EDITORIAL

THE EFFECT OF INSULIN UPON RESTING MEMBRANE POTENTIAL

An electrical potential is evoked by insertion of a glass micro-electrode, containing 3 M KCl into a living cell. As the tip of the micro-electrode commences to penetrate the cell, there is an abrupt deflection of the base-line, visualized with an oscilloscope or other recording device, which remains stable while the micro-electrode tip is inside the cell. There is an equally abrupt return to the original base-line as the micro-electrode tip is advanced or withdrawn to a position outside of the cell. Fulfillment of certain technical criteria permits designation of these cellular electrical potentials as "resting membrane electrical potentials" (RP). Complex equipment and careful attention to technic are required so that the potential may be accurately magnified and artifactual electrical impulses eliminated.

The complexity of the mechanism(s) comprising these electrical potentials precludes, at this time, a single hypothesis explaining the effect of insulin upon these potentials. Nevertheless, these recently developed methods and concepts of cell electrophysiology suggest a unique approach to the investigation of insulin action upon cells. Also, a new vantage point for the study of diabetes mellitus may be emerging, particularly of value in the investigation of cellular defects which may play a role in the pathogenesis of this disease.

Zierler,[1,2] in 1959, reported that 0.1 U./ml. of insulin, in vitro, increased the resting membrane electrical potential (RP) of rat skeletal muscle. More recently, it has been shown that a smaller concentration of insulin (.01 U./ml.) produces the same increment of RP (hyperpolarization) with skeletal muscle from hypophysectomized rats.[3]

Beigelman and Hollander[4,5] demonstrated, in vitro, a significant relationship between concentrations of insulin in the 1-1,000μU./ml. (1×10^{-3} — 1×10^{-6} U./ml.) range and increased RP of rat epididymal adipose tissue. This insulin effect is reversible, the adipose tissue RP diminishing towards baseline levels as insulin is replaced by insulin-free control solution. Rat age is very important, insulin causing little or no change of adipose tissue RP in old rats. Other proteins, including gelatin, serum albumin, insulin antiserum, and ACTH, have no effect on adipose tissue RP.[6] Insulin antiserum completely blocks the increase of rat adipose tissue RP associated with insulin.

In 1960, Miller and Constant[7] demonstrated elevation of RP in ocular ciliary body epithelium with 0.1-0.3 U./ml. of insulin. This insulin effect upon ciliary body epithelium was observed following decrease of RP, these low potentials occurring spontaneously or in response to intravenous glucose injected before enucleation. The rise of ciliary body RP induced by insulin was, essentially, a return to control levels of RP.

It is of particular interest that increase of RP by insulin in the three tissues studied, skeletal muscle, adipose tissue, and ciliary body epithelium, was unaffected by omission of glucose from the medium. This observation eliminates the likelihood that insulin-induced increase of RP is secondary to glucose transport into the cell. Insulin effect on RP may be mediated by an alteration of the gradient or flux between intracellular and extracellular ions. Such a gradient of potassium ion (K^+) has been widely accepted as the determinant of RP, at least in nerve and muscle.[8] However, no clear-cut quantitative or temporal relationships can be demonstrated between K^+ transport and insulin effect on skeletal muscle RP.[1,2] Insulin increases intracellular potassium of rat epididymal adipose tissue without need of glucose in the medium.[9] Marked, nonphysiological alterations of extracellular K^+ are required to change adipose tissue RP significantly, and these changes are much smaller than those associated with insulin.[10] Similarly, enormous changes of extracellular K^+ are required to alter thyroid tissue RP.[11] An ion other than K^+ may determine RP in some tissues. A preliminary report suggests the principal action of insulin upon skeletal muscle is extrusion of intracellular sodium, the changes of K^+ and RP being ancillary.[12] Possibly, changes in amino acid, monosaccharide, and ion transport caused by insulin are secondary to a primary insulin effect on the cell manifested by the change in RP. Perhaps, the mechanism of RP is similar to that proposed by Grundfest,[13] the membrane functioning as a "bio-electric generator." Preliminary evidence indicates that, in vitro, physiological concentrations of epinephrine and norepinephrine increase rat adipose tissue RP.[6] A vital relation exists between these

hormones and intracellular substrate which provides energy required to maintain membrane transport systems.[14] These hormones may affect RP by an identical or similar mechanism.

As investigations of insulin and RP continue, one may properly reflect upon the nature and origin of cell membrane RP. There is evidence that differences exist between RP of "nonexcitable" and "excitable" tissues. Tissues are defined as "nonexcitable" if they do not readily respond to electrical stimulation with an action potential as do "excitable" tissues. The RP of nonexcitable tissues, including fat, thyroid, ciliary body epithelium, corneal epithelium,[15] testis,[16] kidney,[17] and liver[17-20] is 25-50 mV. RP of excitable tissues, nerve and muscle, is considerably higher (70-95 mV). It is not possible to relate RP of nonexcitable tissues directly to gradients between extracellular and intracellular K^+ as may be done for excitable tissue. Nonexcitable thyroid and hepatic tissues have much lower electrical resistance and conductivity than excitable nerve or muscle.[11,21] These important differences between the RP of these two types of tissue suggest that each may be a distinct electrophysiological entity.

This brief discussion emphasizes that new concepts, some as yet unformulated, will be required for interpretation of data obtained with these new electrophysiological technics. Correlation of such electrophysiological investigations with biochemical, histologic, electron microscopic, and clinical studies are already yielding promising results.

REFERENCES

[1] Zierler, K. L.: Effect of insulin on membrane potential and potassium content of rat muscles. Amer. J. Physiol. *197*: 515, 1959.

[2] Zierler, K. L.: Hyperpolarization of muscle by insulin in a glucose-free environment. Amer. J. Physiol. *197*:524, 1959.

[3] Hazlewood, C. F., and Zierler, K. L.: Insulin induced hyperpolarization in skeletal muscle from hypophysectomized rats. Fed. Proc. *23*:410, 1964.

[4] Beigelman, P. M., and Hollander, P. B.: Effect of insulin upon resting electrical potential of adipose tissue. Proc. Soc. Exp. Biol. Med. *110*:590, 1962.

[5] Beigelman, P. M., and Hollander, P. B.: Effect of insulin and rat weight upon rat adipose membrane resting electrical potential (REP). Diabetes *12*:262, 1963.

[6] Beigelman, P. M., and Hollander, P. B.: Effects of hormones upon adipose tissue membrane electrical potentials. Proc. Soc. Exp. Biol. Med. *116*:31, 1964.

[7] Miller, J. E., and Consant, M. A.: The measurement of rabbit ciliary epithelial potentials in vitro. Amer. J. Ophthal. *50*:855, 1960.

[8] Hodgkin, A. L.: The ionic basis of electrical activity in nerve and muscle. Biol. Rev. *26*:339, 1951.

[9] Gourley, D. R. H., and Davidson, M. B.: Effect of insulin upon potassium content of adipose tissue. Fed. Proc. *22*:388, 1963.

[10] Beigelman, P. M., and Hollander, P. B.: Effects of electrolytes upon adipose tissue membrane electrical potentials. Proc. Soc. Exp. Biol. Med. *115*:14, 1964.

[11] Woodbury, D. M., and Woodbury, J. W.: Correlation of micro-electrode potential recordings with histology of rat and guinea-pig thyroid glands. J. Physiol. *169*:553, 1963.

[12] Moore, R. D.: The ionic effects of insulin. Abstracts of the Ninth Annual Meeting of the Biophysical Society, San Francisco, Feb. 24-26, 1965, p. 122.

[13] Grundfest, H.: The nature of the electrochemical potentials of bioelectric tissues, in "Electrochemistry in Biology and Medicine," T. Shedlovsky, ed., New York, Wiley, 1953.

[14] Maffly, R. H., and Edelman, I. S.: The coupling of the short-circuit current to metabolism of the urinary bladder of the toad. J. Gen. Physiol. *46*:733, 1963.

[15] Kikkawa, Y.: The intracellular potential of the corneal epithelium. Exp. Eye Res. *3*:132, 1964.

[16] Mio, S.: Electrophysiological studies on frog and toad testes. I. Effects of various hormones and drugs. Hirosaki Med. *9*:644, 1958.

[17] Li, C.-L., and McIlwain, H.: Maintenance of resting membrane and potentials in slices of mammalian cerebral cortex and other tissues in vitro. J. Physiol. *139*:178, 1957.

[18] Toida, N., Tamai, T., and Takagisi, T.: Electrophysiological studies of the liver by intracellular recording with a microelectrode. Part. I. Electrical potential from a rat liver in situ. Kyushu J. Med. Sci. *9*:163, 1958.

[19] Limberger, J.: Messung von Membran potentialen normaler Leber Parenchymsellen und hepato-cellularer Leber-Carcinoneder Ratte. Z. Krebforsch. *65*:590, 1963.

[20] Beigelman, P. M., Coraboeuf, E., and Guilbault, P.: Electrical potentials of liver. Life Sci. *3*:1343, 1964.

[21] Schanne, O., and Coraboeuf, E.: Personal communication.

PAUL M. BEIGELMAN, M.D.
Los Angeles, California