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At the Cutting Edge

# Steroid hormone actions at the plasma membrane: induced calcium uptake and exocytotic events

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## Calcium uptake and exocytosis

Calcium as an intracellular second messenger is not a new concept, although the subject is undergoing a resurgence in interest, in part reflecting our current appreciation of calcium channels and their role in $Ca^{2+}$ influx, as well as the regulation of cytoplasmic $Ca^{2+}$ movements by phosphoinositide metabolism (Michell, 1986). Perhaps the most intriguing aspect of the topic is that the agonists are not restricted to a single structural class, but include neurotransmitters, peptide hormones, and steroid hormones (see Table 1). Parallels in signal transduction between divergent structural classes of regulatory substances have been proposed before (Szego, 1978). The current work will attempt to focus more closely on systems where agonist-mediated changes in cellular calcium culminate in an exocytotic event.

### *Neurotransmitters*

Neurotransmitters and their analogs are a class of compounds that are perhaps the best known mediators of $Ca^{2+}$ channel regulation. As recently reviewed by Miller (1990), neurotransmitter activation of calcium channels and the concomitant influx of the divalent cation results in the exocytosis of additional neurotransmitters. Conversely, activation of $K^+$ channels reduces $Ca^{2+}$ influx and inhibits the exocytosis of neurotransmitters.

Carbachol is a parasympathomimetic drug that stimulates the exocytosis of chromaffin granules from bovine adrenal cells. Elevation of external $K^+$ to depolarize the cell membrane, or augmentation of intracellular $Ca^{2+}$ by application of the ionophore A23187, leads to similar exocytotic events (Millman and Strittmatter, 1990). Thus, regardless of the primary route for enhancing intracellular $Ca^{2+}$, convergence is found in the endpoint of exocytosis.

### *Peptide hormones*

Vasopressin is a small peptide hormone known to regulate water movement. In 1976 (Pietras et al.) it was reported that vasopressin stimulated calcium uptake in frog urinary bladder, and that this could be correlated with the exocytosis of lysosomal enzymes; the mechanisms involved were not characterized at that time. Parathyroid hormone (PTM) is another peptide that affects renal epithelia, predominantly by stimulating reabsorption of calcium. Backsai and Friedman (1990) have reported that PTH not only activates $Ca^{2+}$ channels in renal epithelial cells but also stimulates exocytosis of microtubule dependent vesicles. The authors postulated that the exocytotic event represented insertion of channels in the plasma membrane. However, in view of the data

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TABLE 1

AGONISTS THAT MEDIATE CHANGES IN INTRACELLULAR $Ca^{2+}$ AND $Ca^{2+}$-DEPENDENT EXOCYTOSIS

| Neurotransmitters | Peptide hormones | Steroid hormones |
|---|---|---|
| Neurotransmitters<br>Miller, 1990 | Vasopressin<br>Pietras et al., 1976 | Estrogen<br>Pietras and Szego, 1975<br>Szego, 1978 |
| Carbachol<br>Millman and Strittmatter, 1990 | Parathyroid hormone<br>Nemere and Szego, 1981a, b<br>Nemere and Norman, 1986<br>Backsai and Friedman, 1990 | Progesterone<br>Moreau et al., 1980<br>Wasserman et al., 1980<br>Deliconstantinos, 1988<br>Osman et al., 1989<br>Thomas and Meizel, 1989 |
| | | $1,25(OH)_2D_3$<br>Matsumoto et al., 1981<br>Nemere and Szego, 1981a, b<br>Nemere et al, 1984, 1986, 1988, 1989<br>Desai et al., 1986<br>Edelman et al., 1986<br>Nemere and Norman, 1986<br>de Boland and Boland, 1987<br>Kurnik et al., 1987<br>Lieberherr, 1987<br>Baran and Kelly, 1988<br>Mezzetti et al., 1988<br>Schwartz et al., 1988<br>Caffrey and Farach-Carson, 1989<br>Lieberherr et al., 1989<br>Lucas et al., 1989<br>Bourdeau et al., 1990<br>Civitelli et al., 1990<br>de Boland and Norman, 1990a, b<br>de Boland et al., 1990<br>Wali et al., 1990 |

that suggest calcium transport in other epithelia occurs by way of membrane-delimited organelles (see below), the phenomenon described may in fact represent PTH-mediated secretion from calcium-bearing vesicles.

*Steroid hormones*

Steroid hormones are best known for their nuclear effects on gene regulation, although there have been various indications for non-nuclear actions over the years. Estrogen, in the form of estradiol-17$\beta$, was reported to enhance calcium uptake in uterine endometrial cells over a 10–30 min period (Pietras and Szego, 1975), although it is not known whether the effects are exerted through calcium channels. The altered calcium levels were, however, correlated to an exocytotic event, the release of lysosomal enzymes (Szego, 1978).

Progesterone is another steroid hormone that has been studied with regard to non-nuclear actions, particularly calcium uptake in target cells. By the use of calcium sensitive dyes, it has been shown that progesterone causes a rapid increase in intracellular $Ca^{2+}$ in oocytes (Moreau et al., 1980; Wasserman et al., 1980), although neither group of workers monitored potential exocytotic events. Progesterone was also found to initiate

rapid calcium uptake in sperm, with the consequent induction of the acrosome reaction (Osman et al., 1989). The acrosome reaction is essentially the exocytosis of a modified Golgi/lysosomal organelle that releases hydrolases enabling sperm penetration of the oocyte zona pellucida and plasmalemma. Although the calcium uptake is not mediated by a voltage operated $Ca^{2+}$ channel, the steroid hormone also stimulates phosphoinositide metabolism (Thomas and Meizel, 1989). Alternative mechanisms for progesterone-mediated calcium increases include inhibition of $Ca^{2+}$-ATPase as reported in synaptosomal preparations (Deliconstantinos, 1988), and the as yet unexplored possibility that a second messenger or receptor operated $Ca^{2+}$ channel may be involved (Thomas and Meizel, 1989).

Both the seco-steroid hormone 1,25-dihydroxyvitamin $D_3$ ($1,25(OH)_2D_3$) and the peptide PTH have been reported to have rapid, non-nuclear actions in the intestinal epithelium (Nemere and Szego, 1981a, b; Nemere et al., 1984; Nemere and Norman, 1986). These actions include calcium uptake into, and lysosomal enzyme release from, isolated cells (Nemere and Szego, 1981a, b), and net $Ca^{2+}$ transport in perfused duodenal loops (Nemere et al., 1984; Nemere and Norman, 1986). Subsequent biochemical studies have linked these observations at least in part by the demonstration that $1,25(OH)_2D_3$-sensitive calcium transport operates through an endosomal-lysosomal pathway (Nemere et al., 1986, 1988, 1989).

Numerous laboratories have expanded upon the theme of non-nuclear effects of $1,25(OH)_2D_3$, particularly with respect to altered calcium and/or phospholipid metabolism. It has been confirmed that the seco-steroid influences calcium levels in rat enterocytes (Lucas et al., 1989) and colonic epithelium (Wali et al., 1990), amphibian kidney tubules (Edelman et al., 1986), muscle cells (de Boland and Boland, 1987), lymphoid cells (Desai et al., 1986), mammary tissue (Mezzetti et al., 1988), hepatocytes, (Baran and Kelly, 1988), and osteoblasts (Lieberherr et al., 1987; Caffrey and Farach-Carson, 1989; Civitelli et al., 1990). In many of these target tissues, phospholipid metabolism has also been documented (Matsumoto et al., 1981; Kurnik et al., 1987; Baran and Kelly, 1988; Schwartz et al., 1988; Lieberherr et al., 1989; Bourdeau et al., 1990; Civitelli et al., 1990; Wali et al., 1990).

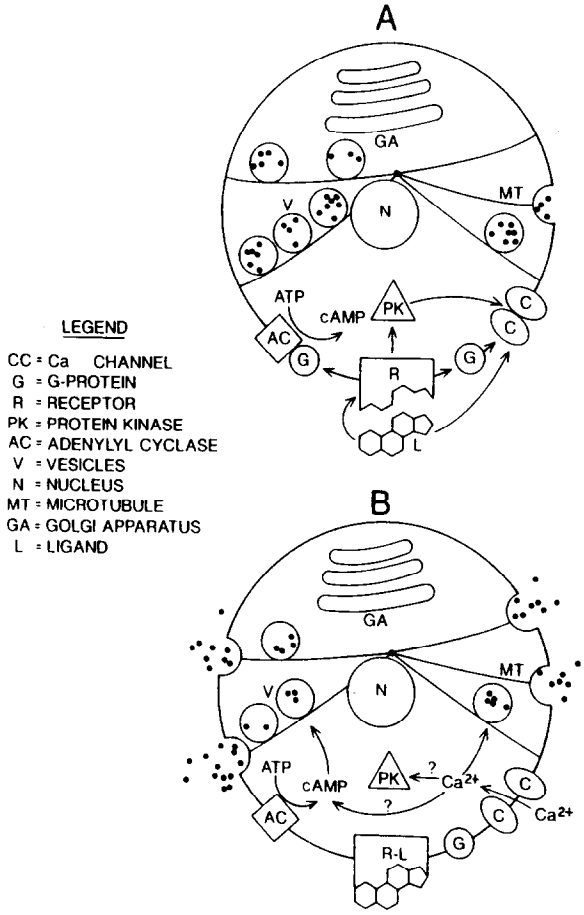


Fig. 1. Potential mechanisms for agonist-mediated $Ca^{2+}$ channel activation and stimulation of exocytosis. (*A*) Among the hypothetical pathways are agonist interaction directly with a channel component or a receptor distinct from the channel. The liganded receptor can then activate a $Ca^{2+}$ channel through G proteins, formation of cAMP or activation of protein kinase(s). The receptor-ligand complex may be rapidly endocytosed and modulate other signal transducers en route to the nucleus. (*B*) $Ca^{2+}$ influx through the channel may promote exocytosis by regulating molecular motors for vesicular movements, act on phosphorylation/dephosphorylation events involved in movement, and/or modulate fusogenic proteins. Alternatively, the channel may be opened through a primary phosphorylation event.

## Transcaltachia, calcium channels, and vesicular calcium transport in intestine

That the non-nuclear actions of $1,25(OH)_2D_3$ and PTH are mediated through alterations in

cytoplasmic calcium levels is also suggested by the addition of the ionophore A23187 to isolated rat enterocytes, which reproduced in part the exocytotic responses (Nemere and Szego, 1981b). More recently, in the perfused duodenal loop of normal chicks, it was shown that $1,25(OH)_2D_3$-induced transcaltachia (the rapid hormonal stimulation of calcium transport) could be mimicked by BAY K8644, an activator of voltage dependent $Ca^{2+}$ channels, applied to the basal-lateral membrane (de Boland et al., 1990). Significantly, the $Ca^{2+}$ channel agonist was without effect at the brush border or apical membrane (de Boland et al., 1990), just as $1,25(OH)_2D_3$ is inactive at the lumenal surface (Nemere et al., 1984). Thus, receptor-mediated induction of transcaltachia may well be mediated through activation of $Ca^{2+}$ channels.

The current interest in the diversity of signal transduction mechanisms has also led to a further evaluation of potential second messengers. Since PTH is known to act on both rat and chick intestinal calcium handling, and the peptide hormone often activates adenylate cyclase, the role of protein kinases in transcaltachia has similarly been addressed. Not surprisingly, vascular perfusion with forskolin, an activator of protein kinase A, or phorbol ester, an activator of protein kinase C, were each found to induce transcaltachia to levels equivalent to those found after seco-steroid stimulation (de Boland and Norman, 1990a). Once again, addition of these second messenger agonists to the brush border surface failed to elicit a transcaltachic response.

In keeping with the model that phosphorylation events may be involved in seco-steroid hormone activity, inhibitors of either protein kinase A or C were found to completely block $1,25(OH)_2D_3$-induced transcaltachia (de Boland and Norman, 1990a). In agreement with the suggestion that transcaltachia involves $Ca^{2+}$ channel activation, nifedipine, an antagonist of $Ca^{2+}$ channels, blocked transcaltachia elicited by $1,25(OH)_2D_3$, forskolin, and phorbol ester (de Boland and Norman, 1990).

Finally, the question remains as to whether influx of extracellular calcium is required for transcaltachia, as opposed to redistribution of the divalent cation from intracellular stores. Vascular perfusion with the ionophore ionomycin induced an increase in calcium transport into the lumen and, thus the vascular effluent (de Boland and Norman, 1990), confirming the stimulatory effect of calcium ionophores reported nearly 10 years earlier (Nemere and Szego, 1981b). Vascular perfusion with the calcium chelator EGTA was found to reversibly inhibit $1,25(OH)_2D_3$-mediated transcaltachia (de Boland and Norman, 1990b); replenishing the divalent cation at the basal-lateral surface allowed stimulated transport to commence, further suggesting that $Ca^{2+}$ from the extracellular environment is necessary for net transport. That the various agonists and antagonists alter intracellular calcium levels was determined in studies with fura-2 loaded intestinal epithelial cells (de Boland and Norman, 1990b). Exposure of dye-loaded cells to $1,25(OH)_2D_3$, phorbol ester, forskolin, ionomycin or depolarizing levels of KCl increased calcium dependent fluorescence relative to controls, whereas addition of $1,25(OH)_2D_3$ with EGTA or nifedipine gave fluorescence levels less than or equal to control.

To summarize the non-nuclear effects of $1,25(OH)_2D_3$ in the chick intestine, it appears that the seco-steroid hormone interacts with a membrane-associated receptor to stimulate protein kinase activity and phosphorylation event(s) that in turn activate voltage operated $Ca^{2+}$ channels at the basal-lateral membrane. Influx of $Ca^{2+}$ stimulates the exocytosis of calcium-bearing vesicles, in other words initiates calcium transport across the intestine. Further, it is now clear that such 'stimulus-secretion coupling' is common to both steroid hormones and other physiological signals.

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