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RECIPROCAL MODULATION OF INSULIN AND INSULIN-LIKE GROWTH FACTOR-I RECEPTOR AFFINITY BY CALCIUM

Robert C. Baxter and Paul F. Williams

Department of Endocrinology, Royal Prince Alfred Hospital, Sydney, NSW 2050, Australia

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In contrast to its stimulation of insulin binding to human placental membranes, calcium inhibited the binding of insulin-like growth factor-I. The effects on receptors for both peptides were half-maximal at 2 mM calcium, and were entirely due to alterations in high affinity binding sites for the respective ligands. Calcium decreased the affinity of insulin-like growth factor-I sites, while stimulating the expression of high affinity insulin sites. Competition by each peptide at the receptor for the other peptide was enhanced by calcium. Modulation by calcium might provide a mechanism to amplify functional differences between the two structurally similar receptors.

IGF-I[a] is a growth-promoting polypeptide from human plasma, related to insulin both structurally (1) and functionally (2). An independently isolated peptide, SM-C[a], is indistinguishable from IGF-I by structural (3) and immunological (4) criteria, leading to the suggestion that the term SM-C/IGF-I be used to designate either peptide (5). Receptors for insulin and IGF-I are physically similar, both consisting of two subunit types of approximately 130,000 and 90,000 molecular weight (6-9). Several human autoantisera against the insulin receptor are also capable of precipitating the IGF-I receptor (10), further indicating the similarity between the two receptor types. Since calcium ions are known to stimulate insulin binding to its receptor by increasing the affinity of the interaction (11), we examined whether this distinctive functional characteristic of the insulin receptor was shared by the IGF-I receptor. We now report that, in contrast to its effect at the insulin receptor, calcium strongly inhibits the binding of IGF-I to its receptor, suggesting a mechanism

a. IGF-I: insulin-like growth factor-I.
SM-C: somatomedin-C.

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by which the functional differentiation between the two hormone-receptor systems might be amplified.

MATERIALS AND METHODS

Insulin and IGF-I receptors were studied in microsomal membrane preparations of human placenta (12), known to be rich in both receptor types (13). Porcine insulin was obtained from Eli Lilly and Co.; SM-C/IGF-I was isolated from human plasma (14) and further purified by hydrophobic interaction chromatography to a potency, determined by radioimmunoassay (15), equal to that of pure human IGF-I (a gift from Professor R.E. Humbel, Zurich, Switzerland). Both peptides were radioiodinated with Na ^{125}I using chloramine-T, to a specific activity of 180 Ci/g for insulin, and 130 Ci/g for SM-C/IGF-I.

Incubations were for 1 h at 22°C in 0.3 ml of 50 mM Na Hepes, 0.25% bovine albumin (pH 7.5) containing various $CaCl_2$ concentrations as indicated. At the end of the incubation period tubes were centrifuged 20 min at 12,000 x g and the pellets washed in 1 ml of cold buffer minus calcium, re-centrifuged, and counted. Values are corrected for non-specific binding measured in the presence of excess unlabeled hormone (3.5% of total for insulin, 4% for IGF-I).

RESULTS

Figure 1 shows the effect of increasing calcium concentrations on the binding of radioiodinated insulin and IGF-I tracers to 60 µg of membrane protein. Insulin binding showed the expected response to calcium, a 3-fold increase from 5.5% binding of tracer without calcium to 17% binding at 10 mM calcium. The inverse pattern was seen for IGF-I tracer binding, a reduction from 34.5% of total tracer bound without calcium, to 14.5% bound at a

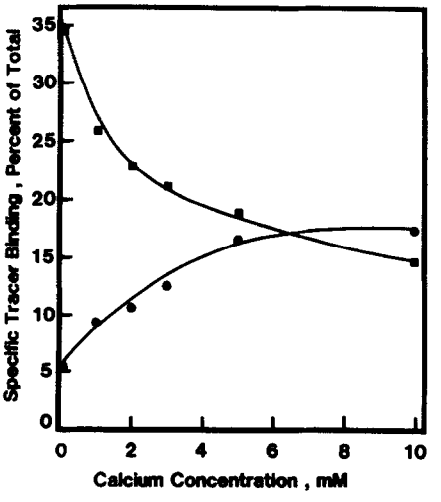

Figure 1 Effect of increasing calcium concentration on the binding of 10 nCi of radioiodinated IGF-I (■) or insulin (●) to human placental membranes (60 µg protein).

concentration of 10 mM. Half-maximal effects on both receptors were seen at a approximately 2 mM calcium.

A previous study from this laboratory indicated that the stimulation of insulin binding by calcium was due to an increase in receptor affinity (11). To determine whether a similar mechanism could be involved in the inhibition of IGF-I binding the effect of calcium was examined on competitive binding curves for the two polypeptides at their own, and at each other's, receptor. Figure 2A shows the displacement of specifically bound IGF-I tracer from placental receptors by increasing concentrations of human IGF-I and porcine insulin. Inhibition of IGF-I binding by calcium was most pronounced at low concentrations of the peptide, and essentially disappeared at higher concentrations. Insulin was over 5000-fold less potent than IGF-I in displacing IGF-I tracer from its receptor, but calcium increased the sensitivity of this receptor towards insulin considerably.

As shown in Table 1, the concentration of insulin required for half-maximal displacement of IGF-I tracer from its receptor was reduced in the presence of 10 mM calcium by 15-fold. At the insulin receptor, displacement

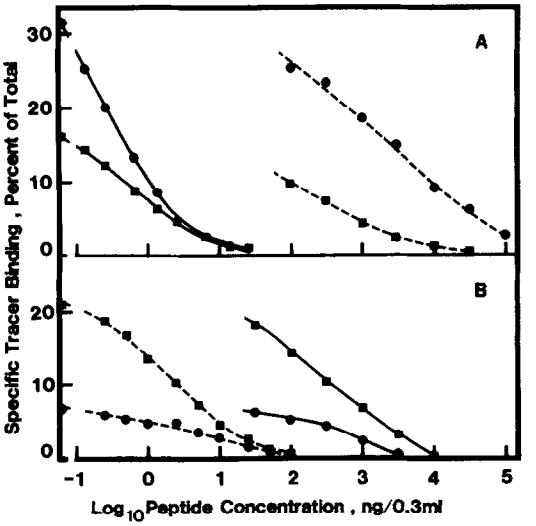

Figure 2 Displacement of bound radioiodinated IGF-I (A) or insulin (B) by increasing concentrations of unlabeled IGF-I (solid lines) or insulin (broken lines) in the absence (●) or presence (■) of 10 mM $CaCl_2$. Incubations contained 75 µg of placental protein. In Panel B, the IGF-I concentrations are expressed as the IGF-I content of a preparation of approximately 1% purity used to generate the IGF-I curves.

Table 1 Concentrations of Peptides Causing 50% Displacement of Specifically Bound Tracer from Placental Receptors.

	IGF-I Receptor Calcium (mM)		Insulin Receptor Calcium (mM)	
	0	10	0	10
Peptide				
IGF-I (ng/ml)	1.7	2.5	2400*	1000*
Insulin (ng/ml)	9700	670	21	7

* Due to insufficient pure peptide, an IGF-I preparation of approximately 1% purity was used to derive these figures. Values are corrected for the IGF-I content of this preparation, assuming that contaminating peptides are inactive.

curves for insulin and IGF-I were parallel (Fig. 2B); the sensitivity towards both peptides was increased to a similar extent by 10 mM calcium (Table 1).

When competitive binding data were subjected to Scatchard analysis (16), curvilinear IGF-I plots were obtained, consistent with the presence of two independent classes of binding site. As summarized in Table 2, the inhibitory effect of 10 mM calcium on IGF-I binding was entirely due to a decrease in the affinity of high affinity sites with no change in their concentration. Lower affinity sites were unaffected by calcium. Insulin binding data were consistent with a single class of low affinity binding site in the absence of calcium. Addition of calcium permitted the expression of a second, high affinity site with little effect on the low affinity binding.

DISCUSSION

The observation that calcium modulates the affinity of placental membrane insulin and IGF-I receptors in opposite ways suggests that calcium may play a

Table 2 The Effect of Calcium on Binding Parameters of IGF-I and Insulin Receptors.

	IGF-I Receptor Calcium (mM)		Insulin Receptor Calcium (mM)	
	0	10	0	10
K_1, L/nmol	17.8	6.3	-	2.7
N_1, pmol/mg protein	0.21	0.20	-	0.32
K_2, L/nmol	0.33	0.32	0.26	0.26
N_2, pmol/mg protein	0.18	0.18	1.08	1.37

K_1 and K_2 are the association constants, and N_1 and N_2 the concentrations, of high and low affinity binding sites respectively.

role in vivo in determining the interaction between these hormones and their target cells. Insulin binding to its placental receptor, although stimulated by calcium, actually causes a decrease in calcium bound at or near the receptor (11). Calcium binding to liver and adipocyte membranes is similarly inhibited by insulin (17,18). In this respect the insulin receptor is analogous to the acetylcholine receptor which, while being activated by calcium, appears to undergo a conformational change on acetylcholine binding which leads to the release of calcium (19). It has been postulated that this agonist-mediated calcium release might result in the opening of membrane ion channels (19). Such a mechanism could similarly account for the action of insulin in the transport of ions and small molecules.

Although IGF-I was less than 1% as potent as insulin at the insulin receptor, calcium increased the sensitivity towards the two peptides to a similar extent (Table 1), suggesting that IGF-I might simply act as a low-affinity insulin analog at this receptor. This accords with the concept that the insulin-like activities of IGF-I are in fact mediated through the insulin receptor (2). In contrast, clear differences were seen between the interactions of insulin and IGF-I at the growth factor receptor. First, curves for IGF-I and insulin competition with bound IGF-I tracer were non-parallel. Second, calcium increased the sensitivity of the IGF-I receptor towards insulin, while lowering its affinity for IGF-I. These observations suggest that if insulin can exert any growth effects through the IGF-I receptor, these actions might be potentiated by calcium ions, while the growth effects of IGF-I should be decreased by calcium. Whether the actions of calcium on the IGF-I receptor, like its effect on the acetylcholine and insulin receptors, are accompanied by binding of calcium to the receptor, and if so, the effect of hormone binding on receptor-bound calcium, remain important questions.

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