INSULIN'S EFFECT ON GLUCOSE OXIDATION INDEPENDENT OF GLUCOSE TRANSPORT.

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SUMMARY

The effects of insulin and spermine to stimulate glucose transport and glucose oxidation were measured. When the insulin -- glucose transport and insulin -- glucose oxidation dose response curves were compared, it was found that insulin increased oxidation to a greater extent than transport, and that half-maximal effects were reached at lower insulin concentrations for transport than for oxidation ($0.15 \pm .06$ vs 0.5 ± 1 ng/ml, $p < .01$). This indicates that all of insulin's effects to increase glucose oxidation are not mediated through simple stimulation of glucose transport. Spermine's ability to promote adipocyte glucose metabolism was analogous to that of insulin, while hyperosmolarity (0.3 M sucrose) stimulated transport and oxidation equally. In the presence of cytochalasin B (which inhibits the ability of insulin, spermine, and hyperosmolarity to increase glucose transport) insulin and spermine could still increase the rate of glucose oxidation whereas hyperosmolarity could not. These results indicate that unlike 0.3 M sucrose, insulin and spermine can promote glucose oxidation independent of their effects to stimulate glucose transport.

INTRODUCTION

It has been suggested that essentially all of insulin's effects on glucose metabolism are secondary to its effects to promote glucose transport (1-3), and the conversion of glucose to CO_2 (oxidation) has long been used to assess this process (4,5). In the presence of insulin it was felt that at relatively low glucose concentrations oxidation rates would be relatively slow, and glucose transport would represent the rate limiting step (6-7). Thus, insulin mediated glucose oxidation is often taken as a measure of insulin mediated glucose transport. In contrast, previous reports have suggested that insulin can increase glucose oxidation, in addition to accelerating transport, by promoting some intracellular step(s) of glucose metabolism (8,9). However these

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studies rely on the differential flow of glucose through metabolic pathways in the presence or absence of insulin (8,9), and may be due to differential saturation of these pathways rather than a specific insulin effect. On the other hand, we have recently reported data consistent with an effect of insulin on intracellular oxidation by showing that insulin can increase the rate of glucose oxidation under conditions where it cannot promote glucose transport (10) In the current studies we have extended and confirmed the idea that insulin stimulates glucose oxidation by increasing both glucose transport and intracellular glucose oxidative steps.

MATERIALS AND METHODS

Porcine monocomponent insulin was generously supplied by Dr. Ronald Chance of the Eli Lilly Co. Bovine serum albumin (fraction V) was purchased from Armour and Co., collagenase from Worthington Biochemicals, {^{14}C}-1-glucose, {^{14}C}-6-glucose, 2-deoxy-1-{^{14}C}-glucose, {^{14}C}-inulin from New England Nuclear, cytochalasin B from Gallard-Schlesinger Chemical Mfg. Co., and spermine from ICN Pharmaceuticals Inc.

Preparation of Isolated Adipocytes: Male Sprague-Dawley rats weighing 120-150 gms were used for all experiments. All studies were performed in the morning on animals who had free access to standard rat chow. Animals were stunned by a blow to the head, decapitated, and epididymal fat pads removed. Isolated fat cells were prepared by shaking at 37°C for 60 minutes in Krebs-Ringer bicarbonate buffer containing collagenase (3 mg/ml) and albumin (40 mg/ml), according to the method of Rodbell (11). Adipocyte counts are performed according to a modification of method III of Hirsch and Gallian (12), in which the cells are fixed in 2% osmium tetroxide in 0.05 M collidine buffer (made isotonic with saline) for 72 hours at 37°C and then taken up in a known volume of 0.154 M NaCl for counting. Counting was performed using a Celloscope Model 112H particle counter with a 400μ aperture.

Glucose Oxidation Studies: The ability of adipocytes to oxidize glucose was determined according to the method of Rodbell (11). Adipocytes were incubated at 37°C with {^{14}C}-1-glucose in Krebs-Ringer bicarbonate buffer, pH 7.4 containing bovine serum albumin (40 mg/ml). Unless otherwise stated the media glucose concentration was 1mM. Following one hour of incubation the generated $^{14}CO_2$ was collected and counted in a liquid scintillation counter.

Glucose Transport Studies: Transport studies were performed by previously described methods (10,13). Unless otherwise stated, isolated adipocytes were incubated with 2-deoxy-{^{14}C}-1-D-glucose (specific activity 2mCi/mM) at a concentration of 1mM in Krebs-Ringer bicarbonate, pH 7.4, containing BSA (10 mg/ml) at 24°C. This assay measures the total uptake of the radiolabeled 2-deoxy glucose and is based on the principle that while 2-deoxy glucose is transported and phosphorylated by the same process as D-glucose it cannot be further metabolized (14). The assay is terminated at the end of 3 mins by transferring 200 μl aliquots from the assay mixture to plastic microtubes containing 100 μl dinonyl phthalate oil. The tubes are centrifuged for 30 secs in a Beckman microfuge, and the assay is considered terminated when centrifu-

gation begins. In experiments in which the stimulatory effect of insulin on uptake was measured, the cells were preincubated with insulin for 45 mins at 24 C. The amount of sugar trapped in the extracellular water space of the cell layers was determined using {^{14}C}-inulin according to the method of Gliemann (15). Extracellular water space is measured in each experiment, and all data of sugar uptake are corrected for this factor. The amount of trapped sugar ranged from 2-10% of the total sugar uptake depending on the conditions of incubation.

RESULTS

Figure 1 summarizes the effect of different concentrations of insulin in stimulating both 2-deoxy glucose uptake (Fig. 1A) and glucose oxidation (Fig. 1B). It is evident that the shape of the dose response curves are somewhat different for both functions. The most striking difference is that insulin

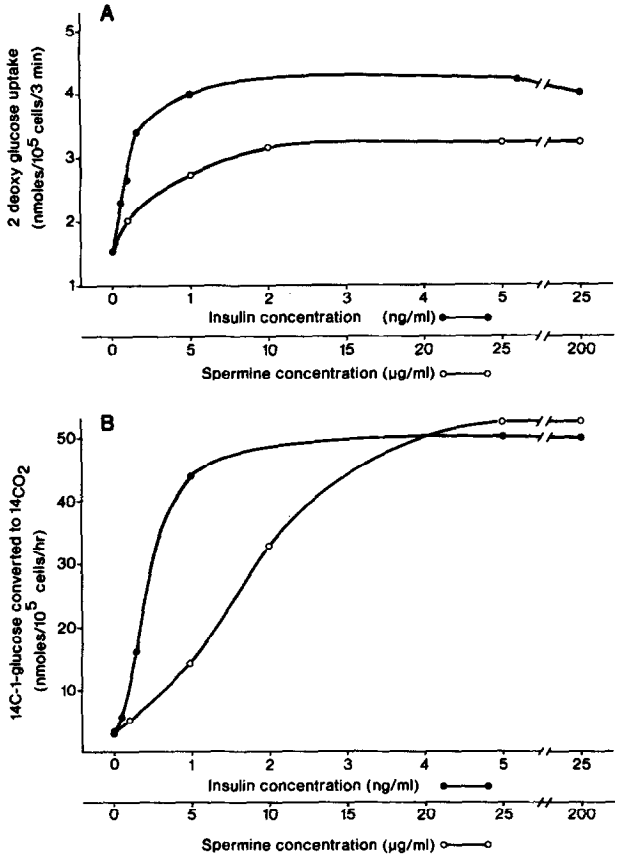

Fig. 1 Ability of insulin (●—●) and spermine (○—○) to promote the conversion of {^{14}C}-1-glucose to $^{14}CO_2$ (A) or to promote the uptake of 2-deoxy-{^{14}C}-1-glucose (B) by isolated rat adipocytes. Hexose concentration was 1mM in all studies.

leads to a 15-fold increase in glucose oxidation, but only about a 3-fold increase in glucose transport. Half-maximal effects of insulin on glucose oxidation occur at 0.5 ± .1 ng/ml, whereas half maximal stimulation of glucose transport occurs at an insulin level of 0.15 ± .03 ng/ml, and these differences are significant ($p < .01$).

Spermine is another known stimulator of glucose transport, and it has been shown that this does not involve interactions with insulin receptors (16). Fig. 1 also demonstrates the effects of spermine on both glucose transport (1A) and glucose oxidation (1B). It can be seen that the dose response curves are again different, and are comparable to the effects of insulin on these two aspects of glucose metabolism (although the magnitude of spermine's effect on transport is less than that of insulin). Thus, maximal concentrations of spermine lead to a 2-fold increase in 2-deoxy glucose uptake (A) and a 15-fold increase in glucose oxidation (B). Furthermore, half-maximal effects occur at a spermine concentration of 4 and 9 μg/ml for 2-deoxy glucose uptake and oxidation respectively.

These data suggest that spermine and insulin can enhance glucose oxidation via mechanisms in addition to promotion of glucose transport. To test these ideas the effects of spermine and insulin to promote glucose oxidation were tested under circumstances where neither agent could increase glucose transport. To accomplish this the glucose transport inhibitor cytochalasin B was used. Cytochalasin B inhibits the facillitated glucose transport system (10,13,17-20), and, in the presence of this agent, glucose can only enter the cell by simple diffusion (10,13,17-20). When cytochalasin B was added to the incubations, 2-deoxy glucose uptake was greatly reduced, and was not stimulated by insulin or spermine (Fig. 2A), indicating that neither of these agents affect the diffusion process. Thus, in the presence of cytochalasin B, these agents could only promote glucose oxidation by stimulating the intracellular oxidation of glucose which had diffused into the cell. When glucose oxidation was measured in the presence of maximally effective levels of cytochalasin B, and at glucose concentrations high enough to allow considerable amounts of glucose

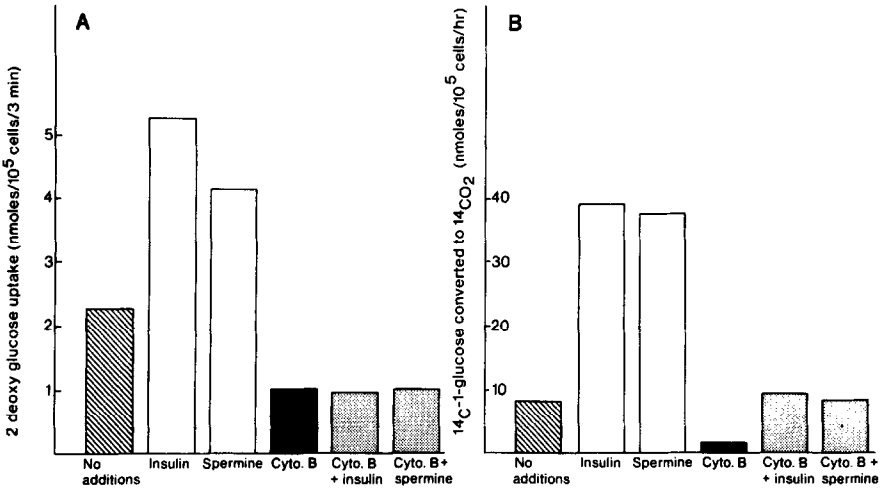

Fig. 2 Ability of spermine (100 µg/ml) and insulin (5 ng/ml), to promote the uptake of 2-deoxy glucose (10mM) (A) or to promote the conversion of {^{14}C}-1-glucose (10mM) to $^{14}CO_2$ (B) in the presence or absence of cytochalasin B (50 µM).

to diffuse into the cell (2B) it can be seen that both spermine and insulin still lead to increases in CO_2 production. Thus, these agents can promote glucose oxidation by stimulating one or more intracellular steps of glucose metabolism. Additionally, we find that oxidants such as cysteine and diamide

TABLE I

EFFECTS OF INSULIN AND SPERMINE TO PROMOTE 2-DEOXY GLUCOSE UPTAKE AND {^{14}C}-6-GLUCOSE OXIDATION

	2-deoxy glucose uptake	C-6 oxidation	C-1 oxidation
Insulin (25 ng/ml)	277[1]	258	1580
Spermine (100 µg/ml)	218	237	1630

[1]Data are expressed as the per cent increase above basal at a total hexose concentration of 1mM

have comparable effects on glucose transport and oxidation as insulin and spermine (data not shown).

It should be noted that in the above studies glucose oxidation was measured with glucose labeled in the number one position, and oxidation of this carbon atom occurs primarily via the pentose pathway (8). In contrast, the number six carbon of glucose is converted to CO_2 primarily via the Krebs cycle and the latter steps of glycolysis (8). When {^{14}C}-6-glucose is used to study oxidation, the stimulatory effects of insulin and spermine are comparable to

TABLE II

EFFECTS OF HYPEROSMOLARITY ON GLUCOSE TRANSPORT AND GLUCOSE OXIDATION[1]

	2-deoxy glucose uptake	C-1 oxidation
no additions	100	100
.3 M sucrose	212	220
insulin (25 ng/ml)	227	730
cytochalasin B	20	11
.3 M sucrose + cytochalasin B	20	12
insulin + cytochalasin B	18	95

[1]These studies were conducted at a hexose concentration of 10mM. The data are presented as percentages of the basal value for each process. It should be noted that the per cent increase due to insulin is not as great as in Fig. 1 and Table I, but that this is due to the higher hexose concentration (10mM vs 1mM) used.

their effects on glucose transport (Table I). Furthermore, these agents do not enhance {^{14}C}-6-glucose oxidation in the presence of cytochalasin B (data not shown). Thus, it would appear that insulin and spermine's ability to promote C-6 oxidation is primarily due to the effect of these agents to enhance glucose transport, while the increase in C-1 oxidation is due to enhanced glucose transport and increased intracellular metabolism.

Hyperosmolarity has been found to promote glucose metabolism (21), and Table II summarizes the effects of 0.3M sucrose on 2-deoxy glucose uptake and {^{14}C}-1-glucose oxidation. It can be seen that both aspects of glucose metabolism are increased equally, and that hyperosmolarity does not enhance C-1 oxidation in the presence of cytochalasin B. Thus, unlike insulin and spermine hyperosmolarity promotes glucose oxidation only by increasing glucose transport.

DISCUSSION

Insulin mediated glucose oxidation is often used as a measure of insulin's effects on glucose transport (1-7). However, the current studies clearly demonstrate that insulin can promote glucose oxidation by more than one mechanism. Insulin stimulates the glucose transport system, and in this way the rate of glucose oxidation is enhanced. Furthermore, insulin can still increase C-1 glucose oxidation under conditions where its ability to stimulate the glucose transport system is blocked. This indicates that insulin can augment one or more steps of intracellular glucose metabolism involved in the conversion of the number one carbon atom of glucose to CO_2. In contrast, the ability of insulin to enhance C-6 oxidation is primarily due to insulin's effects to increase glucose transport. Identical results are obtained when spermine, cysteine, or diamide are used. In contrast, hyperosmolarity enhances glucose oxidation solely by increasing the rate of glucose transport. Thus, the effects of 0.3M sucrose to increase 2-deoxy glucose uptake and {^{14}C}-1-glucose oxidation were comparable, and this agent could not promote oxidation when its ability to stimulate transport was blocked.

In conclusion, these studies show that insulin can promote glucose oxidation independent of its ability to stimulate glucose transport. Consequently, conclusions concerning the glucose transport system based on measurements of glucose oxidation must be cautiously interpreted. It will be of interest to more precisely localize insulin's intracellular effect(s) on oxidation, and to see if other agents which are known to increase glucose oxidation do so solely by increasing glucose transport (22).

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