Phenformin Increases Insulin Binding to Human Cultured Breast Cancer Cells

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SUMMARY

The effect of the hypoglycemic biguanide, phenformin, on the binding of insulin to MCF-7 cells, an in vitro line derived from a human breast cancer, has been investigated. Cells incubated for 24 h in the presence of 1.0 μg/ml of phenformin bound 62.2 ± 8.1% (mean ± SE) more ^{125}I-insulin than did controls. The phenformin effect was dose-dependent over the concentration range of 0.1 μg/ml to 10.0 μg/ml. The increased binding was due to an increase in receptor number without a change in binding affinity. This demonstration of increased receptor number in response to phenformin exposure provides support for the hypothesis that one action of phenformin is to enhance tissue sensitivity to insulin. DIABETES 29:329–331, April 1980.

The mechanism underlying the hypoglycemic effect of biguanides, such as phenformin, that are still used in the treatment of non-insulin-dependent maturity-onset diabetes mellitus in various parts of the world, are incompletely understood.[1,2] There is evidence, however, that suggests that phenformin acts by increasing the physiologic responsiveness of human peripheral tissues to circulating insulin.[3–6] An effect of biguanides on the binding of insulin to the target cell, the initial step in insulin action,[7] was the possibility investigated in this study.

In order to exclude the possible interference of changing levels of circulating hormones and metabolites that could occur in in vivo studies, and to focus directly on the drug-target cell interaction, an in vitro model system was chosen. The MCF-7 cell line, derived from a human breast cancer, is suitable for studies of insulin action because it is responsive to physiologic concentrations of insulin, and because it has both well characterized, high affinity insulin receptors and an insulin degrading capacity.[8]

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METHODS AND MATERIALS

The MCF-7 cell line, derived from a human breast carcinoma and placed into tissue culture at the Michigan Cancer Foundation, was a gift from Dr. M. Lippman (NIH, Bethesda, Maryland). The cells were grown in continuous monolayer culture in Falcon tissue culture flasks using Eagle's Minimal Essential Medium (MEM) plus essential amino acids, penicillin, streptomycin, and 10% fetal calf serum (FCS). The insulin receptors, insulin degrading capacity, and insulin responsiveness (increased acetate, thymidine, and leucine incorporation in response to physiologic concentrations of insulin) have been previously characterized.[8]

Porcine insulin was purchased from Sigma (distributed by Mascia e Brunelli, Milan, Italy) and labeled with ^{125}I from the Amersham Radiochemical Center (Amersham, Great Britain) to a specific activity of 95–120 μCi/μg by the chloramine T method.[9] Phenformin (N-beta-phenetylbiguanide) was a gift from Boehringer Biochemia (Milan, Italy).

Binding studies were performed on cells 4 days after their implantation into tissue culture flasks, while they were in a state of semiconfluence (approximately 1.5×10^6 cells/flask). Twenty-four hours before the binding study, the medium in the flask was changed and phenformin was added.

To prepare cells for binding studies, the procedure of Osborne et al.[8] was followed. Briefly, the cells within the flasks were rinsed with Eagle's MEM (without FCS), rinsed again with a solution of 150 mM NaCl:0.02% EDTA, and then harvested by a 5-min incubation in the same solution. The harvested cells were diluted with Eagle's MEM (without FCS), centrifuged at 250 × *g* for 3 min, resuspended in phosphate buffered (pH 7.6) physiologic saline, recentrifuged, and finally resuspended in the binding assay buffer (100 mM HEPES, 120 mM NaCl, 1.2 mM $MgSO_4$, 2.5 mM KCl, 15 mM $NaC_2H_3O_2$, 10 mM glucose, 1 mM EDTA, and 1% bovine serum albumin, pH 7.6) to a final concentration of 1×10^6 cells/ml.

Within an experiment, two to four flasks were pooled to obtain the cell suspension for each experimental group. All incubations were performed at 22°C. Cell viability, as determined by trypan blue exclusion, was typically greater than 90% at the termination of the incubation period. The concentration of ^{125}I-insulin was 0.4 ng/ml, and that of the unlabeled insulin was 100 μg/ml.

For the separation of bound and free hormone, triplicate 100-μl assay aliquots were layered over 200 μl of cold binding buffer in microfuge tubes, and centrifuged in a Beckman microfuge (Beckman Instruments, Palo Alto, California) for 2 min. The supernatants were collected for determination of insulin degradation by precipitation of intact hormone with 10% trichloroacetic acid. Cell pellets were then washed, the microfuge tips excised, and the radioactivity counted. Specific binding was calculated as the difference between the binding of the ^{125}I-insulin in the absence and presence of excess unlabeled insulin.

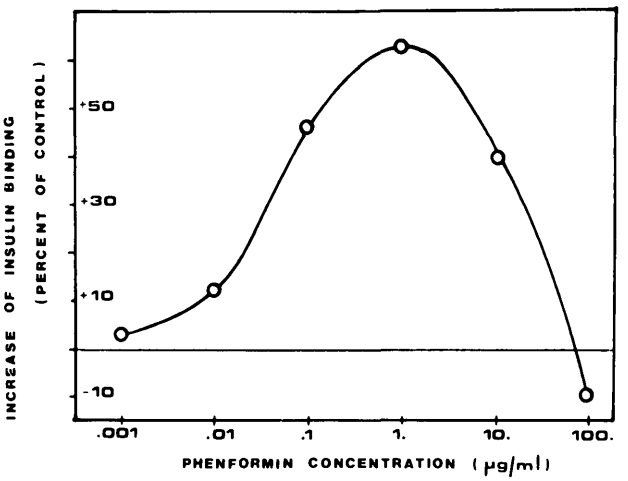

FIGURE 2. Effect of phenformin concentration on specific insulin binding to the MCF-7 cells. Cells were preincubated with phenformin for 24 h.

RESULTS

In a preliminary experiment, the binding of insulin was measured in cells that had been preincubated with 1.0 μg/ml of phenformin for 0, 3, 12, 24, and 48 h. An increase in insulin binding was detectable after 3 h of exposure to phenformin, and was maximal after 24 h (although there was no significant difference between the 12-, 24-, and 48-h time points). For convenience, 24 h was used as the standard period of preincubation with phenformin in the experiments that followed.

Cells incubated in the absence of phenformin specifically bound ^{125}I-insulin maximally between 4–6 h, and maintained a plateau until 8 h. Cells preincubated with 1 μg/ml of phenformin for 24 h produced a similarly shaped binding curve that demonstrated increased binding at all binding assay time points (Figure 1). In a series of seven separate experiments, preincubation of MCF-7 cells with 1 μg/ml of phenformin for 24 h produced an increase in insulin binding at a steady state of 62.2% ± 8.1 (mean ± SE, range of 35–96%) over the untreated cells. The degradation of ^{125}I-insulin was also slightly increased in cells preincubated with phenformin (22–30% of total in untreated cells; 25–36% of total in phenformin-treated cells).

At 24 h of preincubation, a small but reproducible effect of phenformin was detectable at 0.01 μg/ml, and a maximal effect was seen at 1.0 μg/ml. A concentration of 100 μg/ml proved to be cytotoxic, causing a lowering of the medium pH and a decrease in insulin binding (Figure 2).

A Scatchard analysis of the data (Figure 3) revealed that the phenformin-induced increase in insulin binding was due to an increase in the number of insulin binding sites without a significant change in binding site affinity.

DISCUSSION

These data demonstrate that preincubation of MCF-7 cells with phenformin significantly increases their ability to specifically bind insulin. The Scatchard analysis indicates that the enhanced insulin binding is primarily due to an increase in the number of insulin binding sites. This effect of phenformin is rapid (detectable after 3 h of drug exposure), and is dose-related over a concentration range (0.01–10 μg/ml) that includes the effective therapeutic concentration range in blood (0.05–0.1 μg/ml).[10]

Several mechanisms have been suggested to be responsible for the antihyperglycemic action of phenformin.

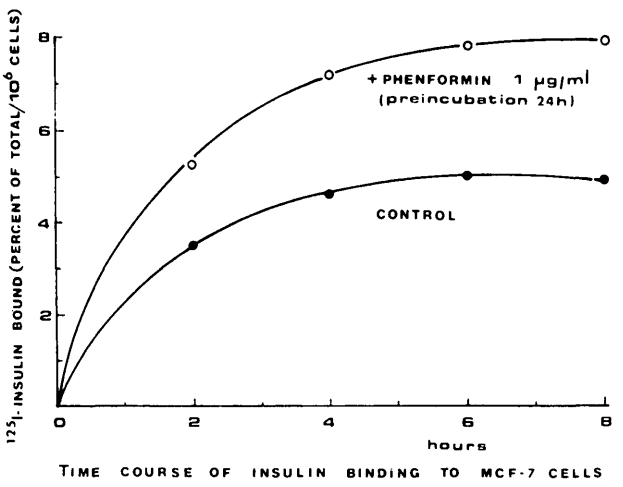

FIGURE 1. Insulin binding to the MCF-7 cells in the presence and absence of preincubation with phenformin.

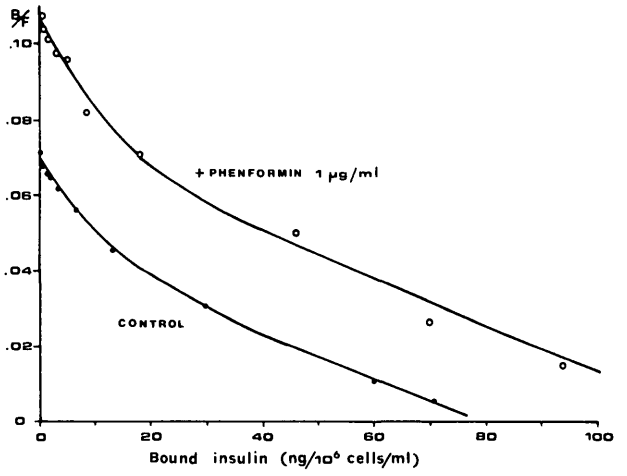

FIGURE 3. Scatchard plot of the binding data in MCF-7 cells with and without 24-h phenformin preincubation. Each point is the mean of three separate experiments.

Among these are decreased glucose absorption from the gut,[11] increased anaerobic glucose metabolism as a consequence of tissue hypoxia,[12] increased glucose metabolism as a consequence of decreased fatty acid oxidation,[13] and enhanced sensitivity of peripheral tissues to circulating insulin.[4-7] The observations reported here lend support to this last hypothesis.

If normal insulin target cells in vivo respond to phenformin by increasing their insulin binding capacity, as do these cells in vitro, then this effect may play a role in the therapeutic action of the drug. The consistency of this hypothesis will require the demonstration (currently under investigation) of an association between the increased insulin binding and the biologic response to insulin in these cells. In addition, the possibility that biguanides may well have multiple sites of action must be recognized.

Recently, the other major class of hypoglycemic agents, sulphonylureas, have been reported to increase the number of insulin receptors in mice[14] and humans.[15,16] These data were obtained in vivo, and thus the possibility that the changes were secondary to alterations in circulating insulin or metabolites cannot be excluded. The data reported here, in contrast, were obtained in vitro, and therefore suggest a direct effect of phenformin upon the target cell.

The process through which cells may increase their effective insulin receptor number in response to phenformin is unknown: structural modifications of the plasma membrane or changes in the insulin receptor turnover rate could conceivably be involved. It is of interest, in this regard, that preliminary experiments (unpublished) with the sulfonylurea glibenclamide (0.1–1.0 μg/ml) have also shown increased insulin binding to the MCF-7 cells, with increments ranging from 15–42% of controls.

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