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RAPID HYPERPOLARIZATION OF RAT SKELETAL MUSCLE INDUCED BY INSULIN

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Summary

It has been proposed that the increase produced by insulin in electrical potential differences across membranes of target cells may be a mechanism by which the cell surface insulin-receptor complex causes at least some of the metabolic effects of insulin. If insulin-induced hyperpolarization is a transducer of common effector responses it must precede those responses. The problem has not been addressed previously, so that rapid responses to insulin have not been sought. Two methods were used. In one method, the bathing solution was changed rapidly so as to include insulin in supramaximal concentrations, and a series of measurements of membrane potentials, E_T, were made. Insulin hyperpolarized by 9.4 mV within 1 min. In the other method, nanoliter amounts of highly concentrated insulin solution were ejected from a micropipette onto the surface of an impaled muscle fiber. In 21 out of 32 insulin injections, hyperpolarization occurred within 1 s; in 11 control injections there was no change. This is the most rapid response to insulin yet reported, and is consistent with the hypothesis that insulin-induced hyperpolarization may transduce effector responses.

In 1957 we reported that insulin made the transmembrane electrical potential difference across muscle more negative [1]. This observation has been confirmed in muscle [2—6], and it has been reported that insulin also hyperpolarizes cell surface membranes of other tissues [7—12].

Since the electrical field strength through a cell surface membrane is a powerful orienting force (about 10^5 V/cm) and since changes in field strength are known to affect some membrane properties, particularly those of potential-dependent ion channels (see Ref. 13 for review), it is possible that hyperpolari-

zation may be a mechanism by which insulin initiates certain of the familiar responses to it, a suggestion first made in 1972 [14].

If hyperpolarization is a transducer of insulin action, one of the criteria is that hyperpolarization must precede other responses to insulin, such as increased glucose uptake, which occurs in adipocytes with a lag time of 40 s [15]. In none of the studies of insulin-induced hyperpolarization, in which measurements were made with intracellular microelectrodes, was it reported that hyperpolarization occurred in less than 10 min, and in some of them the measurements were not given until 20 or 30 min after insulin addition. This does not mean that hyperpolarization did not occur earlier; it was evidently not the design of those experiments to see how early it did occur. Recently, Cheng et al. [16] reported that insulin hyperpolarized rat adipocytes within 5 min, measured by distribution of [^{3}H]triphenylmethylphosphonium between intra- and extracellular fluid.

We suspected that insulin-induced hyperpolarization might occur in less than 1 s, in analogy with the hyperpolarizing action of peptide secretagogues for pancreatic acinar cells, which Petersen and Philpott [17] found occurred rapidly after microiontophoresis of peptide upon the cell surface. We have made preliminary efforts to microiontophorese insulin, but have not yet found satisfactory conditions. Pending solution of the problems of microiontophoresis we turned to two other methods and demonstrated, by the first method, that insulin hyperpolarizes rat skeletal muscle within 1 min, and, by the second, that it hyperpolarizes within about 1 s.

Methods

The muscle used in these experiments was caudofemoralis from rats weighing 90—120 g (Charles River, Wilmington, MA). The muscle is about 4 cm long, 2.5 mm wide, and 1 mm thick. It was placed in a chamber at rest length, its ends passed through two water-tight pouches so that only the middle 14 mm of the muscle was in the compartment in which measurements were made. The volume of this compartment was 3 ml. The entire muscle was bathed in a Krebs-Ringer bicarbonate/pyruvate solution, pH 7.4, at room temperature, and was gassed intermittently with 95% O_2/5% CO_2.

Membrane potentials were measured with a system including 3 M KCl-filled glass electrodes (10—30 MΩ, 0—5 mV tip potential) leading to Ag-AgCl electrodes and a W-P Instruments Model 707 Micro-Probe System. Recording was either on a storage oscilloscope or an x-y pen-writer.

Insulin was introducted into the chamber by one of two methods. (a) After a series of impalements in the absence of insulin, the 3 ml solution bathing the middle compartment was sucked out and replaced by 3 ml of solution of the same composition plus insulin. A series of impalements began at once, usually going no deeper than the first three fibers. Because the object of these experiments was to find out how rapidly insulin could act, it was desirable to minimize the time required for insulin to diffuse to fibers beneath the surface to reach an effective concentration. For this reason, all concentrations of insulin in these experiments were greater than that required to produce maximum hyperpolarization response to the hormone. The concentrations

ranged from 1 to 100 mU/ml. The concentration of insulin in arterial blood in man, attained in response to a glucose meal, is about 0.1 mU/ml. This concentration produces approximately half-maximum hyperpolarization of rat caudofemoralis muscle (unpublished observations). (b) After a series of impalements in the absence of insulin, a glass capillary pipette filled with a concentrated solution of insulin, or of the vehicle without insulin, was introduced into the chamber by means of a micromanipulator. The capillary pipette, of 1 mm outer diameter, with an orifice of 50—150 μm, was inserted into a W-P Instruments injection-type electrode holder, connected by vinyl tubing to a Hamilton gas-tight syring mounted on the frame of the shielded cage in which the apparatus was sited. The volume delivered varied from about 0.006 to 0.024 μl. The connecting tubing was flexible to damp out vibrations that would otherwise be transmitted from the syringe to the pipette. This property also conferred compliance so that there was a lag between the turn of the piston and delivery of solution from the pipette tip. The instant of ejection was recognized visually, through a dissecting microscope, by the onset of a change in level of the meniscus in the pipette. The total volume ejected was estimated from the final change in meniscus level. Calibration of pipette volume was about 0.018 μl per 100 μm length of pipette at full diameter.

Krebs-Ringer bicarbonate/pyruvate solution had the following composition (in mM): Na^+, 145; K^+, 4.7; Ca^{2+}, 1.2; Mg^{2+}, 1.2; Cl^-, 122; HCO_3^-, 27, $H_2PO_4^-$, 1.4 SO_4^{2-}, 1.2; pyruvate, 1. The solution was gassed with 95% O_2, 5% CO_2 (pH was 7.4). Temperature was about 20°C (not controlled).

Insulin (crystalline porcine) was a gift from Mr. Ronald Davis, Eli Lilly and Co., Indianapolis. It was assayed by the manufacturer at 24.5 units/mg and zinc content 0.6%. For experiments in which the bathing solution was changed to one containing insulin, insulin was prepared from stock solution containing 1 mg of insulin per ml 0.01 M HCl. Stock solutions were diluted in Krebs-Ringer bicarbonate/pyruvate solution to give the final concentration of insulin indicated in the appropriate section of Results. For experiments in which insulin was injected, insulin was dissolved in 0.01 M HCl, then neutralized to approx. pH 6.0 with NaOH. The insulin concentration in the micropipette was either 2.5 or 5 mg/ml. In controls for the latter experiments, the solution injected contained approx. 0.005 M HCl, 0.005 M NaOH and 0.0005 M $ZnCl_2$.

Results

Hyperpolarization produced by rapid substitution of a bathing solution containing insulin

After a series of measurements of the membrane potential of a number of muscle fibers, insulin was added to the muscle by rapid substitution of a solution containing insulin at the concentrations indicated in Table I, except in two cases in which a 10-fold concentrated solution of insulin was injected rapidly into the Krebs-Ringer bicarbonate/pyruvate solution bathing muscle to achieve the desired final concentration upon vigorous mixing. In all cases, addition of insulin was made while a surface fiber was impaled, in the hope that, if there was a rapid response to insulin, it could be detected in the impaled fiber. However, in all cases, the disturbances accompanying the addi-

TABLE I

HYPERPOLARIZATION OF RAT CAUDOFEMORALIS MUSCLE BY INSULIN

Data are means ± S.E. Figures in parentheses are numbers of fibers impaled. Data for first 4 min include data for first minute. Degrees of freedom for tests of significance: for individual experiments, based on number of impalements; for grand means, based on number of muscles. Values are expressed in mV.

Insulin concentration (mU/ml)	Control ($-E_R$)	Insulin	
		First minute ($-\Delta E_R$)	First 4 min ($-\Delta E_R$)
100	66.1 ± 1.87 (22)		6.0 ± 2.83 (15) *
100	63.0 ± 4.67 (5)		20.1 ± 5.45 (23) **
100	69.8 ± 1.61 (12)	6.5 ± 2.91 (10) *	6.5 ± 2.91 (10) *
100	70.7 ± 2.28 (16)	9.1 ± 3.10 (5) *	10.3 ± 2.46 (21) **
10	67.8 ± 2.69 (18)	7.0 ± 2.82 (4)	10.2 ± 2.86 (16) **
1	66.7 ± 1.61 (12)		11.0 ± 2.60 (25) **
1	71.9 ± 2.56 (16)	11.5 ± 2.68 (5) *	11.5 ± 2.68 (5) *
1	70.1 ± 1.49 (27)	12.9 ± 1.89 (5) **	9.2 ± 1.95 (21) **
Mean	68.3 ± 1.03	9.4 ± 1.24 **	10.6 ± 1.53 **

* $0.01 < P \leq 0.05$.
** $P \leq 0.01$.

tion of insulin or change of solution dislodged the probe electrode from the fiber. Therefore, the effect of insulin had to be sought statistically by impaling a series of superficial fibers as rapidly as possible. There was a delay of at least 20 s after inital exposure to insulin before the first series of impalements could be started.

Resting membane potentials, E_r, were measured in eight muscles within 4 min or less after addition of insulin. In each muscle, insulin hyperpolarized significantly, by 6—20 mV. The mean hyperpolarization for the eight muscles was 10.6 ± 1.53 mV. In five of the muscles, fibers were impaled during the first minute; hyperpolarization was by 6.5—12.9 mV. In four muscles, hyperpolarization was statistically significant. In the fifth muscle, the mean hyperpolarization was within the range of the others, but the number of impalements was too small to allow satisfactory distinction from chance. Because the concentrations of insulin were supramaximal, the magnitudes of insulin-induced hyperpolarization were averaged over all the concentrations. The mean hyperpolarization for the five muscles was by 9.4 ± 1.4 mV within 1 min, a highly significant effect. There was no difference between the magnitude of hyperpolarization during the first minute and that over the first 4 min. In the two muscles for which data are reported for the first minute after addition of 100 mU insulin/ml, measurements were in fact completed in 30 and 40 s.

Hyperpolarization produced by local injection of insulin

Among five muscles there were 11 injections of the control solution near the surface of a muscle fiber at the site of impalement. There was no change in the membrane potential; the mean change after 1 s was 0.00 ± 1.07 mV (S.D.), ±0.32 mV (S.E.).

Among 11 muscles there were 32 injections of insulin. In 21 of these, within 1 s there was hyperpolarization greater than 3 S.E. of responses to

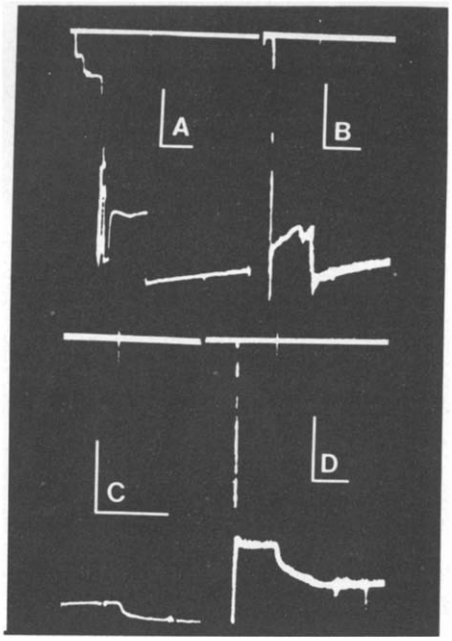

Fig. 1. Hyperpolarizing response to insulin injection. Calibration: Vertical, 20 mV; horizontal, 5 s. Upper line in each tracing is 0 mV; insulin injection indicated by marker. (A and B) Step response to maximum hyperpolarization. (C and D) Rapid onset of response with slower increase to maximum. The initial downward deflection in A, B and D occurred when the electrode tip was on a fibers surface. In C the impalement occurred a few seconds before the portion of the trace reproduced here. In B, C and D the electrode was in a surface fiber. In A the electrode passed through a surface fiber and possibly through the second fiber; the record at the time of injection was from a second or third fiber from the surface.

control injections. Of the 11 cases in which hyperpolarization did not occur within 1 s, in four there was hyperpolarization in 1—35 s. All of the failures to hyperpolarize within 1 s occurred in experiments in which the injection pipette was positioned first, and a fiber was impaled near the injection pipette. There was no case in which hyperpolarization did not occur within 1 s when the sequence was reversed; the injection pipette was brought close to the site of an existing impalement.

For the population of 21 injections in which hyperpolarization occurred within 1 s, the mean hyperpolarization was by 8.5 ± 1.74 (S.E.) mV, which differed from controls ($P < 0.001$). For the total population of 32 injections of insulin, the mean hyperpolarization was by 5.6 ± 1.34 (S.E.) mV, which also differed from controls ($P < 0.001$). Fig. 1 illustrates abrupt hyperpolarization in response to injected insulin. The top two responses reached maximum almost at once. The bottom two responses were rapid in onset, but increased more slowly to maximum.

Discussion

Even in the first set of experiments, in which the bathing solution was changed to one containing insulin, it was possible to demonstrate insulin-

induced hyperpolarization more rapidly than has been reported previously. When insulin was injected near the site of impalement, hyperpolarization occurred within 1 s in two-thirds of the trials. Part of the delay was due to transit of insulin from the tip of the pipette to recognition sites on the muscle fiber surfaces. When the injecting pipette was brought to the site of impalement it was an easy matter to bring the two together within about 30—50 μm. However, when the injection pipette was positioned first, since the tip of the microelectrode is not visible, caution prevented bringing the microelectrode so close to the injection pipette. It is likely, therefore, that the failures were due to longer transit distances in these cases.

There were two types of response to injected insulin. One was a step to maximum hyperpolarization. The other was rapid in onset, but increased more slowly to maximum. Since large step responses did occur, it is likely that in those cases there was not much dispersion of transit times to occupy as many specific binding sites as was necessary for full response, whereas in the other cases it is likely that there were some longer transit times.

Since hyperpolarization is an early response to insulin, almost immediate on the time scale by which metabolic effects are usually measured, the possibility is supported that hyperpolarization may be a mechanism by which insulin initiates other responses.

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