Insulin-like Effect of Zinc on Adipocytes

L. COULSTON AND P. DANDONA

SUMMARY

In view of the known effect of zinc on the crystalline nature of insulin, we have investigated whether zinc alters the biologic potency of this hormone. Using the rate of lipogenesis by rat epididymal adipocytes as an index of the biologic potency of insulin, we have shown that zinc exerts a potent stimulatory effect upon lipogenesis in vitro similar to, but quite independently of, insulin and that it has an additive effect with that of insulin when both are incubated together. This effect of zinc on adipocytes and the biologic potency of insulin, hitherto unreported, is of significance at the biologic, pharmacologic, and clinical level. DIABETES 29:665–667, August 1980.

It is well known that the addition of zinc promotes the formation of hexamers from insulin monomers.[1] It is possible that such an alteration in the crystalline form of insulin may result in some change in its biologic potency. The addition of zinc is already known to alter the configuration of proteins and to alter their biologic activity; for example, the addition of zinc to denatured alcohol dehydrogenase leads to a reconstitution of the dimeric form from its monomers and to the restoration of its activity which would otherwise not occur.[2]

We have therefore investigated the possibility that zinc may modify the biologic activity/potency of insulin in vitro. We have used the rate of lipogenesis by adipocytes from the rat epididymal fat pad as an index of the biologic potency of insulin.

MATERIALS

Analytic grade zinc chloride, nickel chloride, cupric chloride, sodium chloride, potassium chloride, sodium bicarbonate, calcium chloride, sodium dihydrogen phosphate, disodium hydrogen, phosphate, and glucose were obtained from B.D.H.* Collagenase was purchased from Worthington Laboratories. Bovine serum albumin (crystallized) was obtained from Armour Pharmaceuticals. Monocomponent Actrapid porcine insulin (Novo, Copenhagen) was used in all experiments requiring insulin. Epididymal fat pads were obtained from cesarian-delivered male Sprague-Dawley rats weighing between 120 and 150 g and kept on an ad libitum diet (Grain Harvesters, Brand 41B).

The composition of Krebs-Ringer-bicarbonate buffer (KRB) used in our experiments was as follows: NaCl, 121 mM; KCl, 4.8 mM; KH_2PO_4, 1.2 mM; $MgSO_4$, 1.2 mM; $NaHCO_3$, 25 mM; and $CaCl_2$, 1.3 mM; pH 7.4.

METHODS

Adipocyte suspensions from rat epididymal fat pads were prepared by the method of Rodbell[3] which utilizes collagenase digestion in KRB (pH 7.4) in a shaking water bath at 37°C for 2 h. Adipocytes were resuspended in KRB with [3-^{3}H]-glucose (0.2 μCi per vial, overall glucose concentration = 0.55 mM) for 2 h at 37°C under an atmosphere of 5% CO_2:95% O_2 in a shaking water bath. Each incubation contained 4×10^4 adipocytes/ml. The reaction was stopped at 2 h by the addition of a toluene-based scintillant followed by vigorous shaking. This procedure lyses the adipocytes and extracts lipids into the toluene phase. Radioactivity in the lipid-toluene phase was measured as described by Moody et al.[4] using a Phillips PW 4540 liquid scintillation counter. This system works as a sensitive bioassay for insulin, giving a dose-response curve between 2.5 and 25 μU/ml.

Based on the assay methodology described above the following further experiments were performed:

(1) Incubation of adipocytes with [3-^{3}H]-glucose without insulin (referred to below as basal metabolism).
(2) Incubation of adipocytes with [3-^{3}H]-glucose and insulin in three doses: 5, 10, and 20 μU ml^{-1}; a group of six tubes was studied for each insulin dose.
(3) Incubation of adipocytes with 0.1, 0.25, 0.5, and 1 mM zinc chloride, cobalt chloride, and cupric chloride.

From the Metabolic Unit and Department of Chemical Pathology, Royal Free Hospital, Pond Street, London, NW3 2QG, United Kingdom.
Address reprint requests to Dr. Dandona.
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* British Drug House.

(4) Incubation of adipocytes with 0.1, 0.2, 0.3, and 0.5 mM zinc chloride alone and with 5 μU ml^{-1} insulin.
(5) Incubation of adipocytes with (a) 5 μU insulin or (b) 0.5 mM $ZnCl_2$ or (c) no additive for periods of 30, 60, 90, and 120 min, respectively, before reaction was stopped by addition of the scintillant.

CALCULATIONS

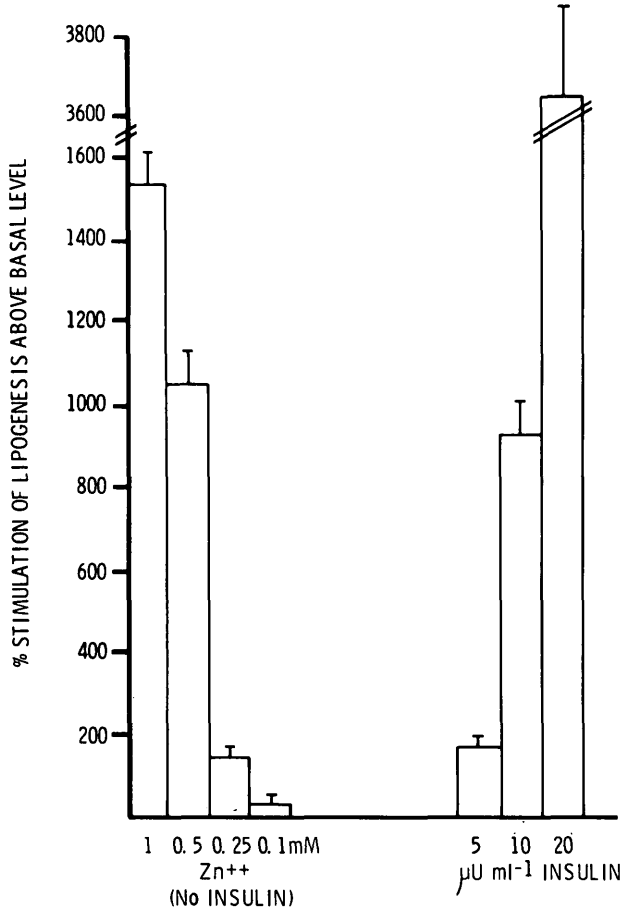
All results are expressed as percent stimulation above the basal metabolism, the latter being the mean counts per minute (cpm) incorporated into the toluene-soluble lipid phase by adipocytes incubated without insulin or other test substances as described above. The significance of differences between two observations was calculated by the Student's *t* test.

RESULTS

Zinc alone at concentrations of 0.25, 0.5, and 1 mM produced a significant increase in stimulation (144%, 1102%, and 1548%, respectively). At a concentration of 0.1 mM zinc, there occurred a consistent but not statistically significant stimulation of lipogenesis by adipocytes. Insulin alone at 5, 10, and 20 μU ml^{-1} produced a stimulation of 172%, 938%, and 3647%, respectively (Figure 1). When the reaction was stopped at various intervals after incubation, it was observed that lipogenesis had been linear in zinc- and insulin-treated adipocytes and in those not treated with either zinc or insulin (Figure 2). Lipogenesis in zinc- or insulin-treated adipocytes was significantly greater at 30 min and continued that way for 2 h.

When adipocyte suspensions were incubated with a combination of 5 μU ml^{-1} insulin and 0.1, 0.2, and 0.3 mM zinc, respectively, there occurred a further significant increase in lipogenesis ($P < 0.01$). However at the higher concentration of 0.5 mM zinc no additional effect of the hormone was observed since the 0.5 mM zinc alone induced the maximal stimulation possible in the system (Figure 3). A similar additive effect of zinc was obtained when 10 μU of insulin was used, provided that the maximal stimulation of the assay

FIGURE 1. Stimulation of lipogenesis by zinc and insulin (mean ± SEM).



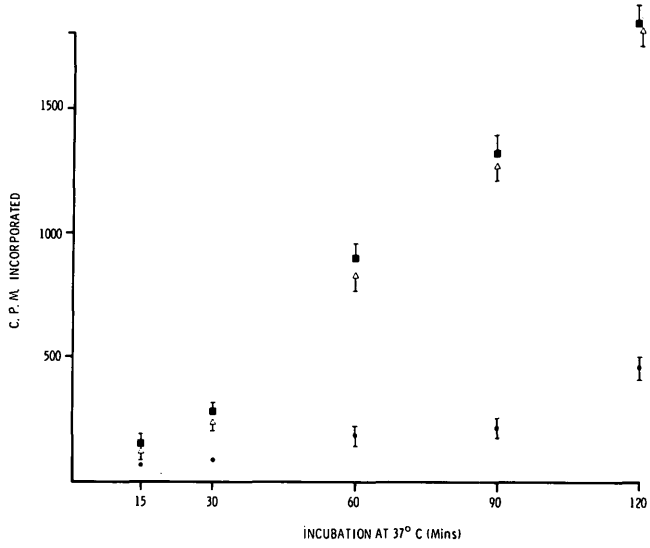
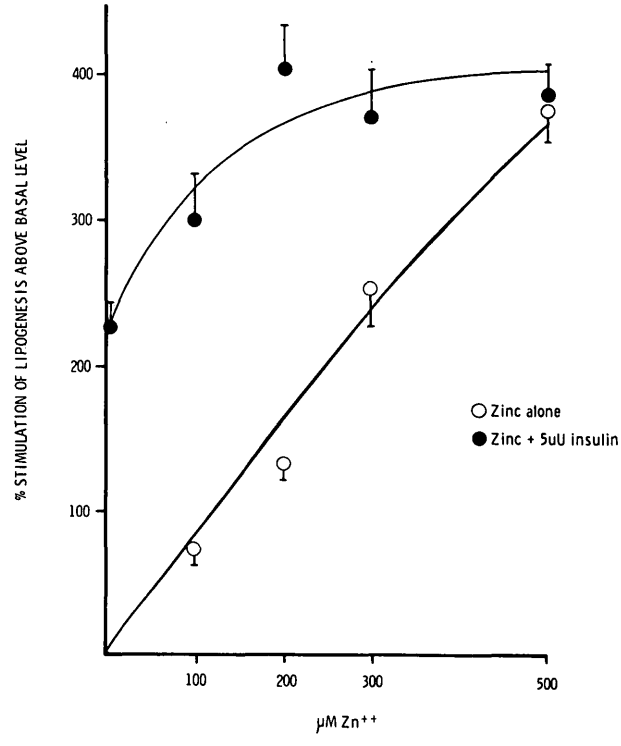


FIGURE 2. Graph showing lipogenesis as cpm incorporated at various intervals at 37°C (mean ± SEM). (●), basal; (■), 0.5 mM Zn^{2+}; and (△), 5 μU ml^{-1} insulin.

FIGURE 3. The additive effect of zinc and insulin on adipocyte lipogenesis (mean ± SEM).



was not achieved with insulin alone. There was considerable variation between the maximal stimulations observed in different assays. However, the consistency of our observations was not altered.

DISCUSSION

Since the hypothesis we set out to test was that zinc may alter the biologic effect of insulin by altering its crystalline nature, we were surprised to find that zinc had a stimulatory effect upon lipogenesis by adipocytes, even in the absence of insulin. This suggests that zinc may interact with the insulin receptor or with an enzyme system at a post-receptor site. That zinc has an additive effect with insulin upon lipogenesis by adipocytes is also clear. Zinc has been shown to enhance the binding of insulin to hepatocyte membranes.[5] Thus, zinc may be important in insulin/adipocyte interaction in two ways: by a direct insulin-like stimulatory effect and by a possible enhancement of insulin binding to its receptor. Whether zinc has a similar additive effect on other cells that respond to insulin is under investigation.

The fact that two other divalent ions, Co and Cu, do not exert this effect suggests that this effect of zinc is fairly specific. Investigations are under way to examine the effect of other related divalent ions like cadmium, nickel, and mercury.

The biologic, clinical, and pharmacologic implications of this effect are important. Since the concentration of zinc found in rat plasma is approximately 20 μmol/L,[8] it may be argued that the concentrations at which we observed the stimulatory effect of zinc were much greater than the concentration that exists in vivo. However, the sensitivity of collagenase-treated adipocytes is not necessarily the same as that which might occur in vivo. Furthermore, zinc concentration in portal plasma may be higher than that in peripheral plasma since the pancreas is rich in zinc, and this may be relevant to the biologic effect of insulin on its major end organ, the liver.

Since the mere addition of zinc markedly alters the biologic effect of insulin, these data emphasize that the biologic effect of any hormone is not dependent merely upon its concentration and, thus, point to the limitation of radioimmunoassays.

At the clinical level, our observations raise the possibility that relative or absolute zinc deficiency may have a role to play in the pathogenesis of maturity-onset diabetes, in which insensitivity to insulin is considered to be an important contributory factor.[6,7] Whether patients with zinc deficiency in general have concomitant glucose intolerance also needs investigation. Furthermore, the fact that insulin is stored in and secreted from β-cells of the pancreas in zinc-associated polymeric crystalline form[9,10] raises the possibility that insulin in portal circulation could be more biologically potent than that in peripheral plasma where insulin exists in a monomeric form.[5] At the pharmacologic level, our data suggest the possibility that the addition of zinc to insulin preparations may significantly enhance the biologic potency of insulin in vivo. Hitherto, the addition of zinc to insulin has been used merely to retard the absorption of insulin from the subcutaneous injection site, to form long-acting insulins, e.g., insulin-zinc suspension (Lente) and protamine-zinc insulin (PZI). We are, at this moment, investigating whether preincubation of insulin with zinc (1) enhances the magnitude and the rate of onset of its effects, and (2) alters its rate of degradation in the human.

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