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ACTIVATION OF PYRUVATE DEHYDROGENASE IN RAT ADIPOCYTES BY CONCANAVALIN A: EVIDENCE FOR INSULIN-LIKE EFFECT MEDIATED BY HYDROGEN PEROXIDE

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SUMMARY: Concanavalin A mimics the action of insulin in stimulating glucose transport and utilization in rat adipocytes. Evidence is presented that the lectin causes a rapid and sustained increase in the active form of mitochondrial pyruvate dehydrogenase in these cells, without altering the total enzyme content. Insulin and hydrogen peroxide have the same effect, but concanavalin A appears to be more potent. Since this lectin also stimulates a pyridine nucleotide oxidase in the plasma membrane of these cells with generation of H_2O_2 like insulin, these data confirm the original evidence of Mukherjee and coauthors that intracellular H_2O_2 represents insulin's transmembrane metabolic signal (messenger). Both the stimulation effects of the lectin or insulin on formate oxidation and pyruvate dehydrogenase activity are independent of medium glucose, but this substrate has a supporting role in maintaining the redox optimum and the PDH response.

INTRODUCTION

Concanavalin A is one of the many different agents which have been found to mimic some major biological effects of insulin. For example, this lectin stimulates glucose transport and oxidation in fat cells, like insulin (1,2). The molecular basis of these cellular responses remained unclear, but the evidence suggests that a common effector system may be involved in the major effects of insulin and concanavalin A. The discovery that insulin activates a pyridine nucleotide oxidase in the plasma membrane of adipocytes with the generation of hydrogen peroxide (3-5) raised the interesting possibility that H_2O_2 may function as a transmembrane signal for the intracellular effects of insulin. Further evidence that H_2O_2 accumulates within insulin-stimulated adipocytes (6) with a profound metabolic consequence such as the stimulation of the hexose monophosphate shunt and regulation of adenylate cyclase activity (4-7) underscores the physiological role of H_2O_2 as insulin's second messenger. The evidence that concanavalin A also induces a peroxidative metabolism in adipocytes in consequence of the activation by it of the plasma membrane NAD(P)H oxidase (8) suggests that H_2O_2 may cause acute cellular responses by modulating some intracellular enzymatic activities. Our evidence for intracellular H_2O_2 in insulin-treated cells (3-6) aroused considerable interest in other laboratories and has been confirmed more recently (10).

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Abbreviations: PDH-Pyruvate dehydrogenase; PDH_a-active form of pyruvate dehydrogenase Con A-Concanavalin A; KRB-Krebs Ringer bicarbonate buffer.

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We present the evidence here that concanavalin A elicts a rapid and sustained activation of pyruvate dehydrogenase, the intramitochondrial enzyme complex which is one rate-limiting step in de novo synthesis of fatty acids and a site of insulin action (11,12). Our data suggest that this insulin-like effect of the lectin is also mediated by H_2O_2.

METHODS AND MATERIALS

Fat cells were prepared from male CD strain rats (150 to 200g) from Charles River Co., Bostin, Mass., by digestion of epididymal fat pads with collagenase (1 mg/ml), in Krebs Ringer bicarbonate buffer (12) pH 7.2 containing 3% bovine serum albumin (KRB) as previously described (4). Glucose oxidation was measured by the rate of $^{14}CO_2$ evolution from [U-^{14}C]D-glucose (0.2 mM)(0.1 µCi/µmol). Similarly, the $^{14}CO_2$ production from Na-[^{14}C]formate (0.1 µCi/µmol) was determined as an index of the intracellularly accumulated H_2O_2: catalase complex (13), according to Mukherjee et al (6), in either Krebs Ringer bicarbonate or -phosphate buffer, pH 7.4 (5), which yeilded similar results.

Pyruvate dehydrogenase activity was assayed in the infranatant fractions of fat pad or adipocyte homogenates according to the published procedure (11,12). After incubation ofcells in the above KRB-buffer for different times at 37°C in the absence or presence of glucose and/or concanavalin A, insulin or H_2O_2, the media were diluted 4X with the same buffer and removed by a rapid centrifugation. The cells were resuspended in a suitably small volume and homogenized in 10 mM potassium phosphate-1 mM EGTA-1 mM dithiothreitol-1% (w/v) bovine albumin. The infranatant fractions (0.2 ml aliquots) were employed for the assay of PDH activity. The final composition of the assay mixture in 17 x 100 mm polypropylene tubes was 11 mM potassium phosphate, 1.1 mM EDTA, 2.8 mM $MgCl_2$, 1.6% serum albumin, 1.2 mM dithiothreitol, 0.16 mM CoA, 1.6 mM NAD^+, 0.08 mM thiamine pyrophosphate, 0.6 mM pyruvate, 0.12 µCi of [^{14}C]pyruvate/ml, pH 7.4. The $^{14}CO_2$ evolved from [1-^{14}C]pyruvate was absorbed in phenethylamine(New England Nuclear) in the filter paper strips inserted in plastic center wells (Kontes Glass Co.), in the same manner as used for glucose oxidation. The PDH reaction was stopped by injecting into the assay medium 1.25 volumes of 0.08 M citrate - 0.04 M phosphate buffer (pH 3.0). After 30 min of further incubation to allow for total $^{14}CO_2$ absorption, the center wells were cut and the radioactivity was counted on a scintillation counter (Intertechnique), using Aquasol-2 (New England Nuclear) as the fluor.

Total PDH content was measured according to Mukherjee and Jungas (12) by supplementing the cell homogenates with 1.25 mM $CaCl_2$, 5 mM $MgCl_2$, and incubated for 40 min at 30°C to fully activate the enzyme through the stimulation of PDH-phosphatase. The determination of PDH activity with aliquots of this preparation revealed the fully activated enzyme under comparable stimulations by Con A, insulin or H_2O_2.

The rates of lipogenesis from glucose were determined by measuring the ^{14}C-incorporation into glyceride glycerol and fatty acid fractions as described by Denton et al (14).

All the radioactive compounds were purchased from New England Nuclear, Boston, Mass. The organic compounds were from Sigma Chemical Co., St. Louis, Mo.. The inorganic compounds were supplied by Fisher Scientific Co., Raleigh, N.C.

RESULTS

Glucose oxidation in adipocytes was substantially increased in the presence of concavalin A as well as of insulin or H_2O_2, as measured by $^{14}CO_2$ production from [U-^{14}C]D-glucose (Table 1). This stimulatory effect on glucose utilization (and therefore, uptake) was accompanied by an enhanced rate of lipogenesis from the endogenous substrates. Con A and H_2O_2 both caused a relatively higher incorporation of radioactivity from glucose carbons into the glyceride fatty acid fraction than into

Table 1

COMPARISON OF THE EFFECTS OF INSULIN, CONCANAVALIN A AND H_2O_2 ON GLUCOSE METABOLISM AND LIPOGENESIS

Experimental Condition	Nanomoles [U-^{14}C]D-glucose utilized/2 hrs/2x10^5 cells			
	$^{14}CO_2$	Total lipids	Glyceride glycerol	Glyceride fatty acids
Control	10.0 ± 1.0	26.0 ± 4.0	7.0 ± 2.0	18.0 ± 4
Insulin (10^{-8}M)	37.0 ± 5.0	49.0 ± 6.0	11.0 ± 2.0	36.0 ± 5.0
Concanavalin A (10^{-7}M)	43.0 ± 5.0	53.0 ± 8.0	12.0 ± 3.0	32.0 ± 3.0
H_2O_2 (5 x 10^{-4}M)	46.0 ± 7.0	52.0 ± 5.0	12.0 ± 3.0	39.0 ± 4.0

The values are averages ± S.E. of 3 experiments in duplicates; the decimal fractions have been ommitted.

Table 2

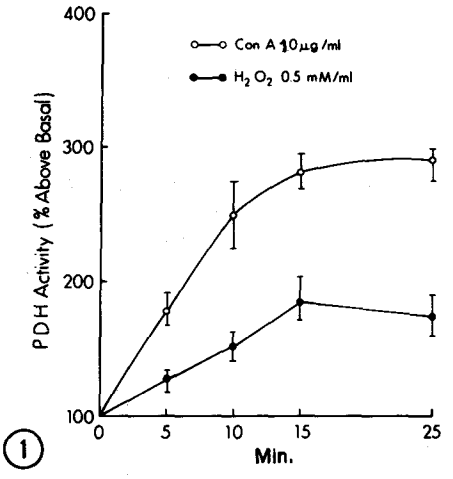
Na[^{14}C]FORMATE OXIDATION IN RAT ADIPOCYTES

Experimental Conditions	Nanomole[^{14}C]formate oxidized/10^6 cells/hr	% of the basal rate
Control	2.4 ± 0.54	—
Insulin (10^{-7}M)	4.3 ± 0.68	180
Concanavalin A (10^{-7}M)	4.6 ± 0.7	190
H_2O_2 (7.6 x 10^{-3}M)	4.1 ± 0.62	170

Fat cells were incubated for 1 or 2 hrs in Krebs Ringer phosphate buffer pH 7.4 (ref.5) containing 3% bovine albumin (final assay volume 1.2 ml) in the presence of Na[^{14}C]formate at 0.2 final concentration. No glucose was added to medium. The values are the averages ± S.E. of 4 duplicate experiments.

the glyceride glycerol fraction, in the same pattern as observed with insulin. From a dose response curve (not illustrated) the maximal response of the cells to the lectin was observed at 10^{-7}M.

The oxidation of Na-[^{14}C] formate in adipocytes was also increased by about 190% of the basal rate in response to Con A (Table 2). The increase in formate oxidation is an index of the peroxidative reaction catalyzed by intracellular H_2O_2:catalase complex (6,13). Exposure of the cells to the lectin caused a pronounced stimulation of pyruvate dehydrogenase activity, as measured in the subsequently washed cells' homogenates. This effect was also maximal at concentrations of the lectin corresponding to the increase in [1-^{14}C]D-glucose oxidation, and was used at 10^{-7}M. The effect was initiated within 3 min and the maximal stimulation was atained in 15 min of exposure of the cells to Con A or H_2O_2 (Figure 1). Although insulin or H_2O_2 had a similar



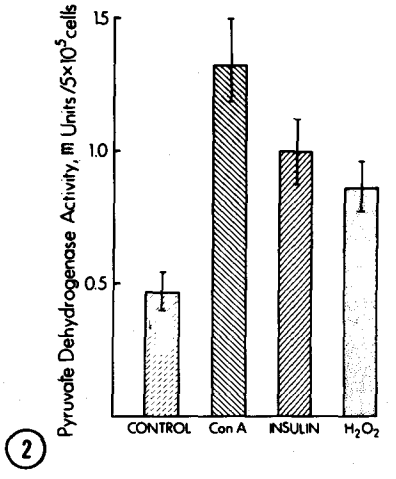


Figure 1: The time course of in vivo (intracellular activation of pyruvate dehydrogenase due to exposure of adipocytes to concanavalin A or H_2O_2. Cells were incubated for 15 min at 37°C with the indicated agents and D-glucose (0.5 mM). The media were removed and PDH activity was determined in the homogenates as described in "Methods".

Figure 2: The relative effects of concanavalin A (10^{-7}M), insulin (10^{-6}M) or H_2O_2 (0.6 mM). The experimental details are the same as in fig. 1 and in "Methods".

stimulatory effect on PDH as Con A, the latter appeared to be more potent, compared on the basis of their maximal effective concentrations (Figure 2).

The total pyruvate dehydrogenase content did not appear to change in the cells due to any of the treatments as observed after a complete activation of the enzyme (Table 3). The lipogenic role of the pyruvate dehydrogenase activity is further reflected by the significantly increased incorporation of [2-^{14}C]pyruvate into fatty acids (Table 3). The enzyme assay was routinely performed with 0.5 mM D-glucose in cell preincubation. The presence of glucose is not required to elicit the PDH response or the increase in H_2O_2 production (6), but had a supportive effect on a sustained response and a higher basal activity (11) apparently due to its effect on the redox optimum for the cells' sensitivity to insulin (6-8; S.P. Mukherjee, unpublished), and the saturation of PDH_a with substrate (12).

DISCUSSION

The previously observed similarities between the effects of concanavalin A and those of insulin on fat cell glucose oxidation (1,2) suggests a useful role of the lectin as a probe for the molecular action of insulin. But the potential metabolic sites sensitive to Con A, e.g., pyruvate dehydrogenase has not been explored. These studies reveal that a major metabolic locus of insulin's action, the intramitochondrial pyruvate dehydrogenase complex is critically responsive to the lectin's interaction with the intact adipocytes. Since the lectin is presumed to exert its effects through its specific receptors on the cell surface (2), such a distal locus as the PDH is like-

Table 3

TOTAL PDH ACTIVITY AND LIPOGENESIS IN FAT PADS IN RESPONSE TO INSULIN, CONCANAVALIN A OR H_2O_2

Incubation of cells	Pyruvate dehydrogenase activity munits/g wet wt		Fatty acid synthesis incorporation of [2-^{14}C] pyruvate nmol/Gm wet wt
	10 min	Total PDH	
	(A)	(B)	(C)
Control	108 ± 22	294 ± 13	280 ± 42
Insulin (10^{-7}M)	182 ± 14	306 ± 14	490 ± 75
Concanavalin A (10^{-7}M)	216 ± 9	298 ± 18	568 ± 52
H_2O_2 (5 x 10^{-4}M)	168 ± 11	306 ± 12	411 ± 66

The column (A) represents PDH activity in fat pads homogenized after 10 min incubation with the agents. The total PDH activity in (B) represents the maximum activity achieved after 40 min incubation of the homogenates at 30°C with the addition of 1.5 mM $CaCl_2$, 5 mM $MgCl_2$, 10 mM D-glucose and 1 unit of hexokinase (Sigma Chemical Co.). For assay of pyruvate incorporation into fatty acids, fat pads were pre-incubated for 30 min in Krebs Ringer bicarbonate buffer, containing 3% albumin, pH 7.4 and 0.3 mM D-glucose (to minimize in vivo hormonal influence) and then transferred to new media (without glucose) and incubated with the indicated agents for 15 min. Then the media containing H_2O_2, insulin or Con A were removed and tissue fragments resuspended with addition of 0.25 mM pyruvate, [2-^{14}C] pyruvate (0.2 µCi/ml). The fatty acids were extracted from the tissue homogenates in 0.4 M $HClO_4$, according to the procedure described by Denton et at (14), and aliquots were counted in the presence of Aquasol-2. Significance: P, control vs. experimentals in (A) >0.02; in (B) N.S.; in (C) insulin >0.01, Con A >0.001, H_2O_2 >0.05.

ly to be mediated by some transmembrane signal. In the case of insulin, a second messenger has long been sought and the discovery of an insulin-responsive H_2O_2-generating NADPH-oxidase in the plasma membrane (3-6) raised a new possibility. It was previously reported by us that Con A also elicits a similar response leading to intracellular peroxidative pathways in adipocytes (8) and an insulin-like redox effect on adenylate cyclase (8,9). The present data which indicate a correlation of H_2O_2 accumulation, enhanced glucose uptake and oxidation, and activation of pyruvate dehydrogenase in cells treated with Con A add further support to an important role of H_2O_2 as a messenger for insulin. The mechanism of PDH activation by low concentrations of H_2O_2 as we (15) and other recent authors (16) observed, is not fully understood at present, but work is in progress. Our evidence suggests that the antilipolytic effect of H_2O_2 (15, 17) may be an important factor because of its regulatory relationship with fatty acid esterification (18), -thus removing the known inhibitory role of fatty acids on PDH (11). It is interesting to note that several agents mimicking the effect of insulin on glucose oxidation in fat cells, including those which promote glucose transport, e.g., Con A (1,2) and phsophatidylinositol (19-21) and those which inhibit insulin-

dependent activation of the hexose carrier system, e.g., thiols or -SH inhibitors (5,6) exert their effect through the activation of the plasma membrane NAD(P)H oxidase. This effect as well as the activation of pyruvate dehydrogenase by H_2O_2, are essentially independent of an operating glucose transport since we observed stimulation of PDH with H_2O_2 as well as with Con A or insulin in the absence of medium glucose. However, it is conceivable that for a net promotion of lipogenesis either by hormones or by agents which cause H_2O_2 production, enhanced glucose transport permits the optimal saturation of the key enzymes with the substrates. In view of the present evidence, the NADPH oxidase and its product H_2O_2 appear to be a major initial key to cellular metabolic stimulation.

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