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Different Mechanisms Are Involved in Intracellular Calcium Increase by Insulin-Like Growth Factors 1 and 2 in Articular Chondrocytes: Voltage-Gated Calcium Channels, and/or Phospholipase C Coupled to a Pertussis-Sensitive G-Protein

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Abstract This study describes the mechanisms involved in the IGF-1 and IGF-2-induced increases in intracellular calcium concentration $[Ca^{2+}]i$ in cultured chondrocytes and the involvement of type 1 IGF receptors. It shows that IGF-1, IGF-2, and insulin increased the cytosolic free calcium concentration $[Ca^{2+}]i$ in a dose-dependent manner, with a plateau from 25 to 100 ng/ml for both IGF-1 and IGF-2and from 1 to 2 μg/ml for insulin. The effect of IGF-1 was twice as great as the one of IGF-2, and the effect of insulin was 40% lower than IGF-1 effect. Two different mechanisms are involved in the intracellular $[Ca^{2+}]i$ increase. 1) IGF-1 and insulin but not IGF-2 involved a Ca^{2+} influx through voltage-gated calcium channels: pretreatment of the cells by EGTA and verapamil diminished the IGF-1 or insulin-induced $[Ca^{2+}]i$ but did not block the effect of IGF-2. 2) IGF-1, IGF-2, and insulin also induced a Ca^{2+} mobilization from the endoplasmic reticulum: phospholipase C (PLC) inhibitors, neomycin, or U-73122 partially blocked the intracellular $[Ca^{2+}]i$ increase induced by IGF-1 and insulin and totally inhibited the effect of IGF-2. This Ca^{2+} mobilization was pertussis toxin (PTX) dependent, suggesting an activation of a PLC coupled to a PTX-sensitive G-protein. Lastly, preincubation of the cells with IGF_1 receptor antibodies diminished the IGF-1-induced Ca^{2+} spike and totally abolished the Ca^{2+} influx, but did not modify the effect of IGF-2. These results suggest that IGF-1 action on Ca^{2+} influx involves the IGF_1 receptor, while part of IGF-1 and all of IGF-2 Ca^{2+} mobilization do not implicate this receptor. J. Cell. Biochem. 64:414–422. © 1997 Wiley-Liss, Inc.

Key words: IGF-1; IGF-2; type 1 IGF receptor; intracellular calcium; phospholipase C; chondrocytes

Insulin-like growth factors (IGFs) are important for the growth and differentiation of cartilage. IGF-1 is thought to act by stimulating chondrocyte proliferation and matrix protein synthesis [Tyler, 1989]. IGF-2 mimics the effects of IGF-1 but is less potent [Froesch et al., 1985]. Most of the mitogenic and anabolic effects of IGF-1 and IGF-2 on cartilage as well as in other tissues are mediated via type 1 IGF receptors [Oh et al., 1993]. Whether or not the high affinity binding of IGF-2 to type 2 IGF receptors is of biological importance remains unclear. A few effects of IGF-2 have been suggested to be mediated via type 2 IGF receptors [Hari et al., 1987; Minniti et al., 1992]. In cartilage, the wild type of IGF-2 and its ^{29}Ser variant have been reported to inhibit in vitro chondrocyte cathepsin B and L activities [De Ceuninck et al., 1995].

Abbreviations used: IGF-1 and -2, insulin growth factors I and 2 ; EGF, epidermal growth factor; $[Ca^{2+}]i$, cytosolic free or intracellular calcium concentration; PLC, phospholipase C; PKC, protein kinase C; $InsP_3$, inositol 1,4,5 trisphosphate; PTX, pertussis toxin; CTX, cholera toxin; U-73122, 1-(6-((17β-3-metoxyestra-1,3,5(10)-trien-17-yl)-amino)-hexyl)-1H-pyrrole-2,5-dione (U-73122); U-73343, 1-(6-((17β-3-metoxyestra-1,3,5(10)-trien-17-yl)-amino)hexyl)-1H-pyrrolidine-2,5-dione; BSA, bovine serum albumin; αMEM, α minimal essential medium; EGTA, ethylene glycol-bis(β-aminoethyl ether)N,N,N′,N′-tetraacetic acid.

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The signal transduction mechanisms involved in IGF-1 and IGF-2 actions are unknown in cartilage and still unclear in other tissues. IGF-1 increases inositol 1, 4, 5, triphosphate ($InsP_3$) formation in porcine thyroid cells [Takasu et al., 1989], rat cardiac monocytes [Guse et al., 1992], and human arterial smooth muscle cells [Bornfeldt et al., 1994], but only Ca^{2+} influx in epidermal growth factor (EGF)-primed BALB/c 3T3 cells [Kojima et al., 1988]. IGF-2 stimulates the formation of $InsP_3$ and diacylglycerol via activation of phospholipase C in the kidney proximal basolateral membranes [Rogers and Hammerman, 1985], while it increases only Ca^{2+} influx in EGF-primed BALB/c 3T3 cells [Nishimoto et al., 1987].

The present study was undertaken to compare the modulation of intracellular calcium concentration in chondrocytes by IGF-1 and IGF-2. It describes for the first time two mechanisms involved in the increased cytosolic free calcium concentration ($[Ca^{2+}]i$) by IGF-1 and IGF-2 in articular chondrocytes in culture and determines whether their signals were transmitted via type 1 IGF receptors. The present data show that only IGF-1 involves a Ca^{2+} influx via voltage-gated calcium channels. In contrast, there is a calcium mobilization from the endoplasmic reticulum induced by IGF-1 and IGF-2 which is mediated by activation of a phospholipase C coupled to a pertussis toxin-sensitive G-protein. Type 1 IGF receptors appeared to be involved in IGF-1-induced Ca^{2+} influx while part of IGF-1 and all of IGF-2 calcium mobilization from the endoplasmic reticulum do not implicate these receptors.

METHODS

Animals and Materials

Fauve de Bourgogne rabbits (21 days old) were obtained from Ruvel, France. Human IGF-1 and IGF-2 were from Genzyme (Boston, MA), and anti-IGF type 1 antibody (IGF-1Rα [1H7]) was from Santa Cruz Biotechnology (Santa Cruz, CA) and Tebu (Le Perray en Yvelines, France). Bovine pancreatic insulin, IGF-free bovine serum albumin (BSA) and all chemicals were from Sigma (St. Louis, MO). 1-(6-((17β-3-Metoxyestra-1,3,5(10)-trien-17-yl)-amino)hexyl)-1H-pyrrole-2,5-dione (U-73122) and 1-(6-((17β-3-metoxyestra-1,3,5(10)-trien-17-yl)-amino)hexyl)-1H-pyrrolidine-2,5-dione (U-73343) were from Biomol Research Laboratory (Plymouth, MA) and Tebu (Le Perray en Yvelines, France). Fetal calf serum (FCS) and Ham's F 12 medium were from Boehringer (Mannheim, Germany), and α-minimal essential medium (αMEM) without phenol red from Eurobio (Paris, France). Fura-2/AM was from Amersham (Les Ulis, France). IGF-1 and IGF-2 were dissolved in 10 mM acetic acid containing 1 mg/ml IGF-free BSA. The solvent had no effect on $[Ca^{2+}]i$.

Isolation and Cell Culture

Articular chondrocytes from 21-day-old rabbits were cultured as previously described [Corvol et al., 1987]. Briefly, shoulder, hip, and knee articular cartilages were dissected out from prepubertal rabbits under sterile conditions, cut into small pieces and digested with 0.05% hyaluronidase, followed by 0.25 % trypsin, and finally 0.2% collagenase [Green 1971]. Chondrocytes were plated out at 5 x 10^4 cells per glass coverslip and cultured for 3 days in Ham's F 12 medium plus 10% FCS until confluent. Cells were incubated in serum-free and phenol red-free αMEM for 24 h before use.

Calcium Measurement

The cells were washed 3 times with Hanks' Hepes, pH 7.4 (137 mM NaCl, 0.441 mM KH_2PO_4, 0.442 mM Na_2HPO_4, 0.885 mM $MgSO_47H_2O$, 27.7 mM glucose, 1.25 mM $CaCl_2$, 20 mM Hepes, 1 mg/ml IGF-free BSA), and loaded with 1 µM Fura-2/AM for 20 min in the same buffer at room temperature. The glass coverslip carrying the cells was inserted into a cuvette containing 2.5 ml Hanks' Hepes, pH 7.4, and the cuvette was placed in a thermostatted (37°C) Hitachi 2000 spectrofluorimeter. Drugs and reagents were added directly to the cuvette under continuous stirring.

The Fura-2 response to the intracellular calcium concentration $[Ca^{2+}]i$ was calibrated from the 340/380 nm fluorescence ratio after subtraction of the background fluorescence of the cells at 340 and 380 nm [Grynkiewicz et al., 1985]. The dissociation constant for the Fura-2/Ca^{2+} complex was taken as 224 nM [Grynkiewicz et al., 1985]. The values for R_{max}, and R_{min} were calculated from measurements using 25 µM digitonin and 4 mM EGTA and enough Tris-base to raise the pH to 8.3 or higher. Each measurement on Fura-2-loaded cells was followed by a parallel experiment with non-Fura-2-loaded cells under the same conditions .

Experimental Protocol

The effects of IGF-1 and IGF-2 (1–100 ng/ml) and insulin (0.025–2 mg/ml) on the intracellular calcium concentration $[Ca^{2+}]i$ in confluent chondrocytes were examined first. We then investigated whether the action of IGFs on $[Ca^{2+}]i$ was due to an influx of Ca^{2+} from extracellular milieu, and/or Ca^{2+} mobilization from intracellular stores using 2 types of blocking experiments: 1) A small excess of EGTA (2 mM) was added to the cuvette medium [Albert and Tashjian 1984] and IGF-1, IGF-2, or insulin was added 1 min later. 2) Cells were incubated for 1 min with 1 μM verapamil, a selective blocker of Ca^{2+} entry via voltage-dependent Ca^{+} channels before adding IGF-1, IGF-2, or insulin. Two inhibitors of phospholipase C were used to determine the part of the $[Ca^{2+}]i$ transient due to Ca^{2+} released from intracellular stores. Cells were incubated with 2 mM neomycin, an indirect inhibitor of PLC via binding to phosphoinositides [Prentki et al., 1986], or 0.5–3 μM U-73122, a direct inhibitor of PLC [Bleasdale et al., 1989] for 2 min before adding IGF-1, IGF-2, or insulin. Third, we examined the possible involvement of a G-protein by incubating the cells for 16 h with 100 ng/ml pertussis toxin (PTX) or 1 μg/ml cholera toxin (CTX) before adding either 25 ng/ml IGF-1 or IGF-2 or 2 mg/ml insulin. Lastly, we attempted to determine whether the effects of IGF-1 and IGF-2 were due to their binding to type 1 IGF receptors in 2 types of experiments. 1) Cells were pretreated for 2 h, 6 h, or 16 h with 5 μg/ml antibodies against type 1 IGF receptor before adding 25 ng/ml IGF-1 or IGF-2. 2) Cells were incubated for 90 s, 10 min, or 60 min with 2 μM insulin before adding 25 ng/ml IGF-1 or 25 ng/ml IGF-2.

Statistical Analysis

The data on the changes in $[Ca^{2+}]i$ were analyzed by one-way analysis of variance. Treatment pairs were compared by Dunnett's method. Differences of $P < 0.05$ were considered significantly different. A value of n represents n different cultures for a specific experiment.

RESULTS

Direct Effects of IGFs and Insulin on Intracellular Calcium

The basal intracellular calcium concentration in confluent articular chondrocytes in culture was 89 ± 7 nM (mean ± S.E., n = 10). Figure 1 shows the transient increases in $[Ca^{2+}]i$ induced by 25 ng/ml IGF-1 and IGF-2 or 2 mg/ml insulin, corresponding to the maximal active concentration of each peptide in this system. $[Ca^{2+}]i$ dropped rapidly after 30 s down to the basal level for IGF-2, but remained above the baseline (15 ± 1%, mean ± S.E., n = 15, $P < 0.001$) for IGF-1 and insulin. The effects of the IGFs were dose-dependent, and a plateau was reached at 25 ng/ml for both IGF-1 and IGF-2 and at 1 mg/ml for insulin (Table I). The IGF-1 effects were twice as great as those of IGF-2 with EC 50% at 12.8 and 6.0 ng/ml, respectively. The effects of insulin were 40% lower than IGF-1 effects. These effects were reproducibly observed in 10 experiments performed with cells from different animals.

Blockade of IGF- or Insulin-Induced Changes in Intracellular Calcium Concentration

A small excess of EGTA (2 mM) slightly decreased the basal $[Ca^{2+}]i$ (15 ± 2%, mean ± S.E., n = 6, $P < 0.001$), while 1 μM verapamil triggered a drop of 18 ± 3 % (mean ± S.E., n = 6, $P < 0.001$). The steady-state values were reached within 20 s for both EGTA and verapamil. A total of 25 ng/ml IGF-1, 25 ng/ml IGF-2, or 2 μg/ml insulin was added 1 min after EGTA or verapamil. Pretreatment by EGTA and verapamil diminished the transient increase of $[Ca^{2+}]i$ induced by IGF-1 or insulin by 43 ± 4% and 40 ± 3% (mean ± S.E., n = 5, $P < 0.001$), respectively. They also totally abolished the sustained plateau phase. By contrast, they did not block the effect of IGF-2 (Fig. 2A,B).

Characterization of the Intracellular Organelle Responsible for Calcium Mobilization

Cells were pretreated for 2 min with 2 mM neomycin, 0.5–3 μM U-73122, or U-73343 (a close but inactive analog of U-73122 [Bleasdale et al., 1989]) before adding 25 ng/ml IGF-1, 25 ng/ml IGF-2, or 2 μg/ml insulin. U-73122 (1–3 μM) itself triggered a transient dose-dependent (1–3 μM) increase in $[Ca^{2+}]i$ (data not shown). The transient $[Ca^{2+}]i$ response to IGF-1 or insulin was partially blocked by neomycin (55 ± 3%, mean ± S.E., n = 5, $P < 0.001$) and U-73122 (53 ± 4%, mean ± S.E., n = 5, $P < 0.001$), while the plateau phase remained unchanged (Fig. 3A,B). In contrast, both 2 mM neomycin and 2 μM U-73122 inhibited the $[Ca^{2+}]i$ response to IGF-2 (Fig. 3A,B). U-73343 (0.5–3 μM) had no

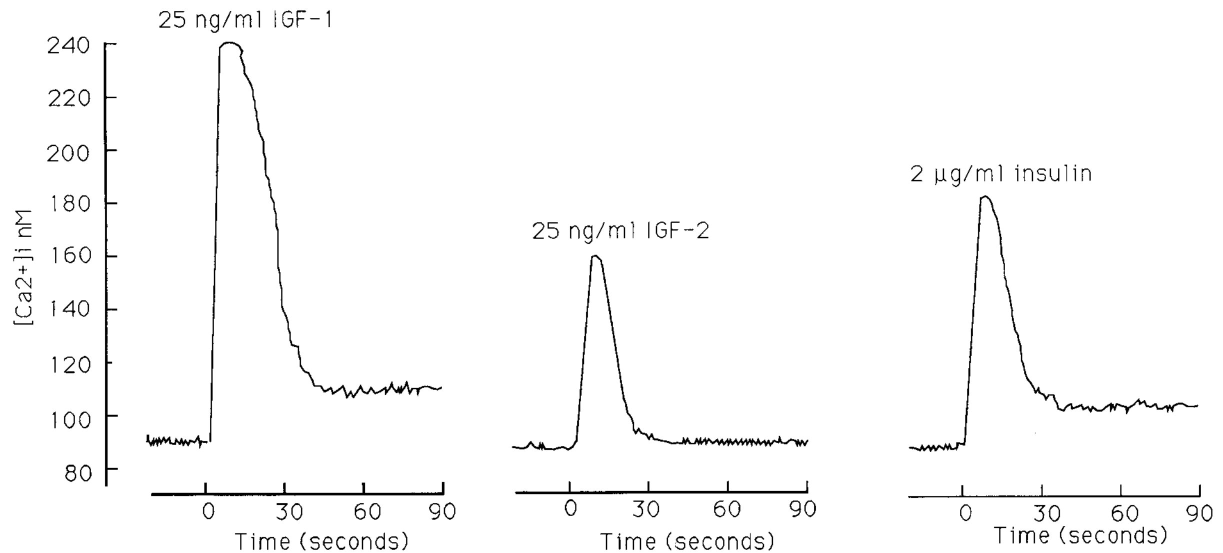

Fig. 1. Effects of 25 ng/ml IGF-1, 25 ng/ml IGF-2, or 2 µg/ml insulin on the intracellular calcium concentration in rabbit confluent chondrocytes in culture. Cells were loaded with 1 µM Fura-2/AM for 20 min. This trace is representative of at least 5 glass coverslips for 10 different cultures.

TABLE I. Dose-Dependent Effects of IGFs and Insulin on the Intracellular Calcium Concentration of Confluent Chondrocytes. Data are the Intracellular Calcium Concentrations 10 s after Adding IGFs or Insulin. Values are Means ± S.E. (n = 10)

Concentration	[Ca2+]i nM		
(ng/ml)	IGF 1	IGF 2	Insulin
0	89 ± 7	89 ± 7	89 ± 7
1	101 ± 6	98 ± 7	nd
5	108 ± 7	107 ± 8	nd
10	142 ± 8[a]	135 ± 8[a]	nd
12.5	166 ± 7[b]	145 ± 7[a]	nd
25	253 ± 6[b]	168 ± 8[b]	102 ± 4
40	248 ± 6[b]	172 ± 7[b]	98 ± 5
50	245 ± 8[b]	165 ± 6[b]	104 ± 7
100	255 ± 9[b]	178 ± 8[b]	101 ± 4
200	nd	nd	99 ± 6
400	nd	nd	138 ± 5[a]
800	nd	nd	164 ± 3[b]
1000	nd	nd	188 ± 5[b]
2000	nd	nd	186 ± 4[b]

Values significantly different from the basal concentration ([a]$p < 0.05$; [b]$p < 0.001$).

effect on the responses to IGF-1, IGF-2, or insulin (data not shown).

Effects of Pertussis (PTX) or Cholera (CTX) Toxins on Intracellular Calcium Response to IGFs and Insulin

Chondrocytes were incubated for 16 h with 100 ng/ml PTX, loaded with Fura-2/AM, and the $[Ca^{2+}]i$ measured. PTX itself did not alter the basal $[Ca^{2+}]i$, but it partially blocked the increase induced by IGF-1 (50 ± 5%, mean ± S.E., n = 5, $P < 0.001$) or insulin (48 ± 4%, mean ± S.E., n = 5) and totally blocked the $[Ca^{2+}]i$ response to IGF-2 (Fig. 4). PTX did not modify the IGF-1- and insulin-induced sustained plateau phase. Incubating the cells with 1 µg/ml CTX for 16 h did not alter the basal $[Ca^{2+}]i$ or the $[Ca^{2+}]i$ responses to IGF-1, IGF-2, or insulin (data not shown).

Possible Involvement of Type I IGF Receptors in the Intracellular Calcium Response to IGFs

In order to see whether the two IGFs exerted their action via the type 1 IGF receptors, 2 series of experiments were undertaken: 1) inactivation of type 1 IGF receptors by specific antibodies; 2) saturation of type 1 IGF receptors by micromolar concentration of insulin. The preincubation of the cells with type 1 IGF receptor antibodies for 2 h, 6 h, or 16 h similarly diminished the $[Ca^{2+}]i$ response to IGF-1 by 50 ± 4% (mean ± S.E., n = 5, $P < 0.001$). Figure 5A only shows data observed after 16h preincubation. The preincubation of the cells for 90 s, 10 or 60 min in the presence of insulin gave identical results (data shown only after 90 s preincubation on Fig. 5A). In contrast, the type 1 IGF receptor antibodies or high concentrations of insulin had no effect on the transient increase induced by IGF-2 (Fig. 5B).

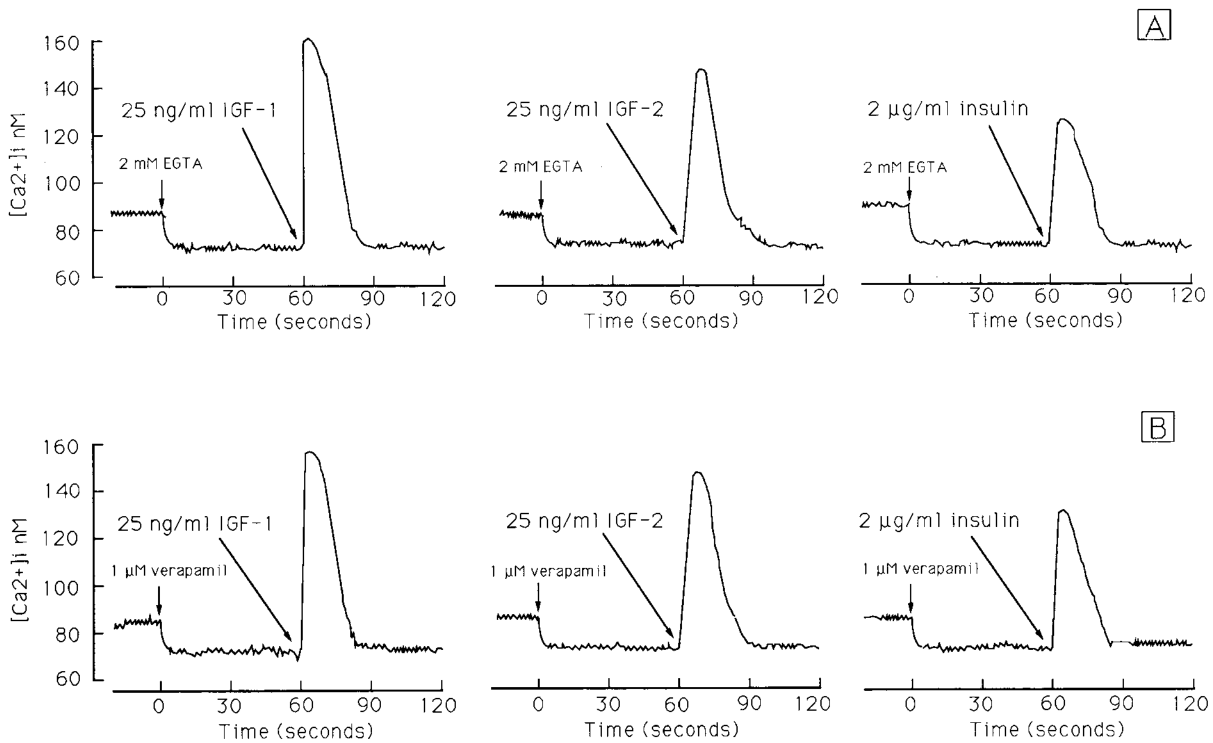

Fig. 2. Effects of EGTA (**A**) and verapamil (**B**) on the response to IGF-1, IGF-2, or insulin. Chondrocytes were incubated for 1 min with 2 mM EGTA or 1 µM verapamil before adding 25 ng/ml IGF-1, 25 ng/ml IGF-2 or 2 µg/ml insulin. This trace is representative of at least 5 coverslips for 5 different cultures.

DISCUSSION

This is, to our knowledge, the first direct evidence that IGF-1 and IGF-2 rapidly increase the cytosolic free calcium concentration within 5 s in articular chondrocytes in culture. The IGF-1 , but not IGF-2, effect involves a Ca^{2+} influx, as indicated by the action of EGTA and verapamil, via voltage-gated calcium channels, found in chondrocytes [Grandolfo et al., 1992]. IGF-1 and IGF-2 induce the mobilization of Ca^{2+} from the endoplasmic reticulum with activation of a phospholipase C (PLC). This effect of IGF-1 and IGF-2 on Ca^{2+} mobilization is in agreement with the data showing that IGF-1 increases $InsP_3$ formation in other cell types such as porcine thyroid cells [Takasu et al., 1989], rat cardiac myocytes [Guse et al., 1992], and in human arterial smooth muscle cells [Börnfeld et al., 1994] and also with the IGF-2-increased formation of inositol 1,4,5 trisphosphate ($InsP_3$) in proximal tubular basolateral membranes of the canine kidney [Rogers et al., 1988]. However, our results on intracellular calcium in chondrocytes differ from those obtained in BALB/c 3T3 fibroblasts with IGF-1 or IGF-2 [Kojima et al., 1988, Nishimoto et al., 1987]. In these cells, IGF-1 or IGF-2 increased Ca^{2+} uptake via a calcium-permeable cation channel only when these fibroblasts were primed with epidermal growth factor (EGF), but not in quiescent or proliferating BALB/c 3T3 fibroblasts. Since EGF acts on several intracellular signal tranduction pathways [Loza et al., 1995; Peppelenbosch et al., 1991, 1992; Casabiell et al., 1993; Cerpovicz and Ochs, 1992; Dean and Boyton, 1995; Dunlop and Clark, 1993; Yeo and Exton, 1995] and there are cross-talks between these signaling pathways, two mechanisms may be responsible for the apparent effect of IGF-1 or IGF-2 on intracellular calcium in EGF-primed BALB/c 3T3 fibroblasts and may explain the apparent lack in cells not primed with EGF. One is that protein kinase C (PKC) activation by EGF may exert a negative feedback control on phosphatidylinositol 4,5 bisphosphate hydrolysis [Loza et al., 1995; Peppelenbosch et al., 1991], masking a possible effect of IGF-1 or IGF-2 on PLC and on the mobilization of Ca^{2+} from intracellular Ca^{2+} stores. The other is that hyperpolarization of the membrane by EGF may lead to increased cytoplasmic Ca^{2+} that is prolonged substan-

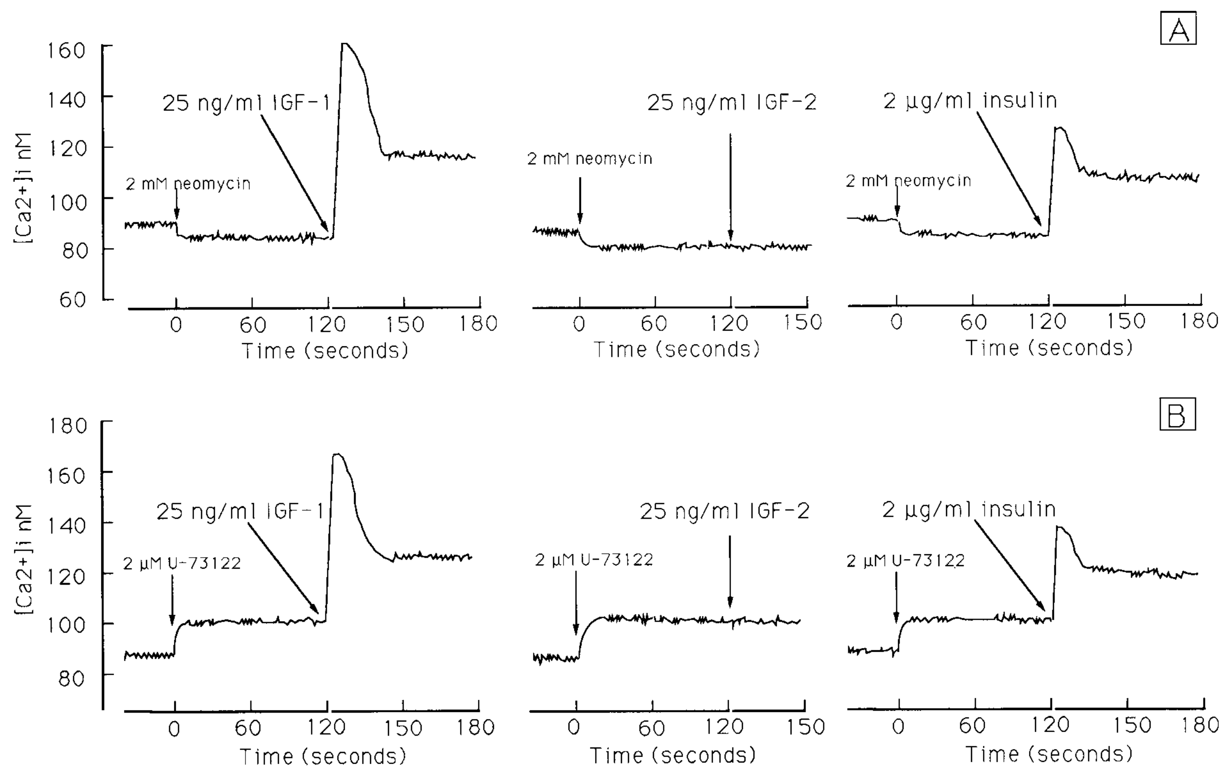

Fig. 3. Effects of neomycin (**A**) and U-73122 (**B**) on the $[Ca^{2+}]i$ response to IGF-1, IGF-2, or insulin. Chondrocytes were incubated for 2 min with 2 mM neomycin or 2 µM U-73122 before adding 25 ng/ml IGF-1, 25 ng/ml IGF-2, or 2 µg/ml insulin. This trace is representative of at least 5 coverslips for 5 different cultures.

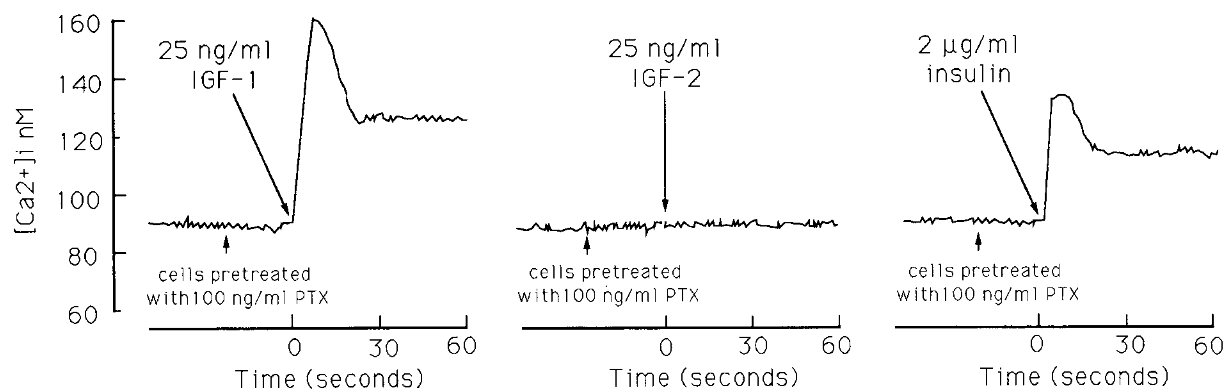

Fig. 4. Effects of pertussis toxin on intracellular calcium responses to IGFs and insulin. Chondrocytes were incubated for 16 h with 100 ng/ml pertussis toxin (PTX) before adding 25 ng/ml IGF-1, 25 ng IGF-2, or 2 µg/ml insulin. Each trace is representative of at least 5 coverslips for 5 different cultures.

tially by capacitive entry mechanisms, adding IGF-1 or IGF-2 may simply enhance the EGF action.

The effect of IGF-1 on $[Ca^{2+}]i$ increase in rabbit articular chondrocytes is partly blocked by pertussis toxin. This blocking effect of pertussis toxin corresponds to the IGF-1-induced Ca^{2+} mobilization without any change in the sustained plateau phase corresponding to the Ca^{2+} influx. This suggests that Ca^{2+} mobilization by IGF-1 involves a G-protein, whose activation is necessary before that of the PLC, and that this G-protein may be a Gq-protein [Taylor, 1990]. The reported IGF-1-increased inositol 1,4,5-triphosphate formation [Guse et al., 1992; Takasu et al., 1989] also suggests that this G-protein is coupled to a PLC that belongs to the PLC β family [Rhee and Choi, 1992]. Fur-

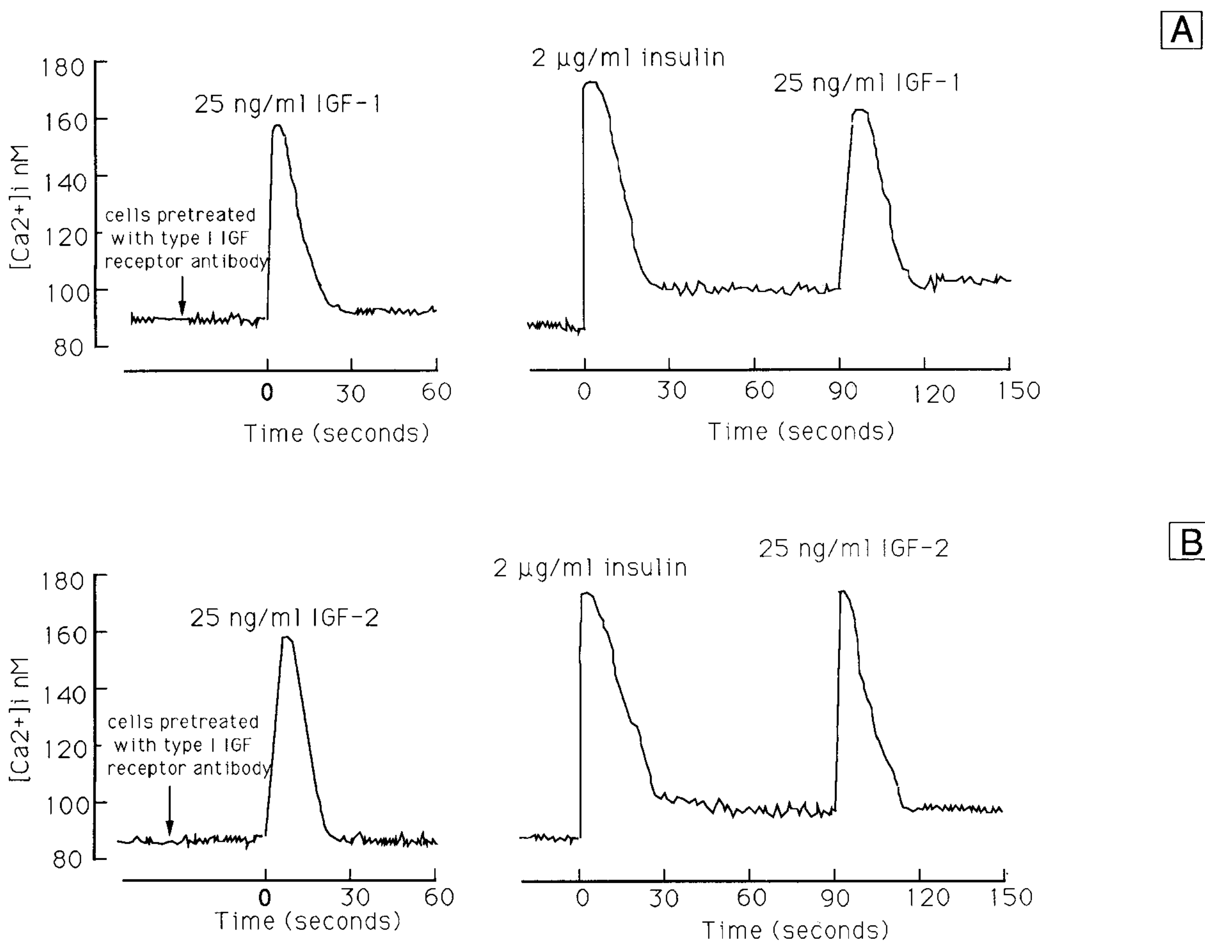

Fig. 5. Possible involvement of type I IGF receptor in the intracellular calcium responses to IGFs. **A:** Chondrocytes were incubated for 16 h with 5 µg/ml type I IGF receptor antibodies or for 90 s with 2 µg/ml insulin before adding 25 ng/ml IGF-1. **B:** Chondrocytes were incubated for 16 h with 5 µg/ml type I IGF receptor antibodies or for 90 s with 2 µg/ml insulin before adding 25 ng/ml IGF-2. Each trace is representative of at least 5 coverslips for 5 different cultures.

ther investigation is needed to characterize the type of PLC and the G-proteins involved in the actions of IGF-1. Neither pertussis toxin nor cholera toxin inhibits the IGF-1-increased Ca^{2+} influx in chondrocytes, suggesting that a G-protein is not involved in the IGF-1 action on Ca^{2+} influx.

Pertussis toxin totally blocked the IGF-2 effect on chondrocyte Ca^{2+} mobilization suggesting that a G-protein coupled to a PLC β is also involved in IGF-2 action. The involvement of a G-protein in IGF-2 transmembrane signaling is still debated. IGF-2 activates a pertussis-toxin (PTX) sensitive G_{i2} protein by functional coupling to purified rat type 2 IGF receptors in phospholipid vesicles and cell membranes [Nishimoto et al., 1989; Murayama et al., 1990]. But the human type 2 receptor neither interacts with a G-protein in mouse L-cell membranes, nor it is coupled to G_{i2} proteins in phospholipid vesicles [Körner et al., 1995].

Three types of receptors can mediate cellular responses to IGFs, the type 1 and type 2 IGF receptors and the insulin receptor. Both types of IGF receptors have been demonstrated in rabbit chondrocytes, but no insulin receptors were found as evaluated by specific binding assays and competition experiments [Jansen et al., 1989]. In rabbit articular chondrocytes, the type 1 IGF receptor binds IGF-1 with high affinity, IGF-2 with 3.7 lower affinity and insulin only at micromolar concentrations [De Ceuninck et al., 1995]. The type 2 IGF receptor binds preferentially to IGF-2, has 100-fold less affinity for IGF-1 and does not bind insulin [De Ceuninck et al., 1995]. Preincubation of the cells with type 1 IGF receptor antibodies, or saturating concentrations of insulin totally abol-

ished the sustained plateau phase and diminished the IGF-1-induced Ca^{2+} spike. These findings mean that, in our experimental conditions, all the Ca^{2+} influx induced by IGF-1 and part of the Ca^{2+} mobilization occur via the binding of IGF-1 to the type 1 IGF receptor. The persistence of a Ca^{2+} mobilization by IGF-1 when type 1 IGF receptor is blocked or saturated suggests that part of this IGF-1 effect is independent of its binding to this type of receptor. However, as the antibodies used are to the human IGF-1 receptor, we cannot exclude the possibility that the concentration of 5 μg/ml (or 10 μg/ml, data non shown) is insufficient to completely block IGF-1 binding to its receptors in rabbit chondrocytes. The data observed with insulin support the concept from the view point of post receptor events, even though in itself this does not directly address receptor binding. Thus, the possibility that part of the IGF-1 effect on Ca^{2+} mobilization occurs trough receptors other than the type 1 IGF receptors remains to be investigated.

The IGF-2 effect on Ca^{2+} mobilization is not modified by preincubation of the cells with type 1 IGF receptor antibodies nor saturating concentrations of insulin indicating that the IGF-2 effect is not mediated by the type 1 IGF receptor. The implication of receptors other than the type 1 IGF receptor in this phenomenon also remains to be determined. The type 2 IGF receptor is the first candidate. But the 300 kDa IGF-2 receptor [Jansen et al., 1989] which appears specific for cartilage could also mediate calcium regulation by IGF-2 in chondrocytes.

In conclusion, these in vitro data may eventually help in understanding cartilage pathophysiology where arthritic chondrocytes are known to be unresponsive to IGF-1 despite normal or increased receptor binding [Dore et al., 1994].

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