which is regulated independently from that of the cell. MVs and plasma transduction are present in MVs, including protein kinase C (PKC) activity, growth factors (1). Enzymes involved in membrane-mediated signal formation, enzymatic modification of the matrix, and activation of local modulate a number of activities in growth plate cartilage, including mineral formation, enzymatic modification of the matrix, and activation of local.

Introduction. Matrix vesicles (MVs) are extracellular organelles that modulate a number of activities in growth plate cartilage, including mineral formation, enzymatic modification of the matrix, and activation of local growth factors (1). Enzymes involved in membrane-mediated signal transduction are present in MVs, including protein kinase C (PKC) activity, growth factors (1). Enzymes involved in membrane-mediated signal formation, enzymatic modification of the matrix, and activation of local modulate a number of activities in growth plate cartilage, including mineral formation, enzymatic modification of the matrix, and activation of local.

Results. PKC at 0.2 h, but by 12 h, the inhibitors blocked the effect of the secosteroid, indicating that production and release of newly synthesized MVs was blocked.

Gq mediates the direct inhibitory effect of 1,25 on MV PKC. GDPβS, but not choleratoxin or pertussis toxin, blocked the effect of 1,25. No other inhibitor modified the action of 1,25. 1,25 inhibits MV PKC via the 1,25-mVDR, as Ab99 abrogated the effect. Moreover, regulation of MV PKC was stereospecific; neither 1β,25(OH)2D3 nor 24R,25(OH)2D3 had an effect. The 1,25-nVDR was not involved as MVs failed to react with a specific antibody to this receptor in western blots. 1,25-nVDR was also not present in PMs, although the cytosol/nuclear fraction remaining after isolating the membranes did contain immunoreactive 1,25-nVDR. Interestingly, arachidonic acid, which is released by GC could be nongenomic receptors. U73122 to inhibit PLC and with GDP βS to inhibit G-proteins. MVs also were incubated directly with 1,25 without pretreatment. (I) Confluent cultures of GC cells were treated for 0.2 to 1.5 hours to assess the nongenomic effect of the secosteroid on MVs resident in the matrix, or for 12 or 24 h to assess the effect of 1,25 on organelle biogenesis. MVs and PMs were isolated from the cultures and the 1,25 dependent isoform in each membrane fraction was determined using PKC isoform-specific antibodies. Regulation of MV production by 1,25 was examined by use of monensin to block protein transport through the Golgi and actinomycin D and cycloheximide to block transcription and translation. The role of the PKC in PMs was assessed using a specific polyclonal antibody (Ab99) (4,5). We also examined whether the effect of 1,25 on MV PKC at 24 h is mediated by phospholipase C (PLC). To do this, cells were treated with 1,25 ± the PLC inhibitor U73122.

(II) To examine the signal transduction pathways involved in mediating the rapid effect of 1,25 on isolated MVs, MVs isolated from cultures not previously treated with 1,25 were incubated directly with 1,25 ± inhibitors or activators of signal transduction pathways shown previously to mediate the activation of PKCα by 1,25 in GC cell layers. MVs were incubated with U73122 to inhibit PLC and with GDPβS to inhibit G-proteins. MVs also were treated directly with arachidonic acid, which is the product of PLA2 action, the arachidonic acid precursor, linolenic acid, and the arachidonic acid metabolite, PGE2, as well as with diacylglycerol, the product of PLC action. Because 1,25 inhibits PKCζ in MVs, we examined the role of signaling pathways that do not mediate the stimulatory effect of 1,25 on PKCζ in PMs. Membrane fractions were incubated with cholera toxin and pertussis toxin; and wortmannin to inhibit PDE.

MVs do not contain DNA or RNA, so any response to the addition of 1,25 by naive membranes would be a priori be nongenomic mechanisms. This would not, however, rule out a role for the 1,25-nuclear VDR (1,25-nVDR). Accordingly, we examined MVs for the presence of the 1,25-nVDR by western blot. The role of the 1,25-nVDR in the response of MV PKC to 1,25 was assessed using Ab99. Specificity of the response was established using 1β,25(OH)2D3 and 24R,25(OH)2D3.

For all experiments, significance among groups was determined by ANOVA and post hoc testing performed using Bonferroni's modification of Student's t-test for multiple comparisons.

Results. 1,25 caused a dose-dependent increase in PM PKC, while PKC in MVs was decreased in cultures treated for 0.2 or 1.5 h (Fig 1). In contrast, after 12 and 24 h of treatment with 1,25, PKC in PMs was unchanged, while that in MVs was increased. This suggests that the early response was probably due to a direct action of the hormone on the membrane and the later effect was due to new MV biosynthesis and altered PKC content. The responsive PKC isoform was PKCζ in the PMs and PKCζ in the MVs. Actinomycin D, cycloheximide, or monensin had no effect on 1,25-dependent PKC activity. We also examined whether the effect of 1,25 on MV PKC to 1,25, as well as with diacylglycerol, the product of PLC action. Because 1,25 inhibits PKCζ in MVs, we examined the role of signaling pathways that do not mediate the stimulatory effect of 1,25 on PKCζ in PMs. Membrane fractions were incubated with cholera toxin and pertussis toxin; and wortmannin to inhibit PDE.

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**References**

48th Annual Meeting of the Orthopaedic Research Society Poster No: 0540

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Support. PHS grants DE-08603 and DE-05937 and the Center for the Enhancement of the Biology/Biomaterials Interface at UTHSCSA.

**Hebrew University, Jerusalem, Israel.**

**Utah State University, Logan, UT.**