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DECREASED EXPRESSION OF LIVER EPIDERMAL GROWTH FACTOR RECEPTORS IN RATS WITH ALLOXAN AND STREPTOZOTOCIN DIABETES

A. Lev-Ran, D.L. Hwang, and G. Barseghian

Department of Endocrinology and Diabetes
City of Hope National Medical Center
Duarte, California 91010

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Male rats (200 g) were rendered diabetic with one intraperitoneal injection of alloxan (150 mg/kg) or streptozotocin (60 mg/kg). In hyperglycemic animals within 3 hours after the injection, the binding of EGF to liver membranes decreased by 43-52%; the maximal drop was by 70% and persisted for the 20 days of the experiment. EGF receptors decreased in number with almost no changes in their affinity. Autophosphorylation of the receptors decreased parallel to the ligand binding. In animals that received lower doses and did not develop diabetes and in animals in whom diabetes was prevented by the injections of glucose (before alloxan) or nicotinamide (before streptozotocin) the binding of EGF to liver receptors remained normal. We conclude that the decreased expression of EGF receptors was caused by diabetes and not by the toxic effects of the diabetogenic compounds on the liver.

EGF receptors, like those to insulin, PDGF and IGF-1, are characterized by their autophosphorylation and tyrosine-kinase activity (1). Changes in the EGF receptors were reported in many situations, mostly related to cell growth, transformation and liver regeneration. Much less is known about their role and regulation in vivo but several hormones were shown to change the expression of the EGF receptors (2-5). Since the liver is the first organ to receive secreted insulin and since EGF is a potent growth factor and liver mitogen, we investigated EGF receptors in experimental diabetes.

MATERIALS AND METHODS

The experiments were performed on male Sprague-Dawley rats. In the first series animals (200 g) after an overnight fast were given a single intraperitoneal injection of alloxan (37.5-150

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mg/kg as alloxan monohydrate) or streptozotocin (30-60 mg/kg). After 3 hours, 1, 5 and 20 days, plasma glucose was determined with a Beckman glucose analyzer, and groups of animals were sacrificed by exsanguination under light ether anesthesia, their livers perfused in situ with cold saline and excised; Golgi and microsomal fractions were isolated (6). A group of 3-4 control animals was always sacrificed on the same day. We determined the binding of [^{125}I]-EGF (2 ng, 40,000 cpm/tube) to the isolated fractions. The results were expressed as the percent specific binding per 0.1 mg protein for the Golgi and per 0.5 mg protein for the microsomal fraction. The competitive binding with cold EGF was studied with the ligand concentrations 0.1-200 ng/tube, the highest concentration used for the determination of non-specific binding. The number and affinity of the receptors were derived from the Scatchard plots. The autophosphorylation of the receptors (Mr 170,000) was determined with γ-[^{32}P]-ATP (7).

In another series of experiments rats were injected in the tail vein with glucose 1000 mg/kg followed immediately with alloxan 150 mg/kg or i.p. with nicotinamide 300 mg/kg followed after 15 minutes by streptozotocin 60 mg/kg. The animals were sacrificed on the 5th day and their liver EGF receptors studied.

RESULTS

All animals given alloxan 150 mg/kg or streptozotocin 60 mg/kg developed overt diabetes with the plasma glucose level increasing from the initial values of 145 $\pm$ 13 mg/dl to a level of 312-448 mg/dl after 3 hours; 408-713 mg/dl after 1 day, 564-800 mg/dl at 5 days and 528-757 mg/dl at 20 days. With lower doses of both diabetogenic chemicals diabetes did not develop and plasma glucose remained below 200 mg/dl.

In animals rendered diabetic with alloxan and streptozotocin, the binding of EGF to its receptors and their autophosphorylation sharply decreased (Figures 1,3). As compared to controls, the maximal drop of the binding to the Golgi fraction was by 70% and to the microsomes - by 75%, all the differences between the control and experimental groups being highly significant. The competition curves and corresponding Scatchard plots (Figure 2) clearly show a decrease in the number of EGF receptors without significant changes in their affinity. Calculation of the number of receptors showed it to be in the controls 2.53 pmol/mg, in the alloxan treated animals 0.85 pmol/mg and in the streptozotocin-treated rats 1.13 pmol/mg. The autophosphoryla-

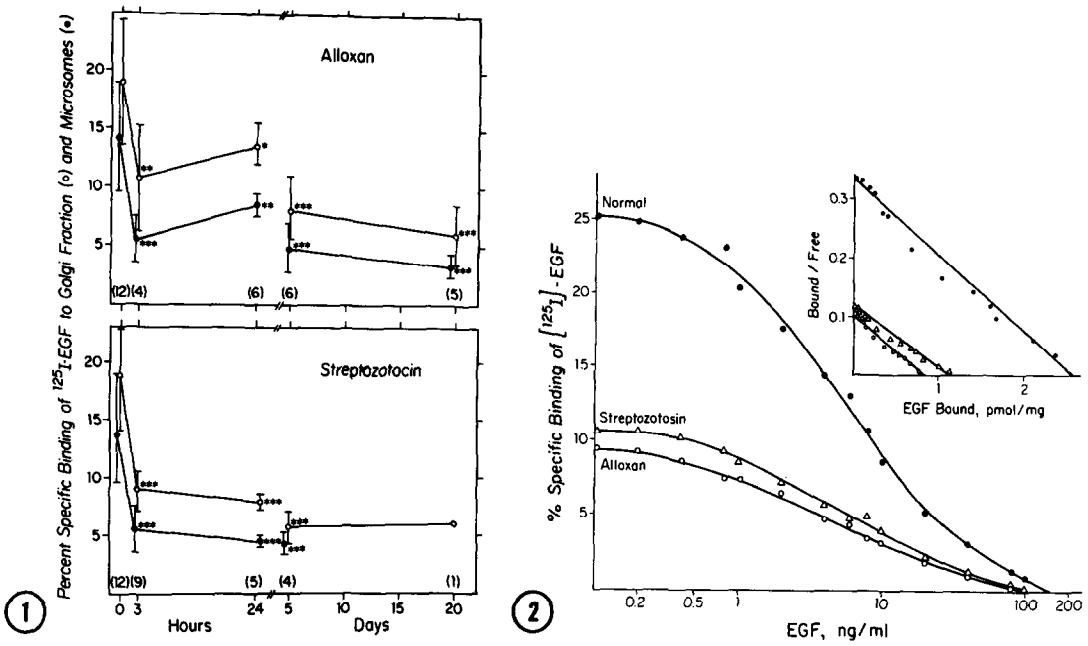

Figure 1. Binding of EGF to liver receptors in rats with alloxan (upper panel) and streptozotocin (lower panel) diabetes. Binding to the microsomal (•) and Golgi (o) fractions. The number in parentheses show the number of animals. Difference from controls: * P=.05; ** P<.02; *** P<.001.

Figure 2. Competition curves and Scatchard plots of EGF binding to the liver microsomes of rats treated with alloxan (o) and streptozotocin (Δ) as compared to the controls (•). Diabetic animals were studied at 5 days.

tion of the receptors decreased concomitant with the drop in the ligand binding (Figure 3).

Rats in whom diabetes did not develop after a lower dose of alloxan or streptozotocin or in which diabetes was prevented by

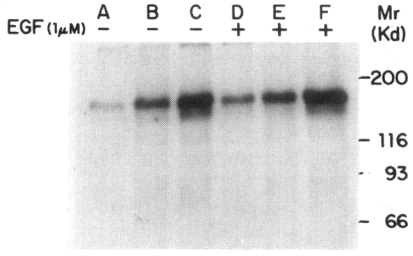

Figure 3. Autophosphorylation of EGF receptors in diabetic and control animals. Lanes A,D- alloxan diabetic rats; B,E- streptozotocin-diabetic rats; C,F- control animals. Radioactivity in Mr 170,000 bands (cpm): A-295; B-598; C-1676; D-554; E-898; F-2650.

the injections correspondingly of glucose and nicotanamide, the binding of EGF to their liver receptors was not decreased as compared to controls.

DISCUSSION

It seems that this is the first report of the changes in the liver EGF receptors in experimental diabetes. Both alloxan and streptozotocin cause diabetes by their direct action on the beta-cells with subsequent hypoinsulinemia. Under these conditions, insulin receptors in the liver and on the isolated hepatocytes, not being down-regulated, increase in number as was shown by most researchers (8-11) with only one dissenting statement (12).

EGF receptors are regulated by many factors. Uterine EGF receptors are stimulated by estrogens (5), mammary EGF receptors undergo changes during gestation and lactation (13). Glucocorticoids increase the EGF binding to C3H10T 1/2 (2) and to HeLa S3 cells (14) as well as to the fetal rabbit lung receptors (15). Liver EGF receptor binding in culture is stimulated by glucocorticoids; this stimulation is inhibited by insulin, though the latter has no effect on the basal binding (16). The normal expression of EGF receptors also requires thyroid hormones (4). We found only one report of the effect of alloxan diabetes on EGF receptors, and it described their decrease in exocrine pancreas, restored to normal by insulin (17).

Our experiments clearly showed that the decrease in the expression of EGF receptors in liver was caused not by alloxan and streptozotocin as chemicals but by diabetes. Since diabetes in this experimental setting means the combination of hypoinsulinemia and hyperglycemia further studies are needed to clarify which of the two factors was instrumental. Unfortunately, it is impossible experimentally to produce chronic

hyperglycemia in normoinsulinemic animals or to induce hypoinsulinemia without concomitant elevation of plasma glucose. Therefore inevitably the approach will have to be an indirect one. In view of the probable important role of EGF receptors, their function in diabetes deserves further study.

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REFERENCES

1. Carpenter, G. (1985) J. Cell Sci. Suppl. 3, 1-9.
2. Ivanovic, V., and Weinstein, I.B. (1981) Nature 293, 404-406.
3. Sadiq, H.F., and Devaskar, U.P. (1984) Bioch. Biophys. Res. Comm. 119, 408-414.
4. Mukku, V.R. (1984) J. Biol. Chem. 259, 6543-6547.
5. Mukku, V.R., and Stancel, G.M. (1985) J. Biol. Chem. 260, 9820-9824.
6. Josefsberg, Z., Carr, B.I., Hwang, D., Barseghian, G., Tomkinson, C., and Lev-Ran, A. (1984) Cancer Research 44, 2754-2757.
7. Fernandez-Pol, J.A. (1981) J. Biol. Chem. 256, 9742-9749.
8. Davidson, M.B., and Kaplan, S.A. (1977) J. Clin. Invest. 59, 22-30.
9. Morenkova, S., and Karelin, A. (1980) Biull. Eksp. Biol. Med. 90, 557-559.
10. Cech, J.M., Freeman, R.B., Caro, J.F., and Amatruda, J.M. (1980) Biochem. J. 188, 839-845.
11. Samson, M., Fehlmann, M., Morin, O., Dolais-Kitabgi, J., and Freychet, P. (1982) Metabolism 31, 766-772.
12. Garcia-Webb, P., and Bonser, A.M. (1985) Biochem. Biophys. Res. Comm. 128, 487-493.
13. Edery, M., Pang, K., Larson, L., Colosi, T., and Nandi, S. (1985) Endocrinology 117, 405-411.
14. Fanger, B.O., Viceps-Madore, D., and Cidlowski, J.A. (1984) Arch. Biochem. Biophys. 235, 141-149.
15. Sadiq, H.F., and Devaskar, U.P. (1984) Biochem. Biophys. Res. Comm. 119, 408-414.
16. Lin, Q., Blaisdell, J., O'Keefe, E., and Earp, H.S. (1984) J. Cell. Physiol. 119, 267-272.
17. Korc, M., Matrisian, L.M., Nakamura, R., and Magun, B.E. (1984) Life Sciences 35, 2049-2055.