ACTIONS OF POLYAMINES ON LIPID AND GLUCOSE METABOLISM OF FAT CELLS

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SUMMARY: Incubation of isolated rat fat cells with insulin, spermidine or spermine stimulated conversion of glucose to carbon dioxide and inhibited lipolysis. The effects of the polyamines were observed in a concentration range of 1.0 to 50 μM. At the higher concentrations of spermidine and spermine, enhanced glucose oxidation and suppressed lipolysis were essentially identical to the maximal effects produced by insulin. Putrescine possessed no insulin-like properties. The rate of glucose utilization was not increased by insulin or spermidine when the glucose concentration was greater than 30 mM. Polyamine-stimulated glucose oxidation was inhibited by phloretin and 3-0-methyl-D-glucose. Spermidine suppressed lipolysis induced by epinephrine or theophylline but the inhibitory effect was absent when lipolysis was stimulated by dibutyryl cyclic AMP. These observations suggest that in fat cells polyamines, like insulin, inhibit lipolysis by suppressing cyclic AMP levels and facilitate glucose transport.

INTRODUCTION: Polyamines, most notably putrescine, spermidine and spermine, influence many cellular processes including those related to cell membranes. Bacterial spheroblasts are protected against lysis in the presence of 1.0 mM spermidine and spermine but not putrescine.[1] Polyamines promote the aggregation of bacteria and spheroblasts while steroidal amines and N-acyltriamines affect the permeability properties of E. coli by causing the membranes to become leaky.[2,3]

Recent studies indicate that insulin affects both transport and some intracellular events by interacting with structures at the surface of the cell membrane.[4-6] At the present time the nature of these interactions and the mode of translation to intracellular processes are not known.

In the present study, we have compared the influence of polyamines and insulin on glucose oxidation and lipolysis in isolated rat adipose

cells. Insulin is known to stimulate glucose oxidation and inhibit lipolysis in this tissue.[7]

MATERIALS AND METHODS: Isolated fat cells were prepared from rat epididymal fat pads by incubation with crude collagenase (1mg/ml) for 1 hour.[7] All procedures were performed at 37° in a modified Krebs-Ringer bicarbonate buffer, pH 7.4, containing 4% dialyzed bovine serum albumin. Glucose oxidation was measured by the conversion of D-glucose-U-^{14}C to $^{14}CO_2$. The effects of insulin and polyamines on the response of fat cells to various lipolytic agents were determined by measuring the release of glycerol into the glucose-free incubation medium.[8]

RESULTS: Figure 1 shows the rate of glucose oxidation when fat cells were incubated in the presence of insulin (1500μU/ml) or spermidine (10^{-4}M). The addition of spermidine or insulin enhanced the rate of glucose oxidation: the effect ranged between 200% and 350% in more than twenty experiments. The effects were linear for at least two hours. A stimulatory effect could usually be detected as early as fifteen min. following the

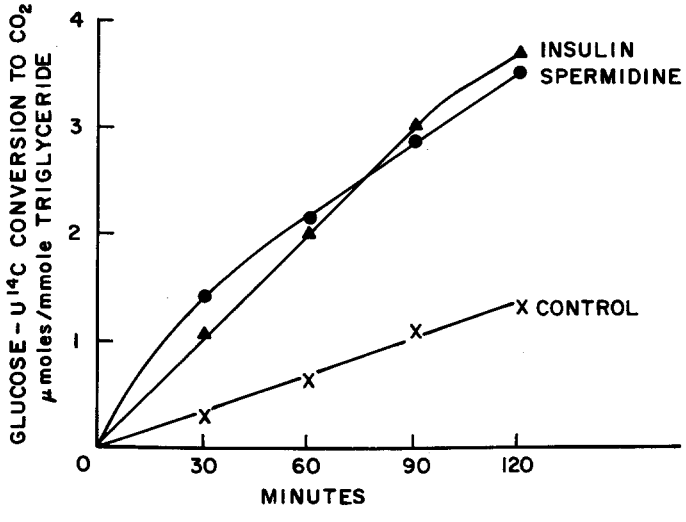

Fig.1. Time course of oxidation of glucose-U-^{14}C by isolated fat cells. Aliquots from a single pool of isolated fat cells were incubated at 37°. The values are the means of four experiments. Each incubation vessel contained 7-14 μmoles of triglyceride, 1.6 x 10^6 dpm as glucose-^{14}C, 0.2mM glucose in a total volume of 2.0 ml. Insulin concentration was 1500μU/ml; spermidine concentration was 10^{-4}M.

addition of the polyamine. Spermidine also increased the conversion of glucose into total lipids in a manner similar to insulin.

The dose-response relationship for the effects of various polyamines on glucose oxidation is shown in Fig. 2. Stimulatory effects of spermidine [$H_2N(CH_2)_4NH(CH_2)_3NH_2$] and spermine [$H_2N(CH_2)_3NH(CH_2)_4NH(CH_2)_3NH_2$] were observed at 1.0 μM and were maximal at 50 μM. At the higher concentrations, enhanced glucose oxidation was essentially identical to the maximal effect produced by insulin. Putrescine [$H_2N(CH_2)_4NH_2$] had no significant influence on glucose oxidation.

Previous studies by Crofford and Renold [9] have indicated that

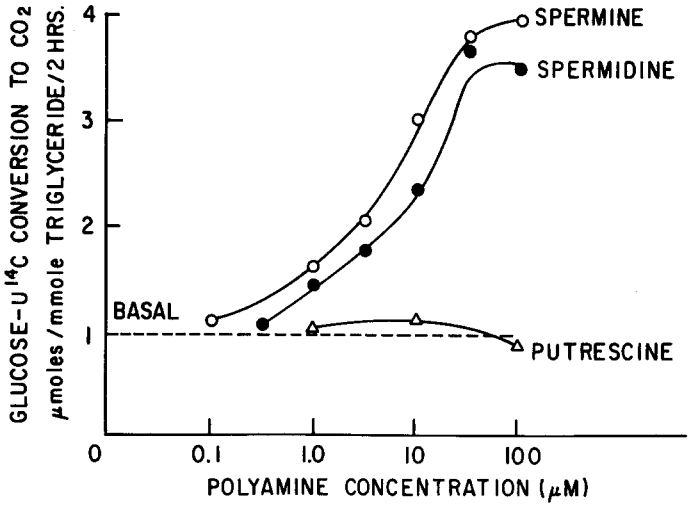

Fig.2. Dose-response relationships for the effects of polyamines on oxidation of glucose. Experimental conditions were as described in Materials and Methods and Fig.1. Fat cells were incubated for 2 hours with glucose-U-^{14}C and various concentrations of putrescine, spermidine or spermine, as indicated in the figure.

facilitation of glucose transport is the principal site of insulin action on glucose metabolism in adipose cells. Glucose travels across mammalian cell membranes by a process which has been termed mediated transport, facilitated diffusion or nonactive transport.[10] This concept of glucose transport is supported by several observations including saturation kinetics, stereospecificity and inhibition of sugar transport by certain compounds

such as phloretin and phlorizin.[11] A series of experiments was performed to determine if polyamines stimulated glucose oxidation by facilitation of glucose transport.

TABLE I

Effect of Glucose Concentration on Glucose Oxidation by Isolated Fat Cells

Glucose Concentration (mM)	Glucose-U^{14}C Conversion to CO_2 (μmoles/mmole Triglyceride)		
	Control	+Insulin	+Spermidine
0.2	0.74	2.44	2.26
1.0	3.64	12.88	11.83
5.0	12.42	22.64	20.74
10.0	19.68	31.12	29.19
30.0	28.02	33.64	32.11
60.0	31.08	32.45	34.56
80.0	30.88	30.96	31.49

Incubation was for 2 hours at 37°. Each incubation vessel contained 3.3×10^6 dpm as glucose-^{14}C. Insulin, when present, was at 1500μU/ml and spermidine was at 10^{-4}M. The results represent the means of three experiments.

The data in Table I indicate that maximal glucose oxidation is attained in control cells when the glucose concentration is greater than 30 mM. At glucose concentrations of 10 mM or less, insulin or spermidine stimulates glucose oxidation while at the higher concentrations these compounds have no effect on the rate of glucose metabolism.

Conversion of glucose to CO_2 was significantly inhibited by 0.1 mM phloretin in control cells and in cells incubated in the presence of insulin or spermidine (Fig. 3). The degree of inhibition was increased when the phloretin concentration was raised to 0.5 mM. These concentrations of phloretin are known to inhibit glucose transport in fat cells but do not affect glucose diffusion and intracellular metabolism.[12]

The effect of the competitive inhibitor, 3-O-methyl-D-glucose, on glucose oxidation was studied in the presence and absence of spermidine (Table II). Methylglucose has transport characteristics which are

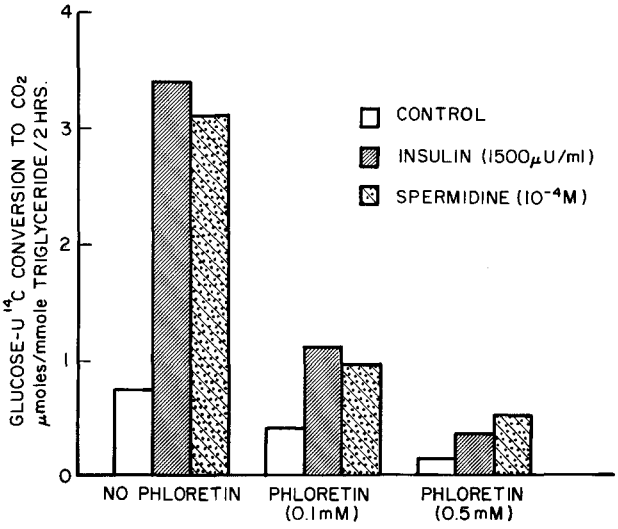

Fig.3. Inhibition of glucose oxidation by phloretin. Experimental conditions were as described in Materials and Methods and Fig.1. Phloretin was dissolved in absolute ethanol. The ethanol concentration of both the control and the phloretin vessels was 0.1 ml of ethanol per 100 ml of medium.

TABLE II

Effect of 3-0-methylglucose on Spermidine-Induced Glucose Oxidation

Concentration of 3-0-methylglucose	Glucose-U^{14}C Conversion to CO_2	
	-Spermidine	+Spermidine (10^{-4}M)
mM	μmoles/mmole Triglyceride	
0	1.08	3.96
1.0	.66	2.91
10.0	.41	1.59

Inhibition of glucose oxidation by 3-0-methyl-D-glucose. Experimental conditions were as described in Materials and Methods and Fig.1. Incubation time was 2 hours.

similar to those of D-glucose but this sugar is not utilized.[13] The oxidation of glucose (0.2mM) was significantly inhibited by 1.0 and 10.0 mM 3-0-methyl-D-glucose in control cells and in cells incubated in the presence of spermidine or insulin.

Fig. 4 shows the effects of insulin and spermidine on lipolysis. Both suppressed lipolysis induced by either epinephrine or theophylline. Spermine, but not putrescine, inhibited lipolysis in similar experiments. The dose-response relationship for the effects of polyamines was similar to that shown for glucose oxidation (Fig. 2). The lipolytic effects of epinephrine and theophylline are mediated by cyclic 3',5'-AMP while the antilipolytic action of insulin is due to suppression of endogenous cyclic AMP levels.[14] Lipolysis induced by dibutyryl cyclic AMP was not suppressed by insulin or spermidine.

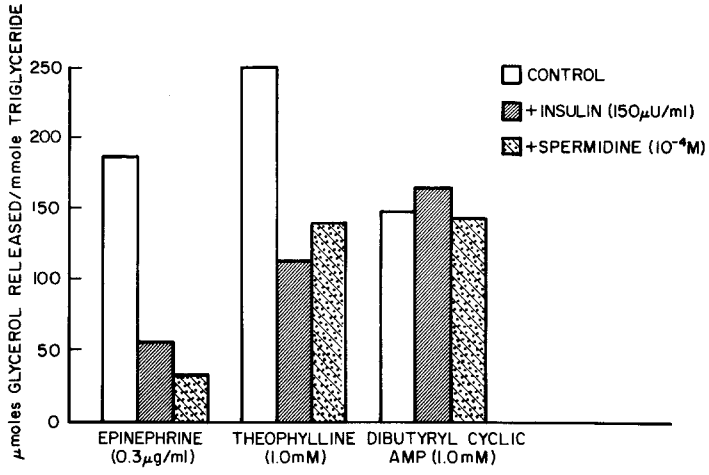

Fig.4. Effect of insulin and spermidine on lipolysis induced by epinephrine, theophylline or dibutyryl cyclic AMP. Aliquots from a single pool of isolated fat cells were incubated at 37° for 2 hours. The values are the means of three experiments. Each incubation vessel contained 10-16 μmoles of triglyceride in a total volume of 2.0 ml. Fat cells were incubated in the absence of glucose.

DISCUSSION: Spermidine and spermine enhance glucose oxidation and inhibit lipolysis in a manner similar to insulin. The mechanism of action of these polyamines is not known, but it does not seem to be a function of ionic strength since putrescine at equivalent ionic strengths was inactive.

Although no direct measurements of glucose transport were made, the data presented in Tables I and II and Fig. 3 are compatible with the

concept that spermidine acts to facilitate glucose transport. At low glucose concentrations, spermidine and insulin enhanced glucose utilization. At high glucose concentrations (maximal glucose transport) spermidine and insulin were ineffective because glucose phosphorylation had become the rate-limiting step in glucose utilization (Table I).[15] The inhibitory effects of phloretin (Fig. 3) and 3-O-methyl-D-glucose (Table II) coupled with our additional demonstration that spermidine did not increase glucose oxidation by disrupted cells, further supports the concept that polyamines facilitate glucose transport.

Spermidine and spermine also mimicked the antilipolytic action of insulin. Spermidine suppressed lipolysis induced by either epinephrine or theophylline. However, the antilipolytic action of spermidine was absent when cells were incubated in the presence of dibutyryl cyclic AMP (Fig. 4). These results suggest that polyamines, like insulin, influence lipolysis by suppressing endogenous cyclic AMP levels.

Previous studies have indicated that polyamines can significantly influence biological membranes. There is evidence that polyamines can stabilize enzymes associated with smooth membranes of the endoplasmic reticulum,[16] and can interact with the ribosomes of the rough membranous endoplasmic reticulum.[17] While it has been shown that polyamines stabilize bacterial cell walls and membranes,[1-3] this report appears to be the first indication that some polyamines can have marked effects on the functions of mammalian cell membranes.

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