INSULIN STIMULATES DEOXYGLUCOSE TRANSPORT IN ADULT RAT HEART CELLS IN THE ABSENCE OF Ca^{2+}

Robert A. HAWORTH, Douglas R. HUNTER, and Herbert A. BERKOFF
Department of Surgery, University of Wisconsin Clinical Sciences Center, 600 Highland Avenue, Madison, WI 53792, USA

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1. Introduction

The role of Ca^{2+} in the insulin-induced stimulation of glucose transport in muscle is controversial. A direct role for Ca^{2+} in this action of insulin has been argued in [1,2]. The evidence is largely circumstantial: many conditions which mimic insulin action with respect to glucose transport also produce either an increased Ca^{2+} influx or a decreased Ca^{2+} efflux; insulin itself has been shown to affect Ca^{2+} fluxes; and finally, insulin-stimulated glucose uptake in muscle has been found to be inhibited when Ca^{2+} was removed from the external medium (review [3]). The latter evidence is the most direct support for a role of Ca^{2+} in the activation process. The hypothesis has been refined by the suggestion [4] that the Ca^{2+} pool involved in glucose-transport activation is not cytoplasmic, but is membrane-bound, since contraction-stimulated glucose uptake does not return to basal levels for a considerable time after contraction has ceased. We have measured insulin-stimulated 2-deoxy-D-glucose uptake in isolated heart cells from the adult rat, and have found a response to insulin similar in magnitude and sensitivity to that of the whole heart. However, we have found that insulin continues to stimulate 2-deoxy-D-glucose uptake into isolated cells in the presence of excess EGTA. Moreover, treatment of the cells with no Ca^{2+} + EGTA + A23187, a Ca^{2+} ionophore, failed to diminish the insulin response. This result suggests that neither internal nor external Ca^{2+} is required for the activation of 2-deoxy-D-glucose uptake in muscle by insulin.

Abbreviations: MOPS, 3-(*N*-morpholino)propanesulfonate; BSA, bovine serum albumin; EGTA, ethylene bisoxoethylenenitrolotetraacetic acid, Na^+ salt

2. Materials and methods

Suspensions of heart cells were isolated from female retired breeder Sprague-Dawley rats as in [5], and suspended at 5 mg protein/ml in a modified Krebs–Henseleit medium containing: 118 mM NaCl, 4.8 mM KCl, 25 mM MOPS (pH adjusted to 7.0 with NaOH), 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 1 mM $CaCl_2$ (unless omitted as indicated), 5 mM Na-acetate, 1 mg BSA/ml, and 2 mM sucrose. Samples (2 ml) of cell suspension were incubated aerobically at 37°C with insulin as shown for 30 min. Each sample was cooled to 21°C for 5 min, and 0.5 μCi [^{14}C]sucrose was added. After 30 s 5 mM 2-deoxy-D-glucose was added, followed 30 s later by 1.25 μCi 2-deoxy-D-[^{3}H]glucose. After incubation at 21°C for the times shown, twin 0.5 ml aliquots of cell suspension were layered onto 0.5 ml bromododecane over 0.1 ml 16% perchloric acid, and centrifuged for 1 min in a Beckman microfuge B bench centrifuge. ^{14}C and ^{3}H activity was measured in the perchloric acid layer (plus pellet), and in an aliquot of the supernatant, by liquid scintillation counting. The pellet counts were corrected for contributions from extracellular (sucrose-containing) fluid, and converted to the rates of 2-deoxy-D-glucose uptake shown.

3. Results and discussion

The uptake of 2-deoxy-D-glucose by cells at 21°C in the presence and absence of insulin was found to be linear for $\geqslant$30 min (fig.1). $CaCl_2$ was present in the medium at 1 mM. Our preparation of heart cells resists this level of Ca^{2+} without undergoing contracture [5]. A dose–response curve under the same conditions is shown in fig.2. A half-maximal effect of

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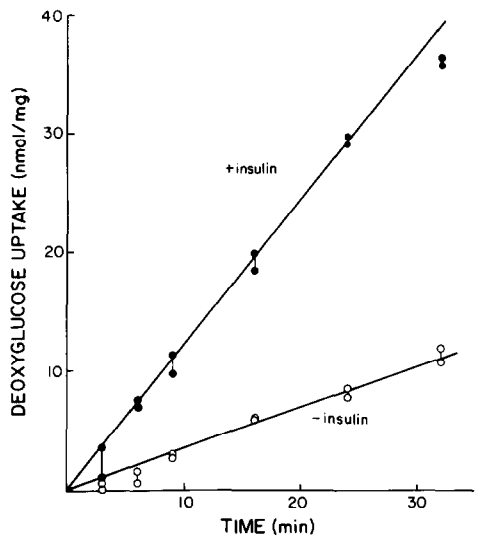

Fig.1. Uptake of 2-deoxy-D-glucose in the presence and absence of insulin at 0.2 μM. Data shown is from a single representative experiment.

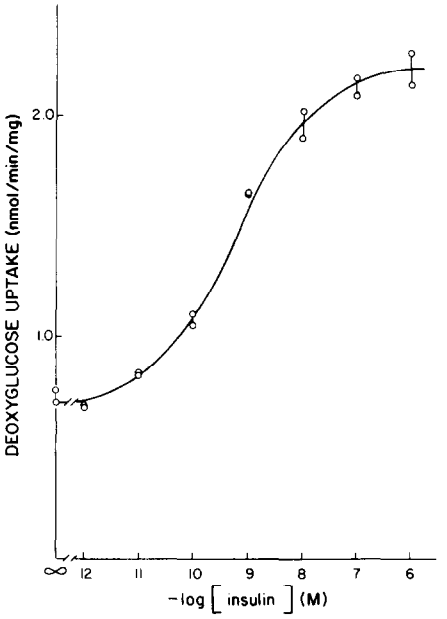

Fig.2. Dose–response curve for insulin-stimulated 2-deoxy-D-glucose uptake. Incubation with labelled 2-deoxy-D-glucose was for 22 min. Data shown is from a single representative experiment.

insulin was achieved at 5.2×10^{-10} M. A second identical experiment gave a half-maximal response at 10^{-9} M. This response to insulin is comparable in both magnitude [6] and sensitivity [7] to the effect of insulin on glucose transport in the intact heart or in adipocytes. The average maximal stimulation found with insulin under these conditions, expressed as the ratio of the average rate of 2-deoxy-D-glucose uptake with and without insulin, was 2.64 (table 1). Insulin effects on glucose metabolism have been observed in preparations of isolated heart cells, even in preparations where cells are not Ca^{2+}-resistant. A small stimulation of glucose uptake by insulin was observed in [8] with rat myocytes, but no effect was observed on glucose oxidation to CO_2. By contrast, a 2-fold increase in glucose oxidation was observed with canine myocytes [9] and Ca^{2+}-resistant rat myocytes [10].

When 2 mM EGTA was added 15 min before insulin addition, the rate of 2-deoxy-D-glucose transport in the absence of insulin was slightly elevated ($p < 0.02$, paired t-test), while the transport rate in the presence of insulin was unaffected (table 1). Omission of Ca^{2+} from the suspension medium made no further significant difference (table 1). This result is unexpected in light of the observation [12] that insulin did not stimulate 3-*O*-methyl-D-glucose transport in guinea pig atria perfused by a Ca^{2+}-free medium + EGTA. No

Table 1
Effect of Ca^{2+} on rates of 2-deoxy-D-glucose uptake

Condition	Rate of uptake (nmol . min^{-1} . mg^{-1})			
	– Insulin	+ Insulin	Ratio	n
1 mM Ca^{2+}	0.73 ± 0.29	1.93 ± 0.59	2.64	8
1 mM Ca^{2+} + 2 mM EGTA	0.90 ± 0.21	1.85 ± 0.18	2.06	3
No Ca^{2+} + 2 mM EGTA	1.14 ± 0.16	2.19 ± 0.41	1.92	4
No Ca^{2+} + 2 mM EGTA + A23187 (2.1 μg/mg)	0.75 ± 0.15	1.77 + 0.34	2.36	4

Values shown are mean ± SD of n different cell preparations

stimulation of glucose uptake by insulin was observed in developing skeletal muscle cells when only trace levels of Ca^{2+} were present in the medium [12]. The rate of 2-deoxy-D-glucose uptake is determined by both the rate of entry and efflux, with the latter strongly affected by the rate of phosphorylation, whereas the rate of 3-*O*-methyl-D-glucose uptake is determined only by the rate of entry and efflux [13]. It might therefore be argued that the stimulation of 2-deoxy-D-glucose uptake by insulin is caused by a Ca^{2+}-insensitive stimulation of the rate of 2-deoxy-D-glucose phosphorylation. We consider this unlikely, since in adipocytes, [14] with 5 mM 2-deoxy-D-glucose in the medium the level of free 2-deoxy-D-glucose in the cell in the presence of insulin was only 0.28 mM under conditions of linear uptake kinetics. Thus, the hexokinase activity did not appear to be rate limiting, and it was concluded [14] that the measurements were representative of the rate of D-glucose transport. Although at variance with previous work on muscle, our result is similar to that found in adipocytes [15], namely that the effect of insulin on adipocyte glucose metabolism was unaffected by the removal of Ca^{2+}, even in the presence of EDTA.

Since it could always be argued that intracellular levels of Ca^{2+} may not be affected by this treatment, we did the experiment also in the presence of 2.1 μg A23187/mg protein, added just after the EGTA. Again, no reduction in the insulin response was seen (table 1). A23187 appeared to restore the basal transport rate, since there was no longer any significant difference from the rate in the presence of 1 mM Ca^{2+} (table 1). A23187 is a Ca^{2+} ionophore which facilitates Ca^{2+} removal from membrane-bounded compartments. The level of A23187 used here is ~10-fold greater than that necessary to complete Ca^{2+} release from spermatozoa <1 min [16]. It is also ~10-fold greater than the level found to stimulate glucose uptake into developing skeletal muscle cells in the presence of Ca^{2+}, an effect attributed to its ability to translocate Ca^{2+} across the sarcolemma [12]. It is therefore difficult to imagine how a Ca^{2+} pool not removed by our treatment would still be sufficiently mobile to be of use in regulation, and the data in table 1 suggest that neither internal nor external Ca^{2+} is required for the stimulation of 2-deoxy-D-glucose uptake in heart muscle by insulin.

One possible explanation for our different results is the involvement of the Ca^{2+} paradox in studies of whole tissue. Ca^{2+}-free perfusion of the heart, followed by reperfusion with Ca^{2+}, causes extensive ultrastructural and functional damage [17]. Just what the critical changes are which occur during the Ca^{2+}-free perfusion are not yet known. These changes could also remove insulin sensitivity, and this would explain the observation in [12]. Our preparation of isolated cells, on the other hand, can withstand exposure to a Ca^{2+}-free medium for much longer than the intact heart without restoration of Ca^{2+} causing contracture [5], and this property may also allow the insulin sensitivity to remain.

Finally, although our cell suspension shows a physiological response to insulin, this does not necessarily mean that the insulin receptor sites are unaffected by the trypsin treatment during preparation of the cell suspension [5]. The insulin receptor is known to be cleaved by trypsin at the level of treatment used here; however, it has also been found in adipocytes that such cleaved receptors are still biologically competent [7]. Our results support this conclusion.

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