Effect of Insulin upon Resting Electrical Potential of Adipose Tissue.* (27588)

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Increase of rat skeletal muscle resting membrane electrical potential by addition of insulin has been reported(1). No such increase of resting electrical potentials (REP), following addition of insulin, was noted in an isolated ventricular heart preparation obtained from 200 g rats(2). The present report concerns the effect of insulin upon epididymal adipose tissue REP.

Methods. The technics employed for utilization of epididymal adipose tissue have been described(3). Segments of epididymal adipose tissue were excised from male Wistar rats and immediately transferred to Krebs-Ringer-bicarbonate buffer solution containing approximately 0.1% gelatin† and, for certain experiments 1 mg/ml glucose. This buffer solution was maintained during preparation of the experiments at room temperature. The rats were in the fed state, and weighed 110-405 g.

The instrument and the experimental technics employed for measurement of resting potentials have been described in detail(4). In each experiment, a segment of adipose tissue was suspended in the medium, maintained constantly at 30°C, between two supports without being stretched. Ninety-five per cent O_2-five per cent CO_2 was bubbled through the medium at a rate of approximately 300 ml/min. The tissue was sufficiently agitated by this action that, by a single adjustment of the micro-electrode tip to the undulating surface of the tissue membrane, repeated electrical contact of tissue with the micro-electrode could occur. Numerous REP's of many areas were recorded in this manner. Also, good REP configurations were obtained, employing the standard technic of penetration of the adipose tissue by the micro-electrode. Dead adipose tissue demonstrated no REP.

In collecting data, an initial 10-15-minute interval of equilibration of the tissue in the buffer medium was followed by a 10-minute period of recording control REP. The control buffer was then replaced by buffer containing insulin and after a 2-minute period of equilibration, three 3-minute periods of REP were recorded. There were 2-minute waiting intervals between these periods. In some experiments, the insulin-containing buffer was replaced by control buffer and the previous experimental procedure followed. In a few experiments, a second "wash-out" with a control buffer was performed and REP's recorded for 2 or 3 intervals of 3 minutes separated by 2-minute waiting periods.

Results. There was considerable variation of pre-insulin control REP among individual rats, varying from +17 to +69 millivolts (Table I). The increase in adipose tissue REP to 1000 micro-units/ml, compared with the pre-insulin control values, is consistent and marked with the mean increase in the last 3-minute period varying from +28 to +37 millivolts. The mean increase during this last 3-minute insulin period was +33 millivolts, computed on the basis of a single value for each of the 5 smaller animals (Table II). This is highly significant as determined by the Student "t" test, the probability ("P") being $<.001$, according to Fisher's Tables (5).

Following the replacement of media containing 1000 micro-units/ml of insulin by control buffer media, there was a decrease of REP. In the final 3-minute post-insulin control periods, the adipose tissue REP increment in young animals varied from +9 to +23 millivolts, as compared with pre-insulin mean control values. The mean increase of REP in this period, computed on the basis of a single value for each rat, was +16. "P"

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† Knox Special Gelatin, 6% conc., no added NaCl, lot # P-111 N.S.

TABLE I. Effects of Insulin upon Resting Electrical Potentials of Adipose Tissue-Glucose Conc. (1 mg/ml).

				Pre-insulin control—10 min.		Insulin—3-min. periods								
						1st			2nd			3rd		
Rat No.	Wt of rat (g)	Wt of adipose tissue (mg)	Insulin conc. (μunits/ml)	No.*	Mean ± SEM†	No.	Mean ± SEM	Change in REP‡	No.	Mean ± SEM	Change in REP	No.	Mean ± SEM	Change in REP
1	110	70	1000	44	44 ± .7	21	54 ± 1.8	+10	24	71 ± 2.4	+27	32	74 ± 2.4	+30
2	130	65	1000	38	17 ± .6	11	37 ± 2.4	+20	6	47 ± 5.0	+30	24	50 ± 2.5	+33
3	125	125	1000	105	40 ± .5	26	60 ± 1.2	+20	28	75 ± 2.2	+35	31	76 ± 2.2	+36
4	130	50	1000	97	54 ± .5	37	80 ± 1.4	+26	26	72 ± 1.1	+18	39	91 ± 1.3	+37
5	130	110	1000	88	48 ± 1.1	30	73 ± 2.6	+25	27	76 ± 2.2	+28	28	78 ± 2.3	+30
6	160	50	10	95	60 ± .9	38	81 ± 1.5	+21	36	87 ± 1.4	+27	27	88 ± 1.7	+28
7	135	35	1	94	69 ± .7	40	60 ± .9	− 9	35	77 ± 1.2	+ 8	37	87 ± 1.1	+18

	First post-insulin control—3-min. periods									Second post-insulin control—3-min. periods								
	1st			2nd			3rd			1st			2nd			3rd		
Rat No.	No.	Mean ± SEM	Change in REP	No.	Mean ± SEM	Change in REP	No.	Mean ± SEM	Change in REP	No.	Mean ± SEM	Change in REP	No.	Mean ± SEM	Change in REP	No.	Mean ± SEM	Change in REP
3	33	66 ± 1.5	+26	38	56 ± 1.2	+16	32	59 ± 1.2	+19	32	68 ± 1.4	+28	35	54 ± 3.1	+14	42	56 ± .9	+16
4	33	84 ± 1.5	+30	40	70 ± 2.2	+16	35	74 ± 1.6	+20	43	70 ± 1.5	+16	40	68 ± 1.2	+14	38	77 ± 1.4	+23
5	26	67 ± 3.0	+19	37	62 ± 2.0	+14	18	67 ± 2.7	+19	26	53 ± 2.0	+ 5	17	57 ± 2.9	+ 9			
6	32	80 ± 1.3	+20	28	76 ± 1.0	+16	36	79 ± 1.0	+19	27	68 ± 1.6	+ 8	21	67 ± 1.2	+ 7			
7	38	75 ± 1.2	+ 6	44	73 ± 1.7	+ 4	28	66 ± 1.9	− 3									

* No. of determinations.
† Mean value of resting electrical potential in millivolts ± stand. error of mean.
‡ Change in Resting Electrical Potential (millivolts).

TABLE II. Effects of Insulin upon Resting Electrical Potentials of Adipose Tissue-Glucose Conc. (1 mg/ml).

Rat No.	Wt of rat (g)	Wt of adipose tissue (mg)	Insulin conc. (μunits/ml)	Pre-insulin control—10 min. No.*	Mean ± SEM†	Insulin—3-min. periods 1st No.	1st Mean ± SEM	1st Change in REP‡	2nd No.	2nd Mean ± SEM	2nd Change in REP	3rd No.	3rd Mean ± SEM	3rd Change in REP
8	330	220	1000	116	18 ± .8	33	0	−18	33	0	−18	34	0	−18
9	360	70	1000	78	16 ± .4	33	0	−16	33	0	−16	34	0	−16
10	380	100	1000	111	56 ± .7	40	56 ± 1.2	− 1	19	53 ± 1.3	− 4	27	53 ± 1.0	− 4
11	355	100	1000	77	37 ± 1.7	24	31 ± 2.6	− 6	36	32 ± 2.5	− 5	36	29 ± 1.8	− 8
12	405	105	1000	91	58 ± .6	38	53 ± 1.3	− 5	35	59 ± 1.2	+ 1	41	61 ± 1.0	+ 3

First post-insulin control—3-min. period

Rat No.	1st No.	1st Mean ± SEM	1st Change in REP
8	15	20 ± 1.2	+2
9	43	14 ± .7	−2

* † ‡ See footnotes, Table I.

was not significant, being >.05,<.1.

There was an increase of adipose tissue REP, in one small animal, to a maximum of +28 millivolts following addition of 10 micro-units/ml of insulin. The final post-insulin control value with 10 micro-units of insulin appeared lower, +7 millivolts, than observed following 1000 micro-units/ml. One micro-unit per ml of insulin appeared to produce no stimulation of adipose tissue REP during the first 3 minutes, but increasing increments of REP appeared in the following two 3-minute periods, the maximum increase being +18 millivolts. It is perhaps also significant that the initial 3-minute post-insulin control period, after removal of one micro-unit of insulin, demonstrated a marked decrease of resting potential, in marked contrast to the results observed with 1000 micro-units/ml of insulin.

There is considerable variation of pre-insulin control REP among individual rats weighing 330-405 g, varying from +16 to +58 millivolts (Table II). In response to 1000 micro-units/ml of insulin, the large rat adipose tissue REP showed either little change or a marked decrease (Table II). Adipose tissue REP's in 2 animals were totally ablated, the decrements being −16 to −18 millivolts. The REP in each was totally restored by replacing insulin with control medium.

In response to insulin, the mean change of REP was −9 millivolts, computed on the basis of a single value for each large rat. According to the Student "t" test, this figure is not statistically significant, the probability ("P") being >.05, <.1 by Fisher's Tables (5).

Tissue tested in Krebs-Ringer-bicarbonate buffer containing no glucose demonstrated little variation of pre-insulin control REP's, mean values ranging from 52-61 millivolts. The alterations of REP following addition of 1000 micro-units/ml of insulin generally paralleled those observed in the presence of glucose (Table III). There was a marked increase of adipose tissue REP in the smaller rats, with REP increase in the last 3-minute period varying from +22 to +33 millivolts.

TABLE III. Effect of 1000 U/ml of Insulin upon Resting Electrical Potentials of Adipose Tissue in Absence of Glucose.

Rat No.	Wt of rat (g)	Wt of adipose tissue (mg)	Pre-insulin control—10 min. No.*	Pre-insulin control—10 min. Mean ± SEM†	Insulin—3-min. periods 1st No.	1st Mean ± SEM	1st Change in REP‡	2nd No.	2nd Mean ± SEM	2nd Change in REP	3rd No.	3rd Mean ± SEM	3rd Change in REP
1	125	110	87	57 ± .6	40	66 ± 1.2	+ 9	35	77 ± 1.6	+20	33	79 ± .8	+22
2	135	50	90	52 ± .7	26	71 ± 1.8	+19	24	91 ± 2.2	+39	24	90 ± 1.3	+38
3	130	105	85	61 ± .8	61	77 ± 2.5	+16	38	89 ± 1.5	+28	32	92 ± 1.9	+31
4	160	35	103	56 ± .6	23	93 ± 3.6	+37	26	80 ± 2.6	+24	22	89 ± 1.9	+33
5	405	140	88	59 ± .7	30	71 ± 1.8	+12	23	76 ± 2.2	+17	6	72 ± 3.7	+13
6	380	65	96	57 ± .6	29	60 ± 1.4	+ 3	42	57 ± .9	0	32	58 ± 1.1	+ 1

Rat No.	First post-insulin control—3-min. periods 1st No.	1st Mean ± SEM	1st Change in REP	2nd No.	2nd Mean ± SEM	2nd Change in REP	3rd No.	3rd Mean ± SEM	3rd Change in REP	Second post-insulin control—3-min. periods 1st No.	1st Mean ± SEM	1st Change in REP	2nd No.	2nd Mean ± SEM	2nd Change in REP
1	47	63 ± .9	+ 6	36	62 ± 1.1	+ 5	31	63 ± 1.5	+ 5						
2	26	80 ± 1.1	+28	21	87 ± 2.1	+35	30	83 ± 1.6	+31	40	76 ± .9	+24	16	63 ± 2.0	+11
3	24	79 ± 2.4	+18	32	77 ± 1.6	+16	39	72 ± 1.4	+11						
4	21	73 ± 1.5	+16	28	69 ± 3.2	+13	23	53 ± 1.0	- 3						
5	20	59 ± .8	0	18	63 ± 2.1	+ 4									

* † ‡ See footnotes, Table I.

Following replacement of the insulin-containing buffer medium with control medium, there was a variable, but definite, decline in REP. The mean increment, during the last 3-minute insulin period, of REP was +31 millivolts, calculated on the basis of a single value for each of the 4 smaller animals. As computed by the Student "t" test, this figure is highly significant, the probability ("P") being <.01 by Fisher's Tables(5). The mean increment during the final 3-minute post-insulin control period was +16 millivolts, computed on the basis of a single value for each small animal. The "P" was not significant, being >.1,<.2.

The 2 large rats tested for response to insulin of adipose tissue REP demonstrated mean increases in the final 3-minute insulin period of +1 to +13 millivolts, respectively. The mean increase of these 2 rats was +7 millivolts, significantly less than that observed in the small animals.

Discussion. The increase of adipose tissue REP obtained with 1000 μ U/ml of insulin, utilizing tissue from rats weighing 110-160 g, ranged from +28 to +37 millivolts. This is considerably greater than the increment, 2.7-9.6 millivolts, obtained by Zierler(1) with striated muscle from 60 g rats. Also, these significant results were obtained with smaller, more physiologic, concentrations of insulin, 1-1,000 micro-units/ml (.001-1 milli-unit/ml) as compared with 0.1 unit/ml (100 milli-units/ml) in the striated muscle experiments. The absence of response to insulin with adipose tissue from heavier, older rats further extends the evidence that the biologic changes of tissue associated with aging are fundamental and profound. The investigator who conducted the REP readings did not know the weight of the rat from which adipose tissue was secured until the particular experiment ended.

The irregular, but definite decrease in REP following replacement of insulin by control buffer medium became greater with passage of time and repeated "washings." Also, these decreases appeared to be greater and seemed to occur earlier following the smaller insulin concentrations. These observations suggest that little or no firm binding of insulin by adipose tissue occurs in relationship to changes in REP.

Zierler(1,6) demonstrated that approximately the same increment of rat skeletal muscle REP occurred with insulin in the absence of glucose as with glucose. The skeletal muscle REP increments, in response to insulin, ranging from +3.3 - +10.0 millivolts, were considerably less than the adipose tissue increments, ranging from +22 - +38 millivolts. Also, the insulin-concentration employed was 1 milli-unit/ml (1000 micro-units/ml) as compared with 100 milli-units/ml in the muscle study. As occurred with glucose, no significant increment of REP was noted in adipose tissue secured from large rats. Also, without glucose, marked reduction of resting electrical potential was demonstrated following withdrawal of insulin from the medium. These data indicate that change in cell REP, associated with insulin, can be independent of glucose transport.

The possibility of a non-specific protein effect is largely discounted because of the extremely small concentrations of insulin employed in these experiments. Also, 0.1% gelatin was incorporated in the buffer medium so that any increase in protein concentration resulting from addition of these very small concentrations of insulin would be, proportionally, minimal.

Summary. Significant increase of cell membrane resting electrical potential (REP) of adipose tissue obtained from young rats occurred in response to 1-1,000 micro-units/ml of insulin. Decrease of adipose tissue resting potentials occurred following replacement of insulin by control buffer. Resting electrical potentials of adipose tissue from older rats, weighing 330-405 g, showed no such increase in response to 1,000 micro-units/ml of insulin. Presence or absence of glucose in the buffer medium appeared to have no effect upon the response of cell membrane REP.

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Sodium - Potassium Activated G-Strophanthin Sensitive ATPase in Cardiac Muscle.* (27589)

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Skou(1,2) demonstrated the existence of an enzymatic activity in peripheral crab nerves which possessed such properties that he considered whether the enzyme might be involved in active transport of Na and K in the nerve fiber. The substrate for the enzyme is ATP, appears to have an absolute requirement for Mg and can be activated further by Na and K. The Na + K active component of the enzyme exhibits a unique susceptibility to inhibition by very low concentrations of G-Strophanthin, a supposedly specific inhibitor of the coupled Na + K transport system(3).

If such an enzymatic activity is a participant of the Na + K active transport system it may be assumed that the enzyme should be present in other tissues where active Na and K transport occurs. The purpose of this investigation was to search cardiac muscle for an enzymatic activity similar to that first described by Skou(1).

Materials and methods. Preparation of enzyme: Two different procedures were employed to prepare the enzyme. In the first procedure rabbit ventricles were homogenized in 9 volumes of ice cold distilled water using an all-glass Potter Elvehjem homogenizer. The water homogenate was centrifuged for 10 minutes at 2000 × *g* and sediment discarded. The supernate was dialyzed against a large volume of ice cold water at 5°C and was changed once within a 24-hour period. This dialyzed preparation represented the source of enzyme and is referred to as the water extract. A small amount of sediment formed during dialysis and was included in the preparation.

The second procedure was that described to me by Skou with some modification; rabbit ventricles were homogenized in 9 volumes of ice cold sucrose containing 0.1% deoxycholate (DOC), .030 mM L-histidine, and .005 M ethylenediamine tetraacetic acid (EDTA), and the homogenate subjected to the differential centrifugation technic(4). The debris-nuclear (500 × *g*, 10 min), mitochondrial (10,000 × *g*, 10 min), and microsomal (72,000 × *g*, 45 min) fractions were isolated using a Lourdes refrigerated Vacu-Fuge. The microsomes were washed once in a medium consisting of .25 M sucrose, .05% DOC, .030 mM L-histidine and .001 M EDTA. All fractions were resuspended in a suitable amount of cold .25 M sucrose containing .030 mM L-histidine, rehomogenized to ensure a fine suspension of enzyme and immediately assayed for ATPase activity.

Reaction mixture: The activity of these preparations was determined at 37°C in a total volume of 2.5 ml containing 0.2 ml of enzyme preparation buffered with imidazole (100 μmoles), histidine (100 μmoles) and brought to pH 7.1 with approximately 50 μmoles of HCl. Incubation time was 60 minutes. The reaction mixture for routine assays was charged with 6 μmoles of Mg and 5 μmoles of ATP which was rendered free of Na and K by passing it through a Dowex 50 cation-exchange column in the tris form and finally buffered to pH 7.0 with trishydroxymethyl aminomethane (tris). The Dowex

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