Wortmannin Converts Insulin But Not Oxytocin From an Antilipolytic to a Lipolytic Agent in the Presence of Forskolin

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Insulin is an important regulator of glucose transport and lipolysis in adipocytes. The present studies compared the effects of insulin in rat adipocytes with the effects of oxytocin and peroxovanadate, which mimic some effects of insulin. The antilipolytic effects of peroxovanadate and oxytocin were unaffected by 500 nmol/L wortmannin, which blocked the antilipolytic action of insulin. However, wortmannin, which is a relatively specific inhibitor of phosphatidylinositol 3-kinase, did block most of the stimulation of glucose metabolism by peroxovanadate while having little effect on that due to oxytocin. Under appropriate conditions, it was also possible to demonstrate a lipolytic action of insulin, especially with low (0.1 to 1 nmol/L) concentrations of insulin after exposure of adipocytes to 50 nmol/L wortmannin. The data provide additional support for the hypothesis that oxytocin and peroxovanadate affect adipose tissue metabolism by mechanisms distinctly different from those involved in insulin action.

SEVERAL HORMONES, including catecholamines, glucagon, corticotropin, and thyrotropin, stimulate lipolysis, but insulin is the only circulating hormone that inhibits lipolysis in mammalian adipose tissue.[1] In addition to inhibiting lipolysis, insulin stimulates glucose uptake by activating and translocating a pool of insulin-sensitive glucose transporters from internal vesicles to the cell surface.[2] These transporters increase glucose uptake in insulin-sensitive tissues such as fat and muscle. The cascade that leads to inhibition of lipolysis and stimulation of glucose uptake is not clearly defined, but is dependent on activation of the insulin receptor kinase by insulin.[3] The activated receptor kinase, in turn, phosphorylates the principal insulin receptor substrates, IRS-1 and IRS-2.[4] The phosphorylated IRSs have been shown to interact with several *Src* homology (SH2) domain–containing proteins.[5,6] Phosphatidylinositol 3-kinase is one of several SH2 domain–containing proteins with activity stimulated by binding to IRS-1 preactivated by insulin.[7]

Another agent that mimics the action of insulin is peroxovanadate, which is generated by the action of H_2O_2 on vanadate.[8,9] Peroxovanadate but not vanadate exerts insulin-like effects in human adipocytes.[8] These effects include stimulation of glucose transport and inhibition of the lipolytic action of isoproterenol in adipocytes.[8-11] Peroxovanadate, like insulin, stimulates tyrosine phosphorylation in adipocytes.[11] However, the extent of tyrosine phosphorylation of proteins by peroxovanadate in adipocytes was much less than that seen with insulin, except for a protein with an apparent M_r of approximately 53,000.[11] Vanadate and peroxovanadate inhibited lipolysis due to isoproterenol in rat adipocytes, but the potency of peroxovanadate was 100-fold greater than that of vanadate.[9]

Oxytocin is another hormone that can stimulate glucose metabolism in adipocytes.[12-13] The effects of oxytocin are probably secondary to activation of phosphoinositide breakdown in adipocytes,[14] which is unaffected by insulin.[14-16] Phosphoinositide breakdown should result in an elevation of intracellular Ca^{2+} and activation of protein kinase C. The insulin-like effect of oxytocin on glucose oxidation has been shown to be dependent on added Ca^{2+}.[13]

Wortmannin inhibits phosphatidylinositol 3-kinase and blocks the antilipolytic effects and the stimulation of glucose transport by insulin.[17-19] The question of whether the insulin-like effects of peroxovanadate and oxytocin are also blocked by wortmannin was the initial focus of the present studies.

MATERIALS AND METHODS

Materials

Bovine serum albumin powder (Bovuminar, lot L59410; containing <0.05 mol fatty acids/mol albumin) was obtained from Intergen (Purchase, NY), bacterial collagenase (C1. *histolyticum* CLS1 238 U/mL, 4196-SN269) from Worthington Biochemical (Freehold, NJ), and CL 316,243 (*R,R*)5-[2-[[2(3-ethylchlorophenyl)-2-hydroxyethylamino]propyl]-1,3- benzodioxole-2,2 dicarboxylate from American Cyanamid (Pearl River, NY). Adenosine deaminase (type VIII from calf intestinal mucosa, 200 U/mg), (−)-N^6-(2-phenylisopropyl)-adenosine, also known as N^6[-*R*-(−)-1-methyl-2-phenethyl]-adenosine (PIA), 8-(4-chlorophenylthio)-adenosine 3′,5′-cyclic monophosphate (8-CPT cAMP), insulin, wortmannin, and other chemicals were from Sigma Chemical (St Louis, MO).

Peroxovanadate was generated by mixing H_2O_2 (1 mmol/L) with incubation buffer at pH 7.4 containing sodium orthovanadate (10 mmol/L). After 15 minutes at room temperature, catalase (final concentration, 0.2 mg/mL) was added and the solution was used after another 15 minutes at room temperature. Catalase-treated peroxovanadate was stable for at least 2 hours at room temperature. Peroxovanadate is used to denote the combination of vanadate plus H_2O_2 followed by catalase treatment to remove peroxide that was not coordinated to the vanadium atom. It is actually a mixture of aqueous peroxovanadium complexes, and the concentration added is based on that of vanadate. There was no effect of catalase on lipolysis or glucose oxidation.

Adipocyte Preparation

Adipocytes were prepared from the epididymal fat pads of three male Sprague-Dawley rats (200 to 300 g) fed ad libitum.[20,21] The fat pads (4 to 6 g) were cut into small pieces with scissors and incubated with collagenase (10 mg in 18 mL buffer divided between four 1-oz polypropylene bottles) for 60 minutes in an orbital shaking water bath (gyratory water bath shaker G76; New Brunswick Scientific). The buffer contained 122 mmol/L NaCl, 5 mmol/L KCl, 2.4 mmol/L $NaHCO_3$, 1.4

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mmol/L $MgSO_4$, 1.4 mmol/L $CaCl_2$, 1.4 mmol/L Na_2HPO_4, and 25 mmol/L HEPES, plus 4% albumin adjusted to pH 7.4 along with 200 nmol/L adenosine and 2 mmol/L glucose for digestion of the tissue with collagenase. The digest was filtered through nylon mesh with gentle pressure, and the adipocytes were washed three times by flotation using albumin-free buffer and then incubated for 10 minutes in four polypropylene bottles containing 7.5 mL buffer per bottle without albumin, glucose, or adenosine for exposure to wortmannin. The cells were then resuspended to a volume of approximately 16 mL in buffer containing 4% albumin plus 0.1 U/mL adenosine deaminase without added glucose or adenosine. The cell suspension in a volume of 0.2 mL was added to 0.8 mL of the same buffer containing the added agents in 17 × 100-mm polypropylene tubes. We generally incubate 35 to 75 mg packed cells (120,000 to 240,000 adipocytes)/mL medium. The cells were shaken in the orbital shaker bath at 37°C for 20 minutes (100 cycles/min).

Assay of Lipolysis and cAMP

At the end of the incubation, 0.1 mL 1N HCl is added to each tube, and then the tubes are heated in a boiling water bath for 1 minute and cooled, and the extracts are neutralized by addition of 0.1 mL 1N NaOH. A 50-μL aliquot of the medium is removed for analysis of glycerol using the one-step enzymatic fluorimetric procedure for determination of glycerol as described by Boobis and Maughan.[22] cAMP level is measured in aliquots of this extract using the radioligand-binding procedure of Brown et al.[23]

Assay of Glucose Oxidation

For studies on glucose oxidation, we incubated adipocytes in the presence of a trace amount (0.2 μmol/L) of D-[1-^{14}C]glucose and measured the release of [^{14}C]CO_2 by collecting the carbon dioxide in the hanging wells containing NaOH via filter papers suspended from serum stoppers that sealed the incubation tubes. Glucose transport is rate-limiting for glucose oxidation at low concentrations of glucose, and this assay has the added advantage of detecting the effects of inhibitory agents on glucose oxidation. Glucose oxidation is expressed as percent conversion of the added glucose 1-^{14}C to carbon dioxide, and approximately 500,000 dpm were present in each tube.

The values in each experiment were based on duplicate samples, and the experiments were generally replicated at least three times. Statistical comparisons were made using Student's *t* test on paired differences.

RESULTS

Metabolic Effects of Insulin Are Reversed by Wortmannin

Recently, it was reported that the antilipolytic action of insulin was inhibited by wortmannin.[17] We were unable to reproduce these findings in the presence of 4% albumin, which is required to bind fatty acids released during lipolysis. However, if adipocytes were incubated with wortmannin for 10 minutes in buffer without albumin and then resuspended in medium containing 4% albumin in the absence of wortmannin, the effects of insulin during a subsequent 20-minute incubation were abolished in a dose-dependent manner (Fig 1). Most of the increase in glucose oxidation due to peroxovanadate was also

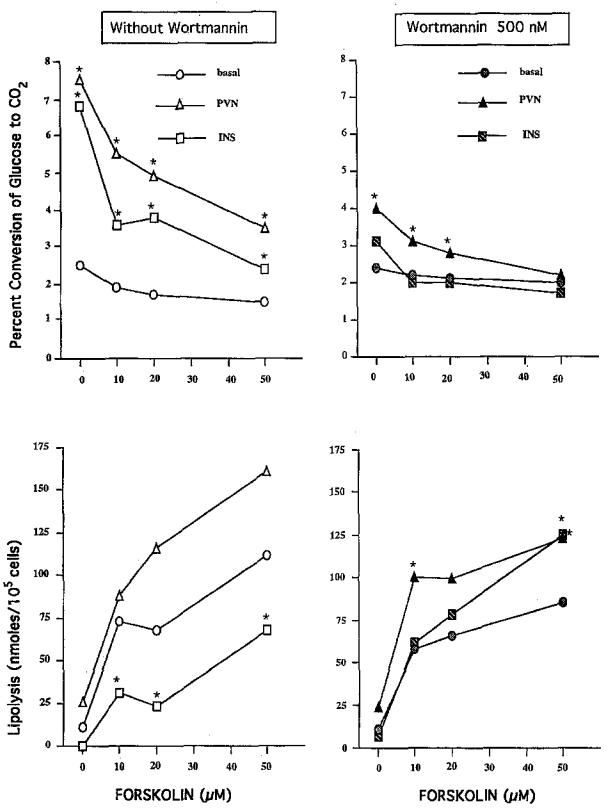

Fig 1. Effect of peroxovanadate and insulin on forskolin stimulation of lipolysis and inhibition of glucose-1-^{14}C oxidation in either the presence or absence of wortmannin. Rat adipocytes were incubated in albumin-free buffer for 10 minutes either without or with 500 nmol/L wortmannin. The buffer was removed, the cells were resuspended in 4% albumin, and then 175,000 cells/mL were incubated for 20 minutes in the presence of 10, 20, or 50 μmol/L forskolin either without, or with 10 nmol/L insulin or 0.1 mmol/L peroxovanadate. Data are the mean of 4 paired experiments. *Statistically significant effects ($P < .05$) of insulin or peroxovanadate based on paired comparisons.

abolished by prior exposure to 500 nmol/L wortmannin, but a small residual stimulation of glucose oxidation due to peroxovanadate was seen after wortmannin (Fig 1).

Peroxovanadate did not mimic the ability of insulin to inhibit lipolysis in the presence of 10 to 50 μmol/L forskolin, a nonspecific activator of all adenylyl cyclase isoforms.[24] In fact, there was a lipolytic effect of peroxovanadate that was reproducibly seen after wortmannin treatment. There was also a significant lipolytic effect of 10 nmol/L insulin in the presence of 50 μmol/L forskolin after exposure of adipocytes to 500 nmol/L wortmannin (Fig 1).

The lipolytic effect of insulin was best seen in adipocytes exposed to only 50 nmol/L wortmannin and then incubated with 0.1 or 1.0 nmol/L insulin (Table 1). Whereas 50 nmol/L wortmannin reversed the antilipolytic action of 1 nmol/L insulin to a lipolytic action, it only reduced the effect of 10 nmol/L insulin by 30% (Table 1).

There was an antilipolytic effect of peroxovanadate against lipolysis due to 100 nmol/L isoproterenol that was unaffected by prior exposure to wortmannin (Table 2). However, under the same conditions, the antilipolytic action of insulin was abol-

Table 1. Lipolytic Effect of Insulin on Lipolysis Stimulated by 50 μmol/L Forskolin in the Presence of Wortmannin

Insulin Concentration (nmol/L)	% Change Due to Insulin	
	Without Wortmannin	With Wortmannin
0.1	+55 ± 27	+60 ± 7*
1.0	−53 ± 13*	+56 ± 14*
10	−52 ± 12*	−36 ± 6*

NOTE. Insulin 0.1 nmol/L = 16 μU/mL. Rat adipocytes were incubated in albumin-free buffer for 10 minutes without or with 50 nmol/L wortmannin. The medium was removed and cells were resuspended in 4% albumin (125,000/mL) and then incubated for 20 minutes in the presence of 50 μmol/L forskolin plus 100 nmol/L PIA. The increase in lipolysis due to forskolin was 100 ± 25 nmol glycerol/10^5 cells in the absence of wortmannin and 85 ± 22 nmol/10^5 cells in the presence of wortmannin. Values are the mean ± SEM of 7 paired replications.

*Significant effects of insulin ($P < .01$).

ished by wortmannin (Table 2). Peroxovanadate inhibition of lipolysis in the presence of 100 nmol/L isoproterenol, which stimulates β_1-catecholamine receptors,[25] involves a mechanism specific to β_1-catecholamine receptors, since peroxovanadate did not inhibit lipolysis due to β_3-catecholamine receptor activation in adipocytes.[26] β_1-adrenergic receptors are desensitized following phosphorylation on serine residues in the carboxy terminus, whereas β_3-adrenergic receptors, which do not contain serines in a favorable context for phosphorylation, do not desensitize by this mechanism.[26] In this regard, peroxovanadate-mediated desensitization of β_1-adrenergic receptors is a consequence of enhanced phosphorylation by peroxovanadate.[26]

Comparison of Oxytocin With Insulin Effects on Glucose Oxidation and Lipolysis in Adipocytes

The data in Table 3 indicate that oxytocin enhanced glucose 1-^{14}C oxidation in adipocytes, but the effect was only 30% of that due to insulin. After wortmannin exposure, the insulin effect was reduced by 85%, whereas the stimulation of glucose oxidation by oxytocin was only reduced by 25%. However, in the presence of isoproterenol, there was no stimulation of glucose oxidation by insulin or oxytocin after prior exposure to wortmannin, whereas the antilipolytic action of oxytocin was now significant (Table 3).

Table 2. Wortmannin Abolishment of the Antilipolytic Action of Insulin But Not That of Peroxovanadate

Additions	Control Values (nmol)	+ Insulin 10 nmol/L	+ Peroxovanadate 0.1 mmol/L
Without wortmannin	25 ± 10	45%*	75%
+Isoproterenol (100 nmol/L)	222 ± 20	60%*	31%*
+Wortmannin	15 ± 6	100%	139%
+Wortmannin and isoproterenol (100 nmol/L)	255 ± 45	91%	38%*

NOTE. Rat adipocytes were incubated in albumin-free buffer for 10 minutes without or with 500 nmol/L wortmannin. The medium was removed, the cells were resuspended in 4% albumin, and 170,000 adipocytes/mL were incubated for 20 minutes in the presence of the indicated additions. Values are the mean ± SEM of 8 paired replications, and the effects of insulin or peroxovanadate are shown as % of control values.

*Significant effects ($P < .05$) based on paired comparisons.

Table 3. Wortmannin Does Not Block the Antilipolytic Action of Oxytocin

Additions	Basal	Δ Due to Insulin 10 nmol/L	Δ Due to EGF 100 nmol/L	Δ Due to Oxytocin 1 μmol/L
Lipolysis (nmol/10^5 cells)				
Without wortmannin	10	−10 ± 7	−3 ± 3	−10 ± 6
+Isoproterenol 10 nmol/L	127	−68 ± 24*	−18 ± 17	−44 ± 25
+Wortmannin	12	−4 ± 2	−0 ± 40	−3 ± 3
+Wortmannin and isoproterenol 10 nmol/L	132	−17 ± 18	−12 ± 10	−55 ± 18*
Glucose-1-^{14}C oxidation to CO_2 (% conversion)				
Without wortmannin	2.0	+2.7 ± 0.6*	+0.5 ± 0.2	+0.8 ± 0.2*
+Isoproterenol 10 nmol/L	2.2	+2.5 ± 0.5*	+0.3 ± 0.2	+0.6 ± 0.2*
+Wortmannin	1.4	+0.4 ± 0.1*	+0.1 ± 0.1	+0.6 ± 0.2*
+Wortmannin and isoproterenol 10 nmol/L	1.4	+0.1 ± 0.1	+0.1 ± 0.1	+0.1 ± 0.1

NOTE. Rat adipocytes were incubated in albumin-free buffer for 10 minutes without or with 500 nmol/L wortmannin. The medium was removed, and the cells were resuspended in 4% albumin (170,000/mL) and then incubated for 20 minutes in the presence of the indicated additions. Values are for 5 paired replications, and the effects of insulin, EGF, or oxytocin are shown as the mean ± SEM of the paired differences.

*Significant effects of insulin, EGF, or oxytocin ($P < .05$).

Basal lipolysis and lipolysis due to isoproterenol were unaffected by wortmannin (Tables 2 and 3). The antilipolytic action of insulin was inhibited by wortmannin exposure, whereas that of oxytocin was enhanced by wortmannin (Table 3). These data indicate that the inhibition of lipolysis in adipocytes induced by oxytocin is relatively insensitive to wortmannin, and suggest that oxytocin acts by mechanisms distinctly different from those involved in the antilipolytic action of insulin. Another difference between insulin and oxytocin was that 2′5′-dideoxyadenosine enhanced the antilipolytic action of insulin in the experiments shown in Table 3 by 65%, confirming the report of Gokmen-Polar et al,[27] while having no effect on that due to oxytocin (data not shown).

Epidermal growth factor (EGF) has been shown to be a more effective stimulator of mitogen-activated protein kinases (ERK-1 and ERK-2) and ribosomal S6 kinases (Rsk-2 and $p70^{S6k}$) than is insulin in rat adipocytes.[28] EGF (100 nmol/L) does not stimulate 2-deoxyglucose uptake or glycogen synthesis in adipocytes.[28] The data in Table 3 confirm that 100 nmol/L EGF has only a small (statistically insignificant) stimulatory effect on glucose oxidation and lipolysis.

To see the reproducible antilipolytic effects of oxytocin in the absence of wortmannin, it was necessary to add oxytocin to adipocytes 60 minutes before addition of lipolytic agents (Table

Table 4. Influence of Preincubating Insulin and Oxytocin for 60 Minutes on Their Antilipolytic Effects

Additions	Basal	+Insulin 10 nmol/L	+Oxytocin 1 μmol/L
Lipolysis (glycerol release, nmol/10^5 cells)			
Without any additions	47 ± 6	14 ± 5‡	25 ± 4†
Δ due to lipolytic agents			
+Isoproterenol 10 nmol/L	+25 ± 6	+45 ± 6†	+32 ± 6
+CI 316,243 1 nmol/L	+22 ± 6	+22 ± 3	+24 ± 5
+Forskolin 50 μmol/L	+19 ± 3	+48 ± 10†	+8 ± 4*
+8 CPT cAMP 0.5 mmol/L	+18 ± 4	+18 ± 3	+24 ± 2

NOTE. Rat adipocytes were incubated for 60 minutes in 3 mL medium containing 4% albumin alone or plus 10 nmol/L insulin or 1 μmol/L oxytocin. At the end of this incubation period, 0.2 mL of the medium containing a mean of 230,000 adipocytes was added to tubes containing 0.8 mL buffer (4% albumin, 0.1 U/mL adenosine deaminase, and 50 μmol/L ascorbic acid) and incubated for 20 minutes with the indicated additions. Values are the mean ± SEM of 6 experiments, and increments in lipolysis due to lipolytic agents are shown as the mean ± SEM of the paired differences.

Significant effects of insulin or oxytocin versus basal are indicated: *$P < .05$, †$P < .025$, and ‡$P < .005$.

4). In these experiments, 10 nmol/L isoproterenol was used to selectively activate β_1-adrenergic receptors,[25] and 1 nmol/L CL 316,243 was used as a selective agonist of β_3-adrenergic receptors.[29] We used forskolin to bypass receptors and directly activate adenylyl cyclase, and a cell-permeable analog of cAMP to directly activate lipolysis. The 60-minute preincubation resulted in a high rate of basal lipolysis that oxytocin inhibited by 47% (Table 4). There was a 58% inhibition of the increase in lipolysis due to forskolin in the presence of oxytocin, but there were no inhibitory effects on lipolysis due to activation of β_1- or β_3-adrenergic receptors or on lipolysis due to cAMP.

Insulin inhibited basal lipolysis to a greater extent (70%) than oxytocin after a 60-minute preincubation. In contrast to what was seen with oxytocin, lipolysis in the presence of forskolin was stimulated if insulin was added 60 minutes before forskolin (Table 4). Lipolysis due to selective β_1-adrenergic receptor stimulation by 10 nmol/L isoproterenol was also increased 80% by insulin, in contrast to the nonsignificant 28% increase seen with oxytocin.

DISCUSSION

Although insulin is usually thought of as an antilipolytic agent, the present results demonstrate that insulin can also enhance lipolysis due to forskolin after inactivation of phosphatidylinositol 3-kinase by wortmannin (Fig 1 and Table 1). A lipolytic action of 6 nmol/L insulin in the presence of epinephrine was seen in adipocytes isolated from epididymal adipose tissue of hypophysectomized rats.[30] The effect of hypophysectomy was similar to that of brief exposure of adipocytes from normal rats to wortmannin, in that insulin did not increase glucose oxidation in adipocytes from hypophysectomized rats.[30] The effect of hypophysectomy was seen within 8 days, but was unique to isolated adipocytes—it was not seen in incubated adipose tissue fragments.[30] Our data suggest that collagenase digestion of epididymal adipose tissue from hypophysectomized rats results in effects similar to those seen after a 10-minute exposure of isolated adipocytes from normal rats to wortmannin, whose primary target enzyme is phosphatidylinositol 3-kinase.[17-18] It is established that most of the effects of insulin on adipocyte metabolism involve wortmannin-sensitive pathways.[17-19,31] However, the activation of pyruvate dehydrogenase by insulin is insensitive to wortmannin.[31]

The mechanism for the wortmannin-insensitive inactivation of catecholamine-stimulated lipolysis by peroxovanadate probably involves enhanced serine phosphorylation of the β_1-adrenergic receptor in adipocytes, which has been demonstrated by Bahouth et al.[26] Further evidence for inhibition of a phosphoserine phosphatase by peroxovanadate was the failure of this compound to inhibit the stimulation of lipolysis by forskolin or β_3-catecholamine agonists.[26] The β_3-catecholamine receptor has no sites for phosphoserine phosphorylation on the carboxy tail, where peroxovanadate increased phosphorylation of the β_1-catecholamine receptor.[25]

Oxytocin is a potent stimulator of phosphoinositide breakdown in adipocytes, which is a process unaffected by insulin.[14-16] Protein kinase C activation by phorbol esters increases the recruitment of glucose transporters to the adipocyte plasma membrane, but this does not result in much of an increase in glucose uptake and metabolism and has no effect on lipolysis.[32] The diacylglycerol derived from phosphoinositide breakdown due to oxytocin could account for the small stimulation (27% that of insulin) of glucose oxidation by oxytocin, which is similar to that seen with phorbol esters.[32] However, it is equally plausible that the elevation of Ca^{2+} by oxytocin secondary to inositol trisphosphate release during phosphoinositide breakdown could account for the increase in glucose oxidation, since the increase in glucose oxidation due to oxytocin in adipocytes is Ca^{2+}-dependent.[13] In contrast, insulin stimulation of glucose oxidation is largely Ca^{2+}-insensitive.[13] The present results clearly support the hypothesis that while Ca^{2+}, diacylglycerol, or both may increase glucose oxidation to a limited extent, little if any insulin stimulation of glucose metabolism involves these second messengers.

It is unclear what is the mechanism for the antilipolytic action of oxytocin, except that it is wortmannin-insensitive and requires a prolonged incubation. The negative effects of EGF on adipocyte lipolysis suggest a lack of involvement of mitogen-activated protein kinases in oxytocin action. Lin and Lawrence[28] found that EGF (100 nmol/L) was more potent than insulin (20 nmol/L) in activating kinases that phosphorylate ribosomal protein S6, S6 peptides, and myelin basic protein, but EGF did not increase glucose transport or incorporation into glycogen in rat adipocytes.

In conclusion, our results provide new insights into the regulation of lipolysis in isolated rat adipocytes. We found a lipolytic action of insulin in the presence of wortmannin, as well as antilipolytic effects of oxytocin and peroxovanadate that are wortmannin-insensitive.

REFERENCES

1. Fain JN, Rosenberg L: Antilipolytic action of insulin on fat cells. Diabetes 21:414-425, 1972 (suppl 2)
2. Birnbaum MJ: The insulin-sensitive glucose transporter. Int Rev Cytol 137:239-297, 1992
3. Taylor SI, Cama A, Accili D, et al: Mutations in the insulin receptor gene. Endocr Rev 13:566-595, 1992
4. White MF, Kahn CR: The insulin signaling system. J Biol Chem 269:1-4, 1994
5. Baltensperger K, Kozma LM, Cherniack AD, et al: Binding of the *ras* activator son of sevenless to insulin receptor substrate-1 signaling complexes. Science 260:1950-1952, 1993
6. Skolnik EY, Batzer A, Li N, et al: The function of GRB2 in linking the insulin receptor to *ras* signaling pathways. Science 260:1953-1955, 1993
7. Kelly KL, Ruderman NB, Chen KS: Phosphatidylinositol-3-kinase in isolated rat adipocytes. J Biol Chem 267:3423-3428, 1992
8. Lonnroth P, Eriksson JW, Posner BI, et al: Peroxovanadate but not vanadate exerts insulin-like effects in human adipocytes. Diabetologia 36:113-116, 1993
9. Fantus G, Kadota S, Deragon G, et al: Pervanadate [peroxide(s) of vanadate] mimics insulin action in rat adipocytes via activation of the insulin receptor tyrosine kinase. Biochemistry 28:8864-8871, 1989
10. Dubyak GR, Kleinzeller A: The insulin-mimetic effects of vanadate in isolated rat adipocytes. J Biol Chem 255:5306-5312, 1980
11. Mooney RA, Bordwell KL, Luhowskyj S, et al: The insulin-like effect of vanadate on lipolysis in rat adipocytes is not accompanied by an insulin-like effect on tyrosine phosphorylation. Endocrinology 124:422-429, 1989
12. Muchmore DB, Little SA, Haen C: A dual mechanism of action of oxytocin in rat epididymal rat cells. J Biol Chem 256:365-372, 1981
13. Bonne D, Belhadj O, Cohen P: Modulation by calcium of the insulin action and the insulin-like effect of oxytocin on isolated rat lipocytes. Eur J Biochem 75:101-105, 1977
14. Lee H, Fain JN: Regulation of oxytocin-induced phosphoinositide breakdown in adipocytes by adenosine, isoproterenol and insulin. Biochim Biophys Acta 1013:73-79, 1989
15. Pennington SR, Martin BR: Insulin-stimulated phosphoinositide metabolism in isolated fat cells. J Biol Chem 260:11039-11045, 1985
16. Augert G, Exton JH: Insulin and oxytocin effects on phosphoinositide metabolism in adipocytes. J Biol Chem 263:3600-3609, 1988
17. Okada T, Kawano Y, Sakakibara T, et al: Essential role of phosphatidylinositol 3-kinase in insulin-induced glucose transport and antilipolysis in rat adipocytes. J Biol Chem 269:3568-3573, 1993
18. Lam K, Carpenter CL, Ruderman NB, et al: The phosphatidylinositol 3-kinase serine kinase phosphorylates IRS-1. J Biol Chem 269:20648-20652, 1994
19. Rahn T, Ridderstrale M, Tornqvist H, et al: Essential role of phosphatidylinositol 3-kinase in insulin-induced activation and phosphorylation of the cGMP-inhibited cAMP phosphodiesterase in rat adipocytes. FEBS Lett 350:314-318, 1994
20. Honnor CR, Dhillon GS, Londos C: cAMP-dependent protein kinase and lipolysis in rat adipocytes. J Biol Chem 260:15122-15129, 1985
21. Kuroda M, Honnor RC, Cushman SW, et al: Regulation of insulin-stimulated glucose transport in the isolated rat adipocyte. J Biol Chem 262:245-253, 1987
22. Boobis LH, Maughan RJ: A simple one-step enzymatic fluorometric method for the determination of glycerol in 20 μl of plasma. Clin Chim Acta 132:173-179, 1983
23. Brown BL, Albano JDM, Ekins RP, et al: A simple and sensitive saturation assay method for the measurement of adenosine 3′:5′-cyclic monophosphate. Biochem J 121:561-562, 1971
24. Taussig R, Gilman AG: Mammalian membrane-bound adenylyl cyclases. J Biol Chem 270:1-4, 1995
25. Granneman JG: Effects of agonist exposure on the coupling of β_1- and β_3-adrenergic receptors to adenylyl cyclase in isolated adipocytes. J Pharmacol Exp Ther 261:638-642, 1992
26. Bahouth SW, Gokmen-Polar Y, Coronel EC, et al: Enhanced desensitization and phosphorylation of the β_1-adrenergic receptor in rat adipocytes by peroxovanadate. Mol Pharmacol 49:1049-1057, 1996
27. Gokmen-Polar Y, Coronel EC, Bahouth SW, et al: Insulin sensitizes β-agonist and forskolin-stimulated lipolysis to inhibition by 2′,5′-dideoxyadenosine. Am J Physiol 270:C562-C569, 1996
28. Lin TA, Lawrence JC Jr: Activation of ribosomal protein S6 kinases does not increase glycogen synthesis or glucose transport in rat adipocytes. J Biol Chem 269:21255-21261, 1994
29. Bloom JD, Claus TH: CL316,243—Antidiabetic, antiobesity, β_3-adrenergic agonist. Drugs Future 19:23-26, 1994
30. Grichting G, Goodman HM: Lipolytic effects of insulin in adipocytes isolated from hypophysectomized rats. Endocrinology 109: 2054-2060, 1981
31. Moule SK, Edgell NJ, Welsh GI, et al: Multiple signalling pathways involved in the stimulation of fatty acid and glycogen synthesis by insulin in rat epididymal fat cells. Biochem J 31:595-601, 1995
32. Muhlbacher C, Karnieli E, Schaff P, et al: Phorbol esters imitate in rat fat-cells the full effect of insulin on glucose-carrier translocation, but not on 3-*O*-methylglucose-transport activity. Biochem J 249:865-870, 1988