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VANADATE STIMULATION OF IGF BINDING TO RAT ADIPOCYTES

S. Kadota, I.G. Fantus, B. Hersh, and B.I Posner

Department of Medicine
McGill University and the Royal Victoria Hospital,
Montreal, Quebec, Canada

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Summary: Preincubation of adipocytes with insulin (10 ng/ml) stimulated binding of IGF-II to maximal levels of 160% above controls. Vanadate also augmented IGF-II binding with an increase of 126% above controls at a concentration of 1 mM. Coincubation of vanadate (1 mM) with a maximal stimulatory dose of insulin (10 ng/ml) produced no additive effect. However, at submaximal doses of insulin (0.1 ng/ml) the effect of vanadate was additive. Amiloride, a potent inhibitor of the insulin receptor kinase, inhibited the effects of both vanadate and insulin. The data are consistent with an effect of vanadate via a similar sequence of steps to that of insulin; perhaps involving activation of the insulin receptor kinase.

A rapid effect of insulin in rat adipocytes is the stimulation of translocation of the type II insulin-like growth factor (IGF) receptor from an intracellular pool to the plasma membrane (1). Though this effect is mediated through the insulin receptor (2) the mechanism involved remains uncertain.

In the past several years it has been observed that vanadate mimics a number of insulin's effects on intact cells including adipocytes (3-6) as well as in the whole animal (7). Thus vanadate stimulates glucose oxidation (4) as well as glycogen synthetase activity in rat adipocytes (5,6). In this study we examined the effect of vanadate and compared it to that of insulin on ^{125}I-IGF-II binding to isolated rat adipocytes. We found that vanadate, like insulin, increased ^{125}I-IGF-II binding to intact adipocytes in a dose-dependent manner.

Materials and Methods

Materials: Porcine insulin was a gift from Connaught-Novo Laboratories (Willowdale Ont.) IGF-II was purified by high pressure liquid chromatography from outdated human plasma as previously outlined (8). It was iodinated with chloramine-T and Na ^{125}I (New England Nuclear, Boston MA) to a specific activity of 150-170 Ci/g, and purified by adsorption to and elution from human placental membranes (8). Bovine serum albumin (Fraction V), 4-(2-hydroxyethyl)-1-piperazine ethanesulfonic acid (HEPES) and sodium orthovanadate were purchased

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from Sigma Chemical Co. (St. Louis, MO). Collagenase (Type I) was from Worthington Biochemical Corp. (Freehold, NJ) and dinonylphthalate from BDH Chemicals Ltd. (Ville St. Laurent, Que.) Amiloride was kindly provided by Dr. Dorion (Merck, Sharp and Dohme, Dorval, Que.).

Adipocyte preparation: Male Sprague-Dawley rats (160-200 g b.wt.) were killed by cervical dislocation, and adipocytes were isolated from the epididymal fat pads by the method of Rodbell (9). In brief, the fat tissues were cut into small pieces with scissors and digested for one hour at 37°C in Krebs-Ringer bicarbonate/HEPES buffer (118.5 mM NaCl, 4.7 mM KCl, 2.5 mM CaCl2, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 24.9 mM $NaHCO_3$, 30 mM HEPES, pH 7.4) containing 20 mg/ml of bovine serum albumin, 5 mM glucose and 1 mg/ml of collagenase. The isolated adipocytes were separated from undigested tissues by filtration through nylon mesh and washed three times with the above buffer.

^{125}I-IGF-II binding: Isolated adipocytes (3.5 x 10^5 cells) were incubated at 37°C in 0.5 ml Krebs-Ringer bicarbonate/HEPES buffer, pH 7.4, containing 20 mg/ml bovine serum albumin with or without insulin and/or vanadate at concentrations noted in the text. After 15 min the incubation temperature was reduced to 15°C and ^{125}I-IGF-II (0.2 ng; 6.6 x 10^4 dpm) was added with or without excess (500 ng) unlabelled IGF-II for an additional 60 min of incubation. Binding was determined by removing aliquots (200 ul) of cell suspension and centrifuging in 500 ul microfuge tubes containing 200 ul of dinonylphthalate as described by Gammeltoft and Gliemann (10). The cell-associated radioactivity in the top layer was determined in an LKB (model 1272) gamma counter. The binding in the presence of excess IGF-II was regarded as nonspecific and was subtracted from total binding to yield specific binding. All determinations were done in duplicate.

Results

The incubation of intact adipocytes with insulin, at doses ranging from 0.01 to 100 ng/ml, resulted in a dose-dependent increase of ^{125}I-IGF-II binding. A maximal increase of 160% above control levels of binding was observed at an insulin concentration of 10 ng/ml (Fig. 1A). Sodium orthovanadate at doses ranging from 10 uM to 1 mM also augmented ^{125}I-IGF-II

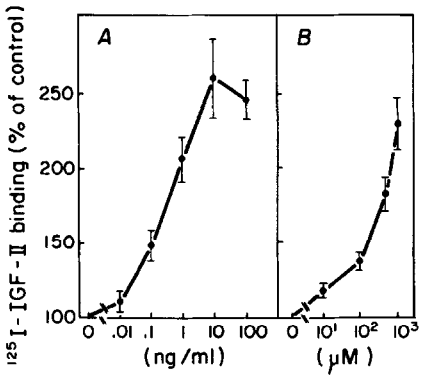

Figure 1. The effect of different doses of insulin (A) and vanadate (B) on the binding of ^{125}I-IGF-II to isolated rat adipocytes. Each value is the mean ± S.E. of 4 separate experiments. The mean specific binding of ^{125}I-IGF-II to control cells was 5.7%.

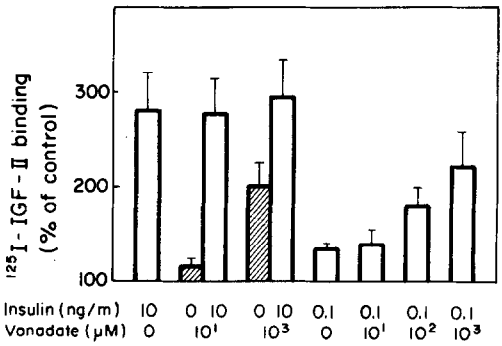

Figure 2. The effect of combining insulin and vanadate on the binding of ^{125}I-IGF-II by rat adipocytes. Each value is the mean ± S.E. of 3 separate experiments.

binding to intact adipocytes in a dose-dependent fashion. The extent of increase above control levels of binding ranged from 18% at 10 uM to 124% at 1 mM (Fig. 1B).

The additive effects of vanadate and insulin on the stimulation of ^{125}I-IGF-II binding were examined (Fig. 2). At maximally stimulatory doses of insulin (10 ng/ml) the addition of vanadate produced no further effect; however, at submaximal stimulatory dose of insulin (0.1 ng/ml), vanadate further augmented the binding of ^{125}I-IGF-II.

The effect of amiloride on the stimulation of ^{125}I-IGF-II binding by both insulin and vanadate was then studied. Amiloride at 1 mM produced significant inhibition of the stimulatory effect of both insulin (10 ng/ml) and vanadate (1 mM) on ^{125}I-IGF-II binding while having minimal effect by itself (Table 1). Ouabain at doses of 0.1 and 1 mM had no significant effect on ^{125}I-IGF-II binding to rat adipocytes (data not shown).

Table 1

Effect of amiloride on ^{125}I-IGF-II binding to rat adipocytes

Additions	% Control
Amiloride	84 ± 4
Insulin	321 ± 32
Insulin + Amiloride	168 ± 23
Vanadate	206 ± 22
Vanadate + Amiloride	112 ± 9

Adipocytes were preincubated with insulin (10 ng/ml), vanadate (1 mM) and/or amiloride (1 mM) and the ^{125}I-IGF-II binding was subsequently performed as described in Materials and Methods. Data are expressed as a percent of ^{125}I-IGF-II binding to adipocytes incubated with no additions (control). Values are mean ± S.E. of five separate experiments.

Discussion

Previous studies have determined that insulin increases ^{125}I-IGF-II binding to intact adipocytes (1,2). This was shown to result from a redistribution of IGF-II receptors from an intracellular pool to the cell surface (1). In this study we have demonstrated that, like insulin, vanadate also augments ^{125}I-IGF-II binding on the cell surface of rat adipocytes. Of interest is the observation that the effect of insulin and vanadate are additive at a submaximal but not at a maximal concentration of insulin. This observation suggests that vanadate acts via a pathway similar to that of insulin.

It is now known that the β-subunit of the insulin receptor is a tyrosine kinase whose activity is stimulated by insulin (11,12). Vanadate has also been demonstrated to augment insulin receptor kinase activity in rat adipocytes (6). The observation of Morgan et al that a cytosolically-injected monoclonal antibody against the kinase abolished insulin action on Xenopus laevis oocytes (13) favors the view that the receptor is important in effecting insulin action. It is possible that both insulin and vanadate increase ^{125}I-IGF-II binding on adipocytes by stimulating the insulin receptor kinase. Vanadate does influence other enzyme activities and has been studied extensively in respect to its inhibition of Na^+-K^+ ATPase (14,15). Since ouabain and amiloride, other inhibitors of this enzyme (16), did not influence ^{125}I-IGF-II binding it would appear that changes in Na^+-K^+ ATPase are not decisive for effecting change in IGF-II binding. Though the effect of amiloride, to inhibit the stimulation of ^{125}I-IGF-II binding produced by insulin and vanadate, is consistent with their action via the receptor kinase (17) we cannot rule out a role for other kinases influenced by amiloride (18).

The suggestion of Corvera and Czech, that dephosphorylation of cell surface IGF-II receptors (by kinase inhibition and/or phosphatase activation) delays their internalization (19), is not necessarily inconsistent with the above considerations. Thus receptor kinase activation could lead to the modification of other kinases or phosphatases more directly impacting on IGF-II

receptors. In this regard it will be interesting to see the effect of vanadate on the phosphorylation state of adipocyte cell surface IGF-II receptors.

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References

1. Wardzala, L.J., Simpson, I.A., Rechler, M.M., and Cushman, S.W. (1984) J. Biol. Chem. 259, 8378-8383.
2. King, G.L., Rechler, M.M., and Kahn, C.R. (1982) J. Biol. Chem. 257, 10001-10006.
3. Ramasarma, T. and Crane, F.L. (1981) Curr. Top. Cell Regul. 20, 247-301.
4. Shechter, Y. and Karlish, S.J.D. (1980) Nature 284, 556-558.
5. Tamura, S., Brown, T.A., Dubler, R.E. and Larner, J. (1983) Biochem. Biophys. Res. Comm. 113, 80-86.
6. Tamura, S., Brown, T.A., Whipple, J.A., Fujita-Yamaguchi, Y., Dubler, R.E., Cheng, K., and Larner, J. (1984) J. Biol. Chem. 259, 6650-6658.
7. Heyliger, C.E., Tahiliani, A.G., and McNeill, J.H. (1985) Science 227, 1474-1477.
8. Goodyer, C.G., De Stephano, L., Guyda, H.J., and Posner, B.I. (1984) Endocrinology 115, 1568-1576.
9. Rodbell, M. (1964) J. Biol. Chem. 239, 375-380.
10. Gammeltoft, S., and Gliemann, J. (1973) Biochim Biophys Acta 320, 16-32.
11. Kasuga, M., Zick, Y., Blithe, D.L., Crettaz, M., and Kahn, C.R. (1982) Nature 298, 667-669.
12. Rosen, O.M., Herrera, R., Olowe, Y., Petruzzelli, L.M., and Cobb, M.H. (1983) Proc. Natl. Acad. Sci. U.S.A. 80: 3237-3240.
13. Morgan, D.O., Ho, L., Korn, L.J., and Roth, R. (1986) Proc. Natl. Acad. Sci. U.S.A. 83, 328-332.
14. Cantley, L.C., Jr., Resh, M.D. and Guidotti, G. (1978) Nature 272, 552-554.
15. Dubyak, G.R., and Kleinzeller, A. (1980) J. Biol. Chem. 255, 5306-5312.
16. Pouyssĕgur, J., Chambard, J.C., Franchi, A., Paris, S., and Van Obberghen-Schilling, E. (1982) Proc. Natl. Acad. Sci. USA 79, 3935-3939.
17. Davis, R.J., and Czech, M.P. (1985) J. Biol. Chem. 260, 2543-2551.
18. Holland, R., Woodgett, J.R., and Hardie, D.G. (1983) FEBS Letters 154, 269-273.
19. Corvera, S., and Czech, M.P. (1985) Proc. Natl. Acad. Sci. USA 82, 7314-7318.