EFFECT OF INSULIN AND INSULIN ANTIBODY
UPON RAT ADIPOSE TISSUE MEMBRANE RESTING
ELECTRICAL POTENTIAL (REP)

By
Paul M. Beigelman and Philip B. Hollander
With the technical assistance of David Shube

ABSTRACT

In vitro penetrations of rat epididymal adipose tissue by glass micro-electrodes, containing 3 m KCl, elicit resting membrane electrical potentials (REP). Adipose tissue REP in young rats is reversibly increased by 1–1000 μU insulin/ml. Insulin antibody, diluted 1/1000–1/4800, significantly inhibits this elevation of REP by insulin.

In vitro studies by Beigelman & Hollander (1962, 1963) demonstrated significant reversible increase of epididymal adipose tissue resting membrane electrical potential (REP), in young male rats with physiologic concentrations of insulin. Recently, specific inhibition of this insulin effect by insulin antibody has been reported (Beigelman & Hollander 1964 b). In this paper, further data are presented demonstrating that insulin stimulation of adipose tissue REP is prevented by insulin antibody.

METHODS

The methods employed in this study have been described in detail previously (Beigelman 1960; Beigelman & Hollander 1962, 1963; Hollander & Webb 1955). Segments of male Wistar rat epididymal adipose tissue were penetrated with glass micro-electrodes,
of 15–30 megohms resistance, and the DC potentials recorded on motion picture film. Frequent change of micro-electrodes was required because of breaking or plugging of the tips. However, some complete experiments were performed with a single micro-electrode.

Eight male Wistar rats, in the fed state, were utilized. Weight ranged from 140 to 235 g. The animals were sacrificed by a blow to the cranium, decapitated, and 50–100 mg segments of epididymal adipose tissue quickly excised. It was important to stretch taut the adipose tissue in the incubation medium during the experiment. The control medium, Krebs-Ringer bicarbonate, usually contained 0.1 % gelatin. In two experiments, control medium and insulin were tested on one segment of adipose tissue and a second bit of tissue was utilized for control medium, insulin antibody, and/or insulin antibody + insulin studies. A single tissue segment was used for experimental and control REP’s in the remaining six experiments. Usually, 3–10 minute periods of recording REP’s were alternated with 2 minute intervals of equilibration. In a few instances, 3–5 minute experimental periods were recorded without intervening periods of equilibration. Occasionally, repeated changes of media, or »washes«, were made during the equilibration period.

The insulin antibody was prepared by the method of Arquilla & Stavitsky (1956). Diluted 1/100, this antibody completely inhibited the effect of 1000 µU insulin/ml upon rat epididymal tissue glucose uptake. Utilizing the method of Grodsky & Forsham (1960), one µU crystalline insulin/ml* could be detected with 1/2000 dilution of this antibody.

**RESULTS**

Rat No. 1 (Fig. 1) demonstrates a significant increase \( P < 0.01 \) of adipose tissue REP, 10 mV, in response to 100 µU insulin/ml. A separate fragment of epididymal adipose tissue from the same rat showed no such increase of REP to the same concentration of insulin, 100 µU/ml, mixed with 1/1000 insulin antibody. One segment of epididymal adipose tissue from Rat No. 2 (Fig. 2) was successively incubated in control medium, 1/2400 insulin antibody, 1/2400 insulin antibody + 100 µU insulin, two changes of control medium, and 1000 µU insulin/ml. The only significant elevation of REP was the 22 mV increase obtained with 1000 µU insulin/ml \( P < 0.001 \). Fig. 3 (Rat No. 3) delineates the response of a single segment of rat epididymal adipose tissue to control medium, 100 µU insulin/ml + 1/4800 insulin antibody, 100 µU insulin + 1/9600 insulin antibody, and two changes of control medium, each change preceded by two »washes«. The REP response to insulin was almost completely blocked by 1/4800 insulin antibody, the mean elevation being only 5 mV \( P > 0.5 \), but not by 1/9600 antibody as the REP significantly increased by 24 mV \( P < 0.01 \). The REP significantly diminished following two successive changes of control media. Rat No. 4 (Table 1) demonstrated a significant, 20 mV, increase of fat

* Supplies of re-crystallized insulin were generously provided by Dr. Lloyd Miller of U. S. Pharmacopia and Dr. Otto K. Behrens, Eli Lilly and Company.

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Effect of insulin and insulin + insulin antibody on rat epididymal adipose tissue REP (millivolts) (180 g rat). Each value represents a single micro-electrode penetration. One segment of tissue incubated in control medium followed by 100 μU insulin/ml. Separate piece of tissue incubated in control medium followed by 100 μU insulin/ml + 1/1000 insulin antibody.

pad REP with 10 μU insulin/ml during the final 3 minutes of incubation. A second segment of adipose tissue showed no significant increase of REP in response to 1/1000 insulin antibody or 1/1000 insulin antibody + 10 μU insulin/ml. Rat No. 5 (Table 1) showed significant increase of REP with 1000 μU insulin/ml, both with and without 1/4800 insulin antibody. Mean adipose tissue REP was 12 mV higher, however, with 1000 μU insulin/ml as compared with the same concentration of insulin + 1/4800 insulin antibody ($P > 0.02$, $< 0.05$). Inhibition of 100 μU insulin/ml by 1/2000 insulin antibody was evident in Rat No. 6. A significant increase of adipose tissue REP was elicited during the final 10 minute period of incubation with 100 μU insulin/ml. Rat No. 7, weighing 235 g, demonstrated a marked, but not statistically significant, increase in mean REP of adipose tissue in the presence of insulin, and complete inhibition of this effect by insulin antibody, also not statistically significant. Rat No. 8 provided the single clear-cut negative result, the significant increase of adipose tissue REP in response to 100 μU insulin/ml being unaffected by 1/1000 insulin antibody.

In Fig. 4, the differences are presented between mean adipose tissue REP,
Effect of insulin, insulin antibody, and insulin + insulin antibody on rat adipose tissue REP (millivolts) (140 g rat). Each value represents a single micro-electrode penetration, and is placed in approximate time sequence that experiments were performed. Single segment of adipose tissue incubated in control medium, 1/2400 insulin antibody, 1/2400 insulin antibody + 1000 µU insulin/ml, and 1000 µU insulin/ml.

for each rat tested, of control compared with insulin antibody, control compared with insulin antibody + insulin, control with insulin, insulin antibody + insulin with insulin, insulin antibody with insulin, and insulin antibody with insulin + insulin antibody. There is significant increase of adipose tissue REP with insulin, as compared with control, insulin antibody, and insulin antibody + insulin. Conversely, insulin antibody + insulin does not differ significantly from insulin antibody or control.

**DISCUSSION**

Unpublished recent studies have shown that *in vivo* rat epididymal fat pad electrical potentials have the same DC configuration and are of the same magnitude as those obtained *in vitro*. Also, measurements of micro-electrode tip resistance performed throughout penetration of rat epididymal adipose tissue demonstrate relatively little increase of resistance during penetration, and no relationship between magnitude of electrical potential and increase of resistance. No potentials are evident in adipose tissue left standing at room
Effect of various dilutions of insulin antibody upon responsiveness of adipose tissue REP (millivolts) to insulin (150 g rat). Each value represents a single micro-electrode penetration, and is placed in approximate time sequence that experiments were performed. Single segment of adipose tissue incubated in control medium, 1/4800 insulin antibody + 100 \( \mu \)U insulin/ml, 1/9600 insulin antibody + 100 \( \mu \)U insulin/ml, and repeated changes of control medium.

The data presented here show that insulin antibody, diluted 1/1000–1/4800, has no effect on adipose tissue membrane resting electrical potential (REP), and significantly inhibits the effect of 10–1000 \( \mu \)U insulin/ml upon adipose tissue REP. This study, therefore, provides further evidence that increase of adipose tissue resting electrical potential (REP) with insulin is a specific effect. Gelatin, corticotrophin, and human serum albumin have demonstrated no effect upon adipose tissue REP (Beigelman & Hollander 1964 b).

A much higher concentration of insulin, 0.1 U/ml, significantly increases REP of rat skeletal muscle, and this may occur without glucose in the medium (Zierler 1959 a,b). Hypophysectomy permits significant rat skeletal muscle REP

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Temperature 2–3 days. Large globules of lipid, manually squeezed from large amounts of epididymal adipose tissue and suspended in Krebs-Ringer bicarbonate medium, were penetrated by micro-electrodes with no DC potentials being obtained. These observations confirm previous investigations by Beigelman & Hollander (1962, 1963) indicating this adipose tissue electrical activity to be cell membrane DC potentials.
**Table 1.**

Effect of Insulin and Insulin Antibody Upon Rat Epididymal Adipose Tissue Rep.

<table>
<thead>
<tr>
<th>Rat No.</th>
<th>Rat Wt. (g)</th>
<th>Medium</th>
<th>Time (min)</th>
<th>No.* of Penetrations</th>
<th>Electrical Potential (Millivolts) Mean±SEM</th>
<th>$P_1^{**}$</th>
<th>$P_2^{***}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Control</td>
<td>5</td>
<td>5</td>
<td>28 ± 4</td>
<td>N.S.***</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>185</td>
<td>10 µU Insulin (Ins)</td>
<td>3</td>
<td>5</td>
<td>28 ± 4</td>
<td>N.S.</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10 µU Insulin (Ins)</td>
<td>3</td>
<td>5</td>
<td>48 ± 4</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>New Tissue</em></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>Control</td>
<td>10</td>
<td>15</td>
<td>50 ± 4</td>
<td>N.S.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Control</td>
<td>5</td>
<td>4</td>
<td>51 ± 3</td>
<td>N.S.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1/1000 Ins Antibody (Ab)</td>
<td>5</td>
<td>4</td>
<td>53 ± 3</td>
<td>N.S.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1/1000 Ins Ab</td>
<td>3</td>
<td>8</td>
<td>51 ± 1</td>
<td>N.S.</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>+ 10 µU Ins</td>
<td>3</td>
<td>7</td>
<td>38 ± 4</td>
<td>N.S.</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>170</td>
<td>1/4800 Ins Ab</td>
<td>5</td>
<td>15</td>
<td>48 ± 3</td>
<td>&gt;0.02, &lt;0.05</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>+ 1000 µU Ins</td>
<td>5</td>
<td>15</td>
<td>60 ± 5</td>
<td>&lt;0.001</td>
<td>&gt;0.02, &lt;0.05</td>
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<tr>
<td></td>
<td></td>
<td>Control</td>
<td>10</td>
<td>13</td>
<td>22 ± 3</td>
<td>N.S.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1/2000 Ins Ab</td>
<td>10</td>
<td>16</td>
<td>19 ± 1</td>
<td>N.S.</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>195</td>
<td>+ 100 µU Ins</td>
<td>10</td>
<td>16</td>
<td>19 ± 1</td>
<td>N.S.</td>
<td></td>
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<td></td>
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<td>Control</td>
<td>3</td>
<td>2</td>
<td>19 ± 3</td>
<td>N.S.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>100 µU Ins</td>
<td>10</td>
<td>17</td>
<td>22 ± 2</td>
<td>N.S.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>100 µU Ins</td>
<td>10</td>
<td>16</td>
<td>31 ± 4</td>
<td>&gt;0.02, &lt;0.05</td>
<td>&lt;0.01</td>
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<tr>
<td></td>
<td></td>
<td>Control</td>
<td>3</td>
<td>4</td>
<td>15 ± 1</td>
<td>N.S.</td>
<td></td>
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<tr>
<td>7</td>
<td>235</td>
<td>Control</td>
<td>10</td>
<td>8</td>
<td>38 ± 4</td>
<td>N.S.</td>
<td>&gt;0.05, &lt;0.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100 µU Ins</td>
<td>10</td>
<td>6</td>
<td>58 ± 13</td>
<td>N.S.</td>
<td>&gt;0.05, &lt;0.1</td>
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<tr>
<td></td>
<td></td>
<td>Control</td>
<td>10</td>
<td>6</td>
<td>41 ± 3</td>
<td>N.S.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1/2000 Ins Ab</td>
<td>10</td>
<td>9</td>
<td>36 ± 3</td>
<td>N.S.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>+ 100 µU Ins</td>
<td>10</td>
<td>13</td>
<td>32 ± 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>165</td>
<td>Control</td>
<td>5</td>
<td>10</td>
<td>14 ± 1</td>
<td>N.S.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1/2000 Ins Ab</td>
<td>5</td>
<td>8</td>
<td>16 ± 1</td>
<td>N.S.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>+ 100 µU Ins</td>
<td>10</td>
<td>21</td>
<td>25 ± 3</td>
<td>&lt;0.01</td>
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<tr>
<td></td>
<td></td>
<td>100 µU Ins</td>
<td>10</td>
<td>19</td>
<td>24 ± 2</td>
<td>&lt;0.001</td>
<td>N.S.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100 µU Ins</td>
<td>10</td>
<td>17</td>
<td>24 ± 1</td>
<td>&lt;0.001</td>
<td>N.S.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Control</td>
<td>3</td>
<td>5</td>
<td>18 ± 1</td>
<td>&gt;0.01, &lt;0.02</td>
<td></td>
</tr>
</tbody>
</table>

* Number of micro-electrode penetrations.

** **$P_1^{**}$ – Probability of difference being significant, compared with initial control for same tissue, calculated by Student's $t$ test.

*** $P_2^{***}$ – Probability of difference being significant, compared with insulin + insulin antibody for same tissue, calculated by Student's $t$ test.

**** N.S. – Not significant.
Change in mean REP (millivolts) for each rat given comparing control to insulin antibody, control to insulin antibody + insulin, control to insulin, insulin antibody to insulin, insulin antibody to insulin + insulin antibody, and insulin antibody to insulin + insulin antibody. Mean change of REP, for all rats tested, given by height of bar. Brackets define Standard Error of Mean (SEM). Numbers above bars indicate numbers of animals. *P* is significance of change, from Fisher's tables, by Student's *t* test. (In one animal, 100 μU insulin/ml + 1/9600 insulin antibody is considered as 100 μU insulin/ml without antibody).

Elevation with a lower concentration of insulin, 0.01 U/ml (Hazlewood & Zierler 1964). Ciliary body epithelium has membrane resting electrical potentials which are rapidly abolished by cyanide (Berggren 1960; Miller & Constant 1963). Infusion of glucose in the intact animal, preceding enucleation, diminishes these ciliary body epithelium REP's, and these decreased REP's or spontaneously elicited low ciliary body REP's are significantly increased by high concentration of insulin, 0.1–0.3 U/ml, with no glucose in the medium (Miller & Constant 1963).

Attempts have been made to associate the insulin effect on REP to extracellular K+ gradient. However, Zierler (1959 b) has shown that no clear-cut quantitative or temporal relationships exist between K+ transport and insulin effect on skeletal muscle REP. Gourley & Davidson (1963) state that insulin, with no glucose in the medium, causes intracellular accumulation of K+ in epididymal adipose tissue. However, the changes in adipose tissue REP associated with great changes in K+-Na+ concentration are much smaller than those obtained with physiological concentrations of insulin (Beigelman & Hol-

The precise nature of adipose tissue REP is not known. However, evidence is emerging that it may differ fundamentally from REP of excitable tissues, such as muscle and nerve, which readily respond to electrical stimulation with an action potential. Recent studies of adipose and other nonexcitable tissues, tissues not responding readily to electrical stimulation with an action potential, suggest that currently accepted hypotheses, such as Hodgkin’s concept (Hodgkin 1951) that the tissue REP is dependent almost entirely upon the gradient between extracellular and intracellular K+, may require modification. Other nonexcitable tissues recently reported to have inherent electrical activity include thyroid (Woodbury & Woodbury 1963), corneal epithelium (Kikkawa 1964), testis (Mio 1958), kidney (Li & Mcllwain 1957), and liver (Li & Mcllwain 1957; Toida et al. 1958, 1960; Limberger 1963; Beigelman et al. 1964 a, b; Coraboeuf et al. 1964). The maximum REP usually reported for these tissues, 60 mV, is considerably lower than nerve or muscle REP. Also, studies of thyroid (Woodbury & Woodbury 1963) REP suggest much lower cell membrane electrical resistance for nonexcitable than excitable tissue.

Other theories may be applicable to REP of nonexcitable tissue. Salminen (1963) has suggested that the REP may represent a secondary transport of K+ into the cell, in response to greater extracellular diffusion of H+ than anion from metabolizing cells. Facets of intracellular metabolism may directly determine REP by providing energy for REP and membrane transport systems. The effect of epinephrine and norepinephrine upon adipose tissue REP (Beigelman & Hollander 1964 a, b) is consonent with the evidence presented by Maffly & Edelman (1963) that an important relationship may exist between these hormones and intracellular substrate to provide energy for REP and membrane transport systems. Grundfest (1953) and Ungar (1963) have suggested that the electrical potential of the cell may be a fundamental characteristic not dependent upon or secondary to ionic gradient.

This cursory examination of the present theoretical status of REP suggests that the specific insulin effect upon REP of nonexcitable tissue, fat, may be quite different, qualitatively as well as quantitatively, from that obtained from an excitable tissue, such as skeletal muscle. It is conceivable that REP of excitable and nonexcitable tissues may differ fundamentally.

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