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SHORT COMMUNICATIONS

Incorporation of leucine into protein by isolated fat cells—Effects of agents which stimulate glucose metabolism and inhibit lipolysis

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WE HAVE investigated the effects of the following agents on the incorporation of [^{3}H]leucine into protein by isolated fat cells: single component porcine insulin;[1] concanavalin A (gift from Dr. E. Davie); D-cysteine (Sigma); ouabain octahydrate (Sigma); vitamin K_5 (General Biochemicals); spermidine hydrochloride (CalBiochem); 3,3′-iminobispropylamine (Aldrich); nystatin (Squibb); and sodium tolbutamide (Upjohn). These agents have been reported to inhibit hormone-stimulated lipolysis[2–12] and to increase glucose transport and/or metabolism[2–10,13–15] of adipose tissue.

Isolated epididymal fat cells from 100–165 g Wistar rats were prepared and counted as previously described.[2] Incubation flasks contained: 2×10^5 to 4×10^5 fat cells; 5×10^5 to 1×10^5 cpm L-leucine-4,5-^{3}H (New England Nuclear); 50 nmoles of L-leucine; either 250 nmoles of pyruvate or 2·5 μmoles of glucose; and the agents to be tested, in a final volume of 0·5 ml of albumin–bicarbonate buffer.[16] After incubation for 1 hr at 37°, proteins were precipitated and lipids extracted with 3 ml of Dole's mixture.[17] The precipitates were washed, heated to 90° for 30 min and washed again with 5% trichloroacetic acid containing 10 mM L-leucine, then trapped on glass-fiber discs (GF/A, Whatman), dissolved at 55° with 1 ml of NCS (Amersham/Searle), and counted in a toluene-based scintillator.

Each treatment was replicated three or four times in an experiment. Every agent was tested in at least two experiments with different pools of fat cells to insure reproducibility.

TABLE 1. INCORPORATION OF LEUCINE INTO PROTEIN*

Additions	Leucine incorporation (nmoles/10^6 cells/1 hr) Expt. 6/5/72	Expt. 8/23/72
None	1·65 ± 0·04	3·64 ± 0·08
Insulin (40 ng/ml)	2·41 ± 0·06†	4·75 ± 0·08†
Concanavalin A (μg/ml)		
0·4	1·69 ± 0·05	
2	1·76 ± 0·11	3·77 ± 0·21
10	2·14 ± 0·04†	4·47 ± 0·09†
50	2·09 ± 0·07‡	4·34 ± 0·06†
250		3·03 ± 0·13‡

* Fat cells were incubated for 1 hr with 5×10^{-4} M pyruvate, 1×10^{-4} M [^{3}H]leucine, and additions as indicated in the table. Incorporation of [^{3}H]leucine into protein is shown as mean ± S.E. of triplicate or quadruplicate incubations. Statistical significance of differences from basal incorporation was measured by the two-tailed *t*-test.[18]

† $P < 0.001$.

‡ $P < 0.01$.

With either 0·5 mM pyruvate or 5 mM glucose as the source of extracellular carbohydrate, 40 ng/ml of insulin consistently stimulated incorporation of leucine into protein, as did concanavalin A, 10 or 50 μg/ml (Table 1). None of the following promoted incorporation: tolbutamide ($3·7 \times 10^{-4}$ to $7·4 \times 10^{-3}$ M); nystatin ($2·2 \times 10^{-5}$ to $5·4 \times 10^{-4}$ M); D-cysteine (1×10^{-4} to 1×10^{-3} M); ouabain (5×10^{-5} to $1·6 \times 10^{-3}$ M); spermidine (1×10^{-5} to 5×10^{-3} M); 3,3′-iminobispropylamine (1×10^{-5} to 1×10^{-3} M); and vitamin K_5 ($4·8 \times 10^{-7}$ to $9·5 \times 10^{-5}$ M). At the higher concentrations tested, concanavalin A, the polyamines, tolbutamide, vitamin K_5 and ouabain significantly inhibited leucine incorporation.

Our results with vitamin K_5 confirm the previous study of menadione by Fain and Rosenberg.[19] We could not confirm our preliminary report[20] that thiols stimulated leucine incorporation. Previously we had precipitated [^{3}H]proteins by fixation to filter paper,[21] a technique liable to error in scintillation counting.[22] Therefore, we have more confidence in the present results.

Despite superficially similar effects on glucose metabolism and lipolysis, some of the other agents act differently from insulin with respect to adenine nucleotide metabolism,[8,10,12,23,24] K^+ transport,[25] and production of profound metabolic depression by supramaximal doses (Refs. 2 and 4 and unpublished results). We show here another disparity between insulin and all the other agents except concanavalin A, which can bind to insulin receptors[9] and did stimulate leucine incorporation. Even this lectin, however, was inhibitory at supramaximal concentrations, unlike insulin (Table 1).

These results imply that stimulation of incorporation of amino acids into protein (a) does not necessarily follow from "insulin-like" effects on glucose and lipid metabolism, and (b) is a more specific index of insulin action than are changes in glucose metabolism and lipolysis, which can be mimicked by non-hormonal agents.

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Division of Endocrinology,
Department of Medicine,
University of Washington,
Seattle, Wash. 98195, *U.S.A.*

TSUNESADA SAKAI
VICTOR LAVIS
ROBERT H. WILLIAMS

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