously described (5). Briefly, chicks were anesthetized with chloropent (0.3 ml/100 g), the duodenal loop was exposed, and the three pairs of blood vessels branching off from the celiac artery ligated prior to cannulation of the celiac artery itself. The arterial perfusion was initiated with modified Gey's Balanced Salt Solution (GBSS) containing 27 mM $NaHCO_3$-5.6 mM D-glucose and oxygenated with 95% $O_2$/5% $CO_2$ at a flow rate of 2 ml/min. An auxiliary pump was used for the introduction of vehicle (ethanol) or test substances plus albumin (0.125% w/v) to the vascular perfusate at a rate of 0.25 ml/min. The intestinal lumen was then flushed and filled with GBSS containing $^{45}Ca^{2+}$ (5 uCi/ml). The intestinal preparation was kept at 27°C and moist under layers of saline-dampened cheese cloth. Each duodenum was perfused with control medium for 20 min after filling the lumen with $^{45}Ca^{2+}$ to establish basal $^{45}Ca$ transport rates. The tissue was then exposed to test substances or re-exposed to control medium for an additional 30 min. The vascular perfusate was collected at 2 min intervals during basal and treatment periods for measurements of $^{45}Ca^{2+}$ levels. The results are expressed as the ratios of the $^{45}Ca^{2+}$ appearing in the control 20 min perfusate divided by the $^{45}Ca^{2+}$ appearing over the subsequent 30 min test period.

## RESULTS

The effects of the $Ca^{2+}$-channel activator, BAY K8644 on intestinal $Ca^{2+}$ transport in normal, vitamin D-replete chicks was investigated. Fig. 1 shows that, similarly to physiological levels of $1,25(OH)_2D_3$, vascular perfusion of duodenal loops with BAY K8644 stimulates $^{45}Ca^{2+}$ transport from the lumenal compartment to the venous effluent. A 70% increase above controls was observed after only 2 min of exposure to the agonist. Within 10 min $^{45}Ca^{2+}$ in the venous effluent rose 140% from controls, reaching a plateau at 30 min with a 200% increase with respect to controls. BAY K8644-stimulation of $^{45}Ca^{2+}$ transport was dose-dependent (Fig. 2). Treatment with 0.5 uM and 1 uM BAY K8644 resulted in $^{45}Ca^{2+}$ transport levels that were lower than those in duodena exposed to 130 pM $1,25(OH)_2D_3$ (20% and 45% increase at 2 min, and 50% and 100% increase at 30 min with respect to controls for 0.5 uM and 1 uM BAY K8644, respectively). The highest concentration of agonist tested (2 uM) resulted in $^{45}Ca^{2+}$ transport levels similar to those obtained with 130 pM $1,25(OH)_2D_3$.

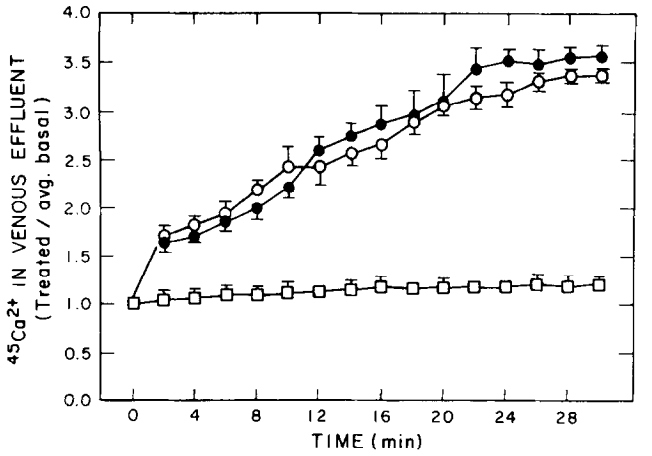


Figure 1.
Effect of $1,25(OH)_2D_3$ and BAY K8644 on the appearance of $^{45}Ca^{2+}$ in the venous effluent of perfused duodena from vitamin D-replete chicks. Each duodenum, filled with $^{45}CaCl_2$ (5 uCi/ml) in GBSS, was vascularly perfused (27°C) for the first 20 min with control medium (GBSS containing 0.125% BSA and 0.05 ul/ml ethanol) and then with 130 pM $1,25(OH)_2D_3$ (O); 2 uM BAY K8644 (●) or control medium ( □). Values are the mean + SD of 4 duodena for each treatment.

In order to determine if the $Ca^{2+}$-agonist affects $^{45}Ca^{2+}$ transport when presented to the brush border membrane surface of the intestinal epithelial cells, 2 uM BAY K8644 was placed in the luminal medium together with $^{45}CaCl_2$. Fig. 3 shows that, analogously to $1,25(OH)_2D_3$, introduction of the $Ca^{2+}$-channel agonist into the lumenal compartment failed to elevate $^{45}Ca^{2+}$ transport above control levels. Also, the $Ca^{2+}$-channel agonist ( 2 uM) failed to stimulate $^{45}Ca^{2+}$ transport when offered to either the vascular or lumenal compartment of vitamin D-deficient chicks for up to 30 min. In agreement with previous observations (2),

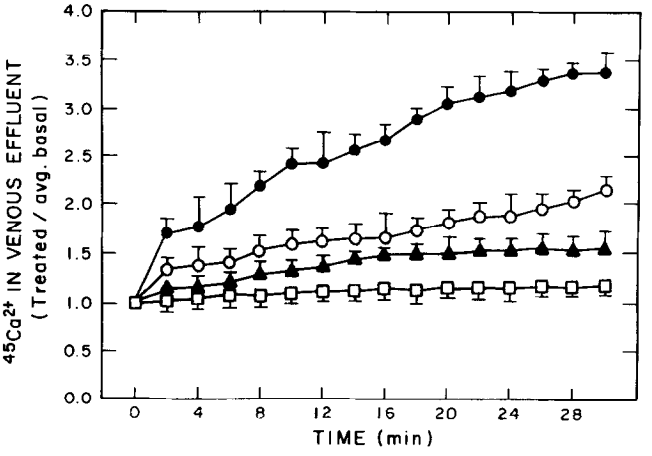


Figure 2. Dose-response effects of BAY K8644 on duodenal $^{45}Ca^{2+}$ transport. Experimental conditions were as described in Fig.1. During the treatment phase, the duodena were perfused with BAY K8644; 0.5 uM (▲); 1 uM (O), 2 uM (●) or control medium ( □). Values are the mean + SD of 3 duodena for each treatment.

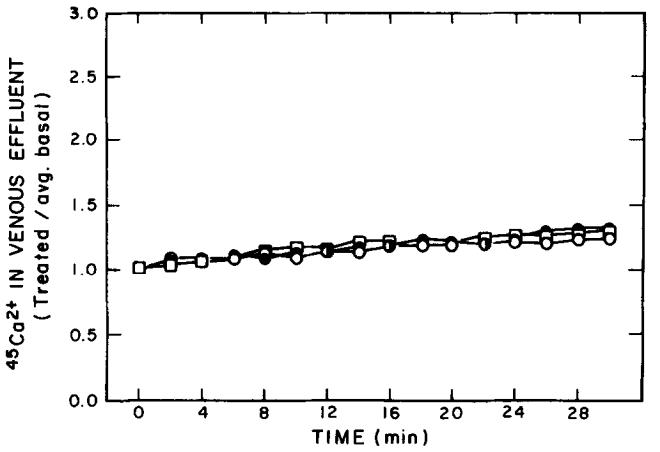


Figure 3. Lack of effect of lumenal BAY K8644 on enhanced appearance of $^{45}Ca^{2+}$ in the venous effluent of perfused duodena. Basal perfusion conditions were described in Fig. 1. The lumen of the duodenum was filled with $^{45}CaCl_2$ in GBSS containing either 2 uM BAY K8644(●); 130 pM $1,25(OH)_2D_3$ (O) or 0.05 ul/ml ethanol (□). Values are the mean + SD of 3 duodena for each treatment.

$1,25(OH)_2D_3$ was also without effects on $^{45}Ca^{2+}$ transport when offered either to the basal lateral membrane or brush border membrane surfaces of vitamin D-deficient chick intestine (data not shown).

Exposure of the basal lateral membrane surface to 1 uM nifedipine, a calcium-channel antagonist, blocked the increase in calcium transport elicited by vascular perfusion of duodena with $1,25(OH)_2D_3$ (Fig. 4).

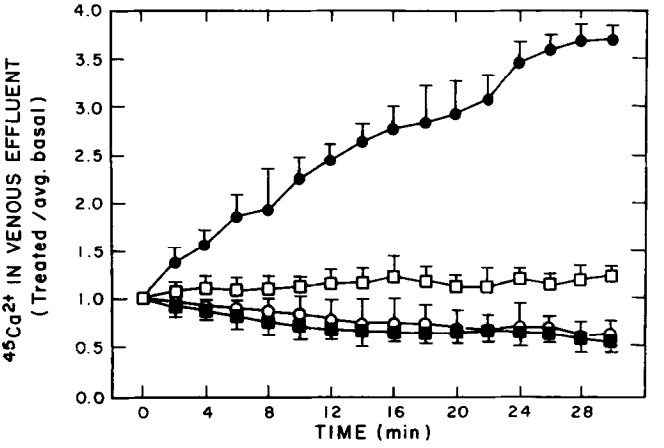


Figure 4. Suppression of $1,25(OH)_2D_3$-induced duodenal $^{45}Ca^{2+}$ transport by the calcium-channel blocker nifedipine. Experimental conditions were as described in Fig. 1. During the treatment phase, duodena were vascularly perfused with 130 pM $1,25(OH)_2D_3$ (●); $1,25(OH)_2D_3$ + 1 uM nifedipine (O); 0.05 ul/ml ethanol (□) or 0.05 ul/ml ethanol + 1 uM nifedipine (■). Values are the mean + SD of 3 duodena for each treatment.

## DISCUSSION

The results of the present study provide the first evidence indicating that the rapid stimulation of intestinal calcium transport (transcaltachia) in normal vitamin $D_3$-replete chicks by $1,25(OH)_2D_3$ is mediated by an activation of basal lateral membrane $Ca^{2+}$-channels. The $Ca^{2+}$-channel agonist BAY K8644 mimicked the effects of the sterol on the transcaltachic response and analogously to $1,25(OH)_2D_3$, enhanced calcium transport was only observed when the basal lateral membrane surface was exposed to the sterol by vascular perfusion. In addition, the dihydropyridine nifedipine completely abolished the rapid increase in $^{45}Ca^{2+}$ transport induced by $1,25(OH)_2D_3$, further suggesting that the vitamin D-metabolite activated voltage-operated $Ca^{2+}$-channels. In agreement with these observations, the existence of $Ca^{2+}$-channels in basal lateral membranes from rabbit ileal epithelial cells has been recently demonstrated (12).

The acute stimulation of duodenal calcium transport evoked either by BAY K8644 or $1,25(OH)_2D_3$ was not observed in vitamin D-deficient chicks. This is in agreement with previous observations (13,14) in which 1-2 h were required to detect enhanced $Ca^{2+}$ transport after administration of $1,25(OH)_2D_3$ to rachitic chicks and may reflect the steroid-hormone receptor-mediated actions of $1,25(OH)_2D_3$ that are known to occur in the intestine (1). It has been shown that a rise in the intracellular $Ca^{2+}$ concentration triggers exocytosis and activation of the plasma membrane $Ca^{2+}$-ATPase and $Na^+$-$Ca^{2+}$ exchanger (15). It is conceivable that the early effects of $1,25(OH)_2D_3$ on $Ca^{2+}$-channel activity may cause a transient increase in intracellular calcium, that in turn would activate exocytosis of $Ca^{2+}$-containing vesicles as well as $Ca^{2+}$ efflux by the $Ca^{2+}$-pump and $Na^+$-$Ca^{2+}$ exchanger resulting in a net increase of $Ca^{2+}$ transport. Further work on the mechanistic aspects of $1,25(OH)_2D_3$ effects on intestinal membrane $Ca^{2+}$ channel activity is currently in progress.

## ACKNOWLEDGMENTS

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