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SHORT COMMUNICATION

Stimulation of the Intracellular Portion of the Human Insulin Receptor by the Antidiabetic Drug Metformin

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ABSTRACT. Our prior work suggested that the antidiabetic metformin must enter the cell to act and that the drug stimulates tyrosine kinase activity. We now report that therapeutic concentrations (~1 μg/mL) of metformin stimulated the tyrosine kinase activity of the intracellular portion of the β-subunit of the human insulin receptor (IPβIRK), the intracellular portion of the epidermal growth factor receptor and pp60-src, but not cAMP-dependent protein kinase. A derivative of metformin unable to lower glucose was ineffective in stimulating IPβIRK. Two derivatives more effective than metformin in patients were also more effective than metformin in stimulating IPβIRK. Higher levels (10–100 μg/mL) of metformin or methylglyoxyl bis(guanylhydrazone) inhibited the tyrosine kinases, and this inhibition may be responsible for the ability of these two drugs to block cell proliferation. BIOCHEM PHARMACOL 55;4:533–536, 1998. © 1998 Elsevier Science Inc.

KEY WORDS. diabetes; tyrosine kinase; proliferation; glucophage; biguanide

Metformin (*N,N*-dimethylbiguanide or *N,N*-dimethylimidodicarbonimidic diamide; commercial name: glucophage) is an oral antihyperglycemic drug used in the treatment of diabetes [1]; however, its mechanism of action is still unknown. For example, the drug's effect on the insulin receptor kinase has been very controversial. After addition of metformin to tissue, some groups found no effect of metformin on the insulin receptor [2, 3], whereas three other groups reported that metformin elevated receptor tyrosine kinase activity [4–6]. In our work [6], we used the *Xenopus* oocyte and a plasma membrane-cortex preparation from these cells to examine metformin action. Our experiments found: (1) metformin must be internalized to act; (2) metformin stimulates an event that occurs within 1 h of insulin addition to whole cells; and (3) metformin stimulates tyrosine kinase activity.

However, metformin stimulation of *in situ* receptor kinase activity may be indirect, since metformin has been reported to have no effect on the isolated insulin receptor kinase [5]. Contrary to this report, we found that therapeutic levels (~1 μg/mL) of metformin directly stimulated three tyrosine kinases (EC 2.7.1.112): the intracellular portion of the β-subunit of the human insulin receptor (IPβIRK),§ the intracellular portion of the epidermal growth factor receptor (IPEGFR), and pp60-src (but not the serine-threonine kinase cAMP-dependent protein kinase A, EC 2.7.1.37). IPβIRK exhibits the major functional properties of the intact insulin receptor [7, 8] and consists of the juxtamembrane, tyrosine kinase, and c terminus domains of the intracellular portion of the human insulin receptor. We also found that high concentrations (10–100 μg/mL) of biguanides (e.g., metformin or the anticancer agent MGBG) can inhibit tyrosine kinase activity and that this inhibition may be responsible for the ability of these drugs to inhibit cell proliferation.

MATERIALS AND METHODS

Measurement of Kinase Activities

To measure insulin receptor kinase activity, IPβIRK (3 U) (Stratagene) and RR-SRC (BioMol) (100 μM) or RCAM-lysozyme (Sigma) (77 μM) were added to [^{32}P]ATP (34 μCi; 100 μM), 2 mM $MnCl_2$, 10 mM $MgCl_2$, 50 mM Tris (pH 7.4) with or without a preincubation in metformin (15 min, 30°). The activity of 100 ng of IPEGFR (Stratagene) was measured in the presence of [^{32}P]ATP (~10 μCi; 80 μM), 500 μM RR-SRC, 50 mM HEPES (pH 7.4), 200 μM Na_3VO_4, and 40 mM $MnCl_2$ (10 min, 30°). pp60-src (3 U) (Upstate Biotechnology) activity was assayed with 100 mM Tris-HCl (pH 7.2), 25 mM $MnCl_2$, 2 mM EGTA, 125 mM magnesium acetate, 250 μM Na_3O_4, [^{32}P]ATP (500 μM; 30 μCi), and 500 μM RR-SRC (10 min, 22°). The activity of the catalytic subunit of protein kinase A (10 U) (Promega) was assayed with 40 mM Tris-HCl (pH 7.4), 20 mM magnesium acetate, [^{32}P]ATP (15 μCi; 200 μM), 130 μM kemptide (10 min, 30°). The reactions were stopped

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§ *Abbreviations:* IPβIRK, the intracellular portion (residues 941-1343) of the insulin receptor; IPEGFR, the intracellular portion (residues 647-1186) of the epidermal growth factor receptor; MGBG, methylglyoxyl bis(guanylhydrazone).

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with 25% trichloracetic acid and addition to P81 paper. After three washes with 75 mM phosphoric acid, radioactivity was quantified by liquid scintillation counting.

RESULTS

Metformin and the Intracellular Portion of the Human Insulin Receptor

At levels equivalent to therapeutic concentrations (~1 μg/mL), metformin stimulated IPβIRK phosphorylation of RR-SRC (Fig. 1A). To confirm that this stimulation is not substrate specific, the effect of metformin on IPβIRK was examined with a second substrate (RCAM-lysozyme) (Fig. 1B) and similar stimulation was observed.

Metformin and Other Kinases

Metformin also stimulated the tyrosine kinase activity of IPEGFR (Fig. 1C) and a soluble tyrosine kinase, pp60-src (Fig. 1D). Similar to the results with IPβIRK, higher concentrations of the drug inhibited these tyrosine kinases. The metformin concentration for peak stimulation of pp60-src occurred at lower drug concentrations than those noted for the receptor tyrosine kinases. Metformin did not stimulate the catalytic subunit of the serine-threonine kinase cAMP-dependent protein kinase A (Fig. 1E). Since the highest metformin concentration inhibited the kinase, these data suggest that metformin stimulation is limited to tyrosine kinases and that metformin can inhibit both serine-threonine and tyrosine kinases.

Use of Derivatives More and Less Effective than Metformin

If the IPβIRK stimulation noted above is important in the *in vivo* action of metformin, then IPβIRK kinase activation should correlate with the known glucose-lowering efficacy of metformin and its derivatives. Monomethylbiguanide is an ineffective derivative of metformin and it was unable to stimulate tyrosine kinase activity of IPβIRK (Fig. 2A). Buformin is a more effective antidiabetic agent than metformin [1] and stimulated IPβIRK over a wider range of concentrations than metformin (Fig. 2B). Methylglyoxyl bis(guanylhydrazone) (MGBG) is a more potent biguanide than metformin [9] and was also a more effective activator of IPβIRK than metformin (Fig. 2C).

DISCUSSION

Metformin stimulated the *in vitro* activity of three tyrosine kinases by 20–40% (cAMP-dependent kinase A was not stimulated). These data are supported by *in vivo* studies showing metformin stimulation of different tyrosine kinases including the insulin [4, 5, 6], IGF-1 [5, 10], and EGF [10] receptors. The stimulation of tyrosine kinases by cationic metformin may be similar to the well-characterized ability of polycations (e.g., polylysine) to stimulate IPβIRK and

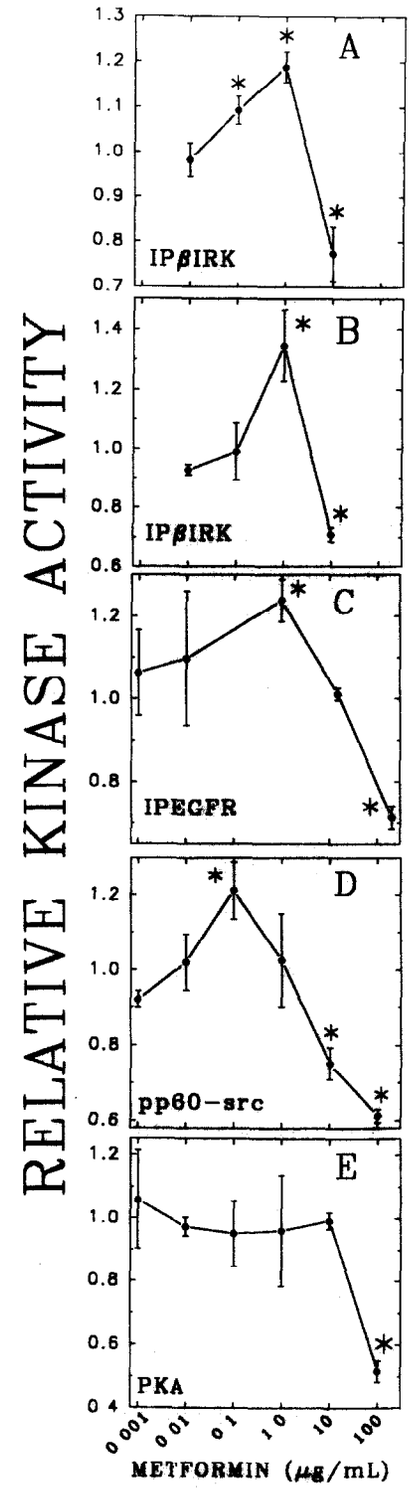

FIG. 1. The effect of metformin on tyrosine and serine-threonine kinases. All data were divided by the control value (asterisk denotes significance at $P < 0.05$). Metformin effects on IPβIRK with the substrate RR-SRC (A) or with the substrate RCAM-lysozyme (B) were similar. Metformin effects on two other tyrosine kinases, the IPEGFR (C) and pp60-src kinase (D), are compared with the drug's effect on a serine-threonine kinase (the catalytic subunit of protein kinase A; E). A involved 6–12 determinations per time point whereas B–E involved 4–6 determinations per time point. Note that 1 μg/mL metformin is 7.7 μM.

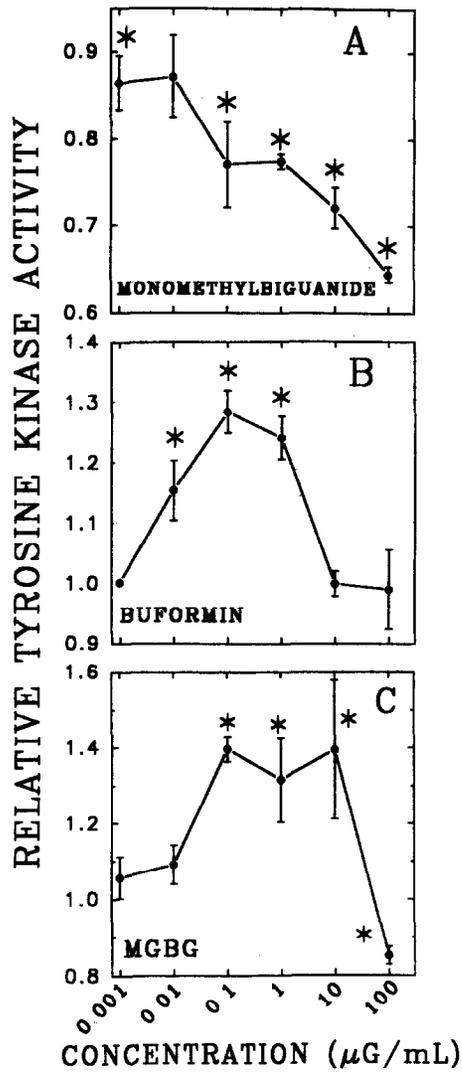

FIG. 2. The effects of monomethylbiguanide (A), buformin (B) and MGBG (C) on IPβIRK activity. RR-SRC was the substrate. Asterisk denotes significance at $P < 0.05$ and each point represents 4–6 determinations.

the intact insulin receptor [e.g., refs. 11, 12]. The inability of others to note the direct effect of metformin on the insulin receptor may be due to the metformin concentration used, the use of detergent-solubilized or partially activated receptors (high basal levels of phosphorylation may obscure metformin stimulation), or the fact that the assay was not sufficiently sensitive to record the small stimulation noted here.

Direct activation of the insulin receptor tyrosine kinase by metformin may be important in diabetes therapy since the relative clinical efficacy of metformin and three derivatives is equivalent to the relative efficacy of these drugs to stimulate IPβIRK. In addition, the concentration of metformin required for activation of tyrosine kinases in the plasma membrane-cortex or the intact cell is similar to that required for *in vitro* metformin stimulation of IPβIRK. For example, with *in vivo Xenopus* preparations, maximal metformin stimulation occurred at 1–10 μg/mL [6]. For rat adipocytes [2], 1–10 μg/mL metformin was optimal, whereas for human adipose tissue [13], 2–4 μg/mL was best. Further evidence is needed to conclude whether direct metformin activation or inhibition of kinases is fully responsible for the multiple, *in vivo* actions of metformin (e.g., enhanced fibrinolysis, glycogen synthesis and glucose uptake, inhibition of PAI-1 production, decreased cell proliferation, blood triglyceride and platelet aggregation, and various effects on microcirculation).

There is a difference between these results and those we previously reported: metformin stimulation of IPβIRK tyrosine kinase activity shown here was less than the two- to threefold increase in tyrosine kinase activity in the plasma membrane-cortex preparation [6]. The rather small 20–30% stimulation of IPβIRK by metformin is not entirely unexpected since metformin is unable to fully mimic insulin action in the intact cell [e.g., ref. 6] and since the plasma membrane-cortex preparation should contain numerous tyrosine kinases that may show different levels of activation by metformin. Furthermore, an activating calcium feedback loop found in the plasma membrane-cortex preparation [6] is not present with the IPβIRK preparation.

Higher concentrations of metformin or MGBG inhibited IPβIRK and tyrosine kinase activity in a *Xenopus* plasma membrane-cortex preparation (50–200 μg/mL metformin; unpublished results, B. J. Stith). This kinase inhibition by higher levels of metformin may be reflected in the fact that higher metformin concentrations inhibit insulin action in human Hep G2 cells (at 13–130 μg/mL metformin) [14], human adipose tissue (at >10 μg/mL) [13] and rat adipocytes (at 100 μg/mL) [2].

Metformin also inhibits the proliferation of HeLa or KB cells [15, 16] and the drug may reduce atherosclerosis through its ability to inhibit proliferation of smooth muscle cells [17, 18]. The biguanide MGBG has been used clinically as an anticancer agent and it inhibits cell division in leukemia cells [19]. We suggest that inhibition of cell division by metformin or MGBG is due to inhibition of tyrosine kinases associated with proliferation.

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