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Oxytocin Action

Mechanisms for Insulin-Like Activity in Isolated Rat Adipocytes

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SUMMARY

We have examined in detail in isolated rat adipocytes the effects of oxytocin on glucose transport, glucose oxidation, and lipogenesis and we have compared these effects with the actions of insulin. For oxytocin and its analogues, dose-response curves for the stimulation of glucose oxidation reveal an order of potency: oxytocin > mesotocin > isotocin > vasotocin > oxypressin ≃ arginine vasopressin. Some of the oxytocin analogues are partial agonists (mesotocin, isotocin, arginine vasopressin); tocinamide and pressinamide are inactive. This order of potency closely parallels the order observed for ocytocin receptors characterized in other tissues and is clearly distinct from the order of potency expected for a vasopressin receptor. The ED_{50} for oxytocin-mediated stimulation of both glucose oxidation and lipogenesis is about 3 nM. Unlike insulin, oxytocin, at concentrations that maximally stimulate glucose oxidation, is unable to stimulate glucose transport. In the presence of a maximally effective concentration of oxytocin (100 nM), the dose-response curve for insulin-stimulated glucose oxidation and lipogenesis is shifted to the right, whereas the effect of insulin on the transport of 3-*O*-methyl-D-glucose is unaffected. Thus, oxytocin appears to confer a state of insulin resistance on the adipocyte, probably acting at a post-receptor site. We conclude that in the adipocyte, oxytocin acts via the same receptor as is present in uterine and breast smooth muscle and that the metabolic actions of oxytocin may be due to mechanisms in common (chemical mediators, phosphorylation-dephosphorylation reactions) with the ones involved in the action of insulin.

INTRODUCTION

Oxytocin is known to mimic many of the effects of insulin in isolated adipocytes. Oxytocin stimulates glucose oxidation, lipogenesis, and glycogen synthesis, whereas it inhibits catecholamine-stimulated lipolysis (1, 2). Oxytocin, like insulin, also stimulates the incorporation of amino acids into protein (3).

The limited data so far obtained indicate that the insulin-like activity of oxytocin is due to oxytocin binding to its own receptor and not to the insulin receptor: displacement of adipocyte-bound [^{3}H]oxytocin is achieved with oxytocin but not with insulin (4–6); [^{125}I] insulin cannot be displaced from fat cells with oxytocin (7); *N*-carbamoyl-*O*-methyl oxytocin, a specific oxytocin antagonist, inhibits oxytocin-stimulated lipogenesis without affecting the insulin response (1), and adipocytes from homozygous diabetes insipidus rats (Brattleboro strain) demonstrate normal insulin-stimulated responses while oxytocin is unable to stimulate glucose oxidation or lipogenesis (6, 8). In this study, we have used oxytocin and a number of oxytocin analogues in adipocyte glucose oxidation assays, to determine the similarity between the uterine receptor and the adipocyte receptor.

Some reports have suggested that, like insulin, oxytocin may be able to stimulate glucose transport in adipocytes (2, 9). However, other data suggest that glucose transport is unaffected by oxytocin (10). We report here that in the concentration range where oxytocin stimulates glucose oxidation and lipogenesis, glucose transport is not stimulated.

Heretofore, the enzymes involved in the mediation of the insulin-like effects of oxytocin have not been studied. Pyruvate dehydrogenase is one mitochondrial enzyme which represents a key step in metabolism of glucose via the tricarboxylic acid cycle or lipogenesis and is stimulated by insulin (11). We have found that oxytocin stimulates pyruvate dehydrogenase.[2] We have also found that oxytocin is able to shift insulin's dose-response curves to the right. These results led us to a new interpretation of the mechanism of action of oxytocin in adipocytes.

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[1] Career Investigator of the Medical Research Council of Canada.

[2] Mukherjee and Mukherjee have recently reported the ability of oxytocin to stimulate pyruvate dehydrogenase in adipose tissue (11a).

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MATERIALS AND METHODS

The following materials were purchased from New England Nuclear Corporation (Boston, Mass.): [U-^{14}C] glucose, 13.9 mCi/mmole; [1-^{14}C]glucose, 13.9 mCi/mmole; 3-*O*-[*methyl*-^{3}H]D-glucose, 80 Ci/mmole; [^{3}H] methoxy inulin, 0.25 mCi/0.7 mg; and sodium [1-^{14}C] pyruvate, 8 mCi/mmole. BSA[3] (Fraction V, Lot T13904) was purchased from Armour Pharmaceutical Company (Tarrytown, N. Y.), and collagenase (4196 CLS 48 M201) from Worthington Biochemical Corporation (Freehold, N. J.). CoA from yeast (Grade 111-S) and cocarboxylase were purchased from Sigma Chemical Company (St. Louis, Mo.), dithiothreitol from Aldrich Chemical Company (Milwaukee, Wisc.). Synthetic oxytocin was generously supplied by Dr. G. Moore, of the University of Calgary. The synthetic oxytocin was considered pure on the basis of amino acid analysis, thin-layer chromatography in several solvent systems, and high-pressure liquid chromatography. It was tested for uterine-contracting activity, and its molar concentration is based on 1 mg of oxytocin having 500 units of contractile activity (12). Mesotocin, isotocin, vasotocin, oxypressin, arginine vasopressin, tocinamide, and pressinamide were kindly provided by Dr. M. Manning (Toledo College of Medicine, Toledo, Ohio) and Dr. W. H. Sawyer (Columbia University, New York, N. Y.). Purity is based on thin-layer chromatography, paper electrophoresis at different pH values, and microchemical and amino acid analyses (13).

Buffers

Krebs-Ringer bicarbonate buffer had the following composition; NaCl, 118 mM; KCl, 5 mM; $CaCl_2$, 1.3 mM; $MgSO_4$, 1.2 mM; KH_2PO_4, 1.2 mM; and $NaHCO_3$, 25 mM; 2% (w/v) BSA was added. The buffer was gassed for 10 min with 95% O_2:5% CO_2, and was then adjusted to pH 7.4 by the addition of 1 N NaOH. Krebs-Ringer phosphate buffer had the following composition: NaCl, 110 mM; KCl, 4 mM; $CaCl_2$, 2 mM; $MgSO_4$, 1 mM; and Na_2HPO_4, 2 mM. The buffer was gassed and adjusted to pH 7.4 as described for the bicarbonate buffer. Homogenization buffer contained 10 mM potassium phosphate, 1 mM EDTA, 1 mM dithiothreitol, and 1% (w/v) BSA (pH 7.4).

Preparation of Fat Cells

Fat cells were prepared by a modification of the collagenase method of Rodbell (14). Male Sprague-Dawley rats (150–300 g) were decapitated, and epididymal fatpads were removed, cut into small pieces, and placed into 30-ml polypropylene bottles containing 3 ml of a solution of collagenase (2 mg/ml) in Krebs-Ringer bicarbonate buffer with 2% BSA (pH 7.4). Incubation proceeded for 30 min at 37° in a shaking water bath (40 cycles/min). Following the collagenase treatment, fat cells that filtered through a silk screen were washed twice with 10 ml of Krebs-Ringer bicarbonate-2% BSA buffer and were resuspended in the same buffer.

The gravimetric method of Dole (15) was used to determine the lipid content of the adipocyte suspension. Since the cellular lipid content is a function of rat size (16), the adipocyte number was estimated from the cell suspension lipid content.

[3] The abbreviation used is: BSA, bovine serum albumin.

Glucose Oxidation

Glucose oxidation was monitored by measuring the production of $^{14}CO_2$ from [U-^{14}C]glucose, following the procedure previously described (6).

Biosynthesis of Triglycerides

Fat cells (0.2 ml; 0.2–2 × 10^5 cells) were added to 30-ml polypropylene bottles containing 1.8 ml of Krebs-Ringer bicarbonate-2% BSA buffer; 0.28 mmole of glucose; 0.2 μCi of [U-^{14}C]glucose; and oxytocin, insulin, or no additional ingredients (basal). The contents of the bottles were gassed for 15 sec with 95% O_2:5% CO_2, capped, and incubated in a metabolic shaker (40 cycles/min) for 1 hr at 37°. Aliquots (250 μl) of the assay mixture were transferred to 0.4-ml polyethylene microcentrifuge tubes (Bio-Rad) containing 100 μl of dinonyl phthalate (17). The cells were separated from the incubation medium by centrifugation (45 sec, Beckman Microfuge B). The tubes were cut at the dinonyl phthalate phase, and the upper phase containing the fat cell pellet was transferred to a 20-ml glass scintillation vial containing 10 ml of toluene (Fisher Scientific Company, Pittsburgh, Pa.) with 0.03% 1,4-bis[2-(4-methyl-5-phenyloxazole)]benzene and 0.5% 2,5-diphenyloxazole. After 12 hr, 1 ml of water was added to the scintillation vials and radioactivity was determined.

Glucose Transport

[1-^{14}C]Glucose oxidation in the presence of phenazine methosulfate. The oxidation of [1-^{14}C]glucose in the presence of phenazine methosulfate is an indirect measure of glucose transport in adipocytes (18). The experimental protocol is similar to glucose oxidation except that the substrate solution contained [1-^{14}C]glucose (0.1 μCi/ml) instead of [U-^{14}C]glucose, and 20 μM phenazine methosulfate was added.

3-O-Methyl-D-glucose. A modification of the procedure described by Livingston and Lockwood (19) was used. Fat cell suspensions (0.3 ml) were incubated in 12 × 75 mm plastic culture tubes in the presence or absence of 25 μl of hormone (1.7 nM insulin or 100 nM oxytocin, final concentrations) at 37° for 30 min. After incubation, 225 μl of cell-free medium (infranatant) was gently removed, and 25 μl of 1 mM 3-*O*-[^{3}H]methyl-D-glucose (20–180 μCi/μmole; see figure legends) in Krebs-Ringer phosphate-2% BSA buffer were added to the remaining cell suspension to initiate the transport assay. The assay was terminated by the addition of 200 μl of cold (0°) aqueous solution of 0.15 M NaCl and 5 mM phlorizin. Cells were separated over dinonyl phthalate (17). Radioactivity was determined in 5 ml of Bray's solution (New England Nuclear Corporation). To determine intercellular trapping of 3-*O*-methyl-D-glucose, 10 μl of [^{3}H]methoxy inulin (40 mg/ml, 0.63 μCi/mg) was added to 0.3 ml of cell suspension. The assay was terminated by centrifugation over dinonyl phthalate, and radioactivity was determined as described above.

Pyruvate Dehydrogenase Assay

Freshly excised fat-pad tissues (100–300 mg) were incubated for 30 min in 20-ml polypropylene bottles containing 2 ml of Krebs-Ringer bicarbonate buffer contain-

ing 0.5 mM $CaCl_2$ and 11 mM fructose, pH 7.4 at 37°. The tissue was then transferred to fresh media of the same composition with or without insulin (5 nM) or oxytocin (100 nM). After 30 min, the tissue was homogenized in a glass tissue homogenizer containing 0.4 ml of ice-cold homogenization buffer, pH 7.4 (11). Aliquots of 200 μl were immediately transferred to 17 × 100 mm Falcon polyethylene tubes containing 0.3 ml of substrate solution [11 mM potassium phosphate, 1.1 mM EDTA, 2.8 mM $MgCl_2$, 1.6 mM β-NAD, 0.08 mM thiamine pyrophosphate, and 0.6 mM sodium [1-^{14}C]pyruvate (0.2 mCi/mmole), pH 7.4]. Prior to initiation of the assay the substrate solution was incubated for 2 min at 37°. The tubes were immediately closed with a Kontes serum rubber stopper holding a hyamine hydroxide-impregnated filter paper (1 × 15 cm) rolled into a suspended central well. Pyruvate oxidation was allowed to proceed for 2 min at 37°. The reaction was stopped by injecting 0.8 ml of citrate-phosphate buffer (0.08 M citric acid and 0.04 M Na_2HPO_4, pH 3.0) into the reaction medium. After 30 min at room temperature, filter papers were transferred to liquid scintillation vials containing 3 ml of ethanol and 12 ml of toluene containing 0.5% 2,5-diphenyloxazole and 0.03% 1,4-bis [2-(5-phenyloxazolyl)]benzene. Radioactivity for all experiments was determined in a Beckman scintillation system (LS-250). Efficiency of counting for ^{14}C was 80% and for ^{3}H, 30%.

RESULTS

Specificity of adipocyte oxytocin receptors. Isolated adipocytes were assayed for peptide-stimulated glucose oxidation in the presence of oxytocin, mesotocin, isotocin, vasotocin, oxypressin, arginine vasopressin, tocinamide, and pressinamide. Figure 1 illustrates dose-response curves for each peptide (except for tocinamide and pressinamide) as a function of maximal oxytocin response (1.6 nmoles of glucose oxidized per hour per 10^5 cells above the basal amount). Of the oxytocin analogues tested, vasotocin and oxypressin were full agonists, whereas mesotocin, isotocin, and arginine vasopressin appeared to be partial agonists. Tocinamide and pressinamide were unable to stimulate glucose oxidation. The slopes of each curve near the ED_{50} were similar; the differences in the maximal responses suggest small differences in the efficacy of the several analogues tested. For the analogues tested, parallelism between the relative potencies (ED_{50}) for stimulation of glucose oxidation and the specific activities previously reported for the uterine bioassay can be seen (Table 1). Thus, the data indicate that the adipocyte receptor exhibits the same specificity as the oxytocin receptor previously characterized in uterine and mammary tissue.

Figure 2 illustrates dose-response curves for adipocyte glucose oxidation and lipogenesis in the presence of insulin and oxytocin. As with insulin, oxytocin's dose-response curves for the two responses were very similar; the ED_{50} values for both oxytocin stimulation of glucose oxidation and for triglyceride synthesis were approximately 3 nM. Oxytocin was not only less potent than insulin on a molar basis, but was unable to achieve as great a response as was insulin (24% of the insulin response for glucose oxidation; 37%, for lipogenesis: Fig. 2).

Oxytocin and glucose transport. Glucose transport was measured by two different methods: (a) [1-^{14}C]glucose oxidation in the presence of phenazine methosulfate (18) and (b) 3-*O*-methyl-D-glucose uptake (19). The uptake of 3-*O*-methyl-D-glucose by adipocytes was not stimulated by oxytocin. Figure 3 illustrates that the basal and oxytocin-stimulated rates of hexose transport could not be differentiated, whereas insulin caused approximately

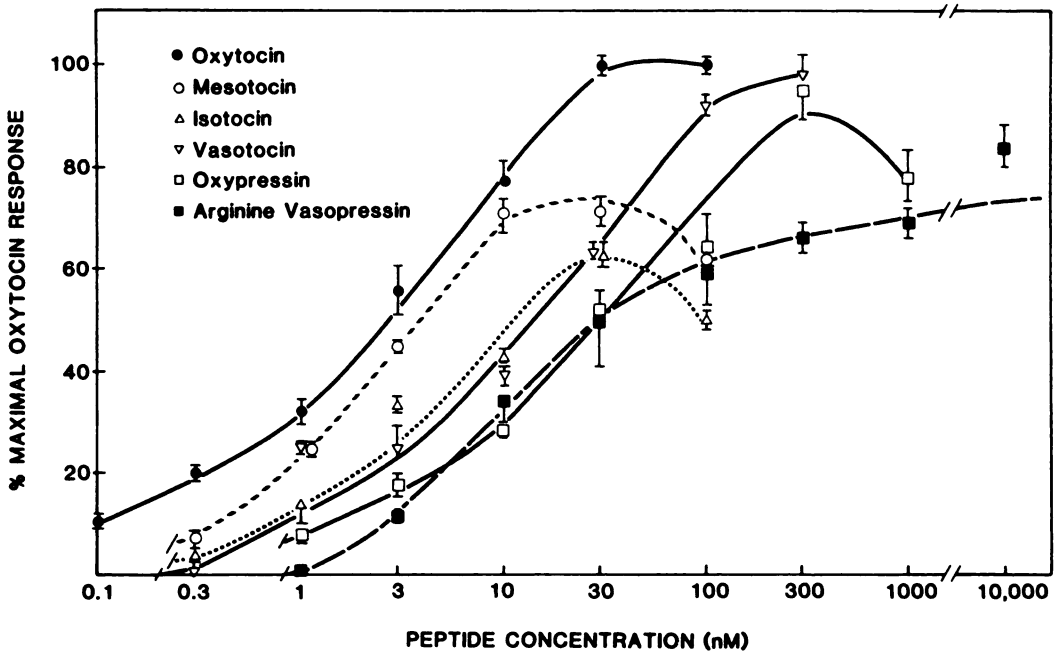

FIG. 1. *Effect of oxytocin and related peptides on the oxidation of [U-^{14}C]glucose by isolated rat adipocytes*

The data are expressed as percentage of maximal oxytocin (100 nM) response above the basal rate of glucose oxidation. The *vertical bars* through the data points represent the standard error of mean (n = 6). Dose-response curves (here and in Figs. 2, 5, and 6) were visually fitted to data.

TABLE 1

Activity of oxytocin and its analogues

Cys-Tyr-X-Y-Asn-Cys-Pro-Z-Gly-NH_2

S——S

Peptide	X	Y	Z	ED_{50}	Activity		
					Fat cell (glucose oxidation)[a]	Uterine contraction[b]	
				nM	*units/mg*	*units/mg*	
Oxytocin	Ile	Glu	Leu	3	500	500	(12, 20)
Mesotocin	Ile	Glu	Ile	2.5	600	458–498	(13)
Isotocin	Ile	Ser	Ile	4.9	315	332–372	(21)
Vasotocin	Ile	Glu	Arg	15	100	130	(22)
Oxypressin	Phe	Glu	Leu	23	65	20	(20)
Arginine vasopressin	Phe	Glu	Arg	20	75	30	(20)
Tocinamide	Ile	Glu	(des-Pro-Z-Gly)	—	0	0	(23)
Pressinamide	Phe	Glu	(des-Pro-Z-Gly)	—	0	0	(23)

[a] Oxytocin's activity was set at 500. The ED_{50} values for the analogues of oxytocin were divided into the ED_{50} for oxytocin (3 nM) and the quotient was multiplied by 500.

[b] Reference source is indicated in parentheses.

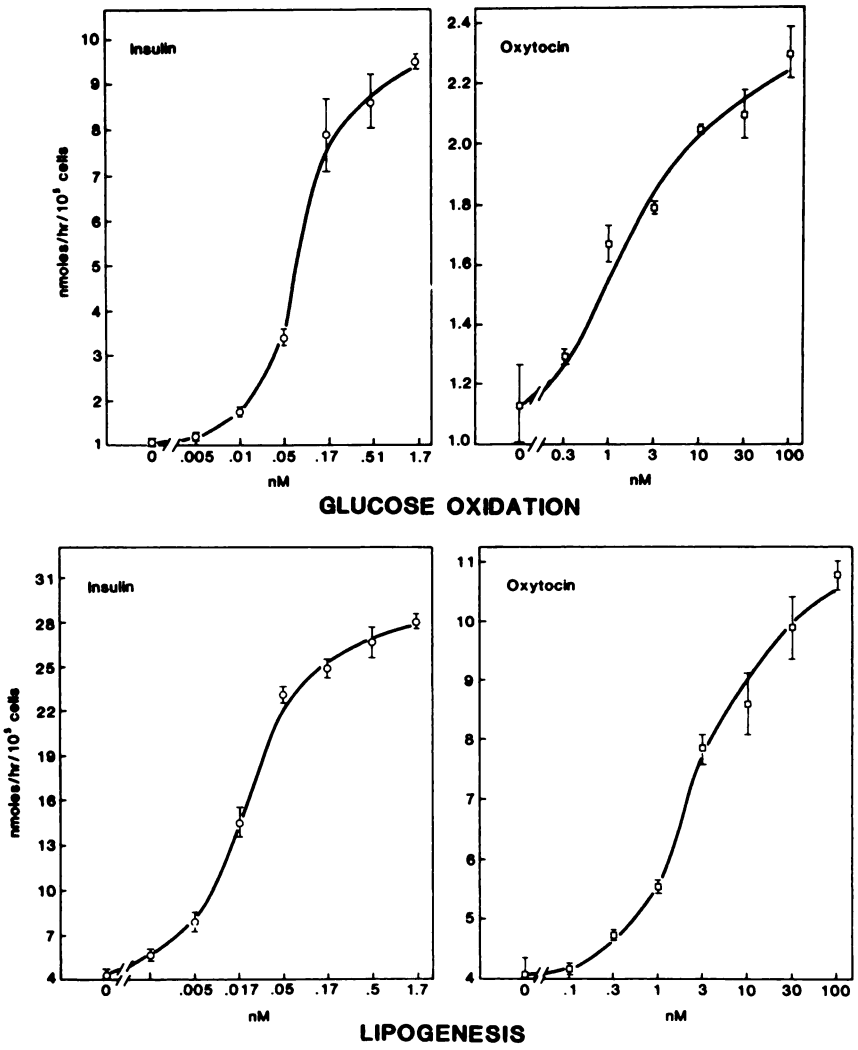

FIG. 2. *Glucose metabolism in isolated adipocytes*

Glucose oxidation (*upper half*) and lipogenesis (*bottom half*) were measured in isolated adipocytes in the presence of variable concentrations of insulin (*left*) and oxytocin (*right*). The *vertical bars* through the data points represent the standard error of the mean ($n = 6$). It should be noted that the scale for oxytocin-stimulated responses is approximately 25% of the scale for insulin-stimulated responses.

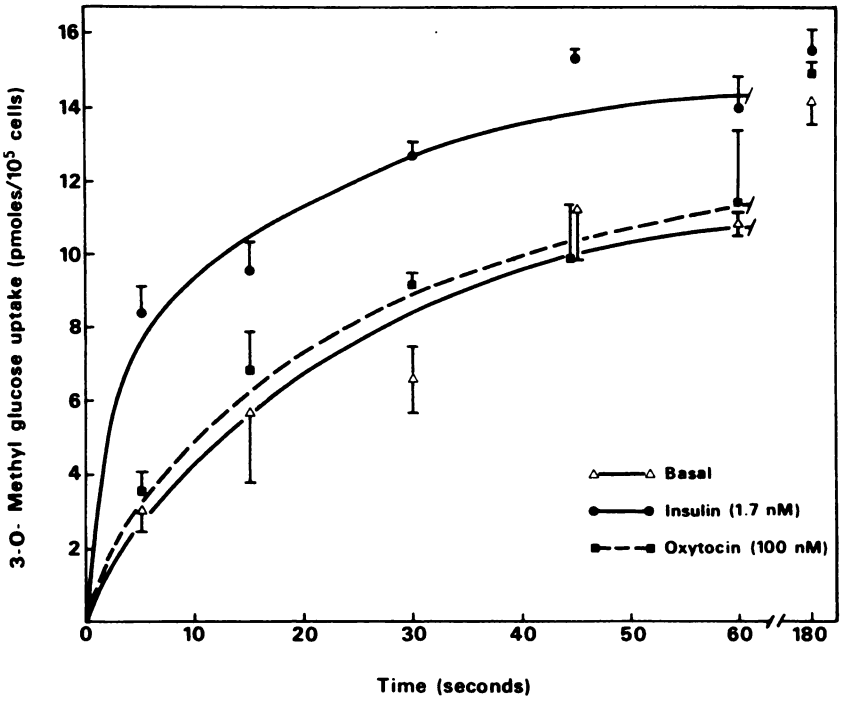

FIG. 3. *Time course of uptake of 3-O-[³H]methyl-D-glucose (1 mM, 20 μCi/μmole) in isolated fat cells from Sprague-Dawley rats*
The uptake was measured at 22° as described under Materials and Methods in the absence of hormone or in cells treated with 1.7 nM insulin or 100 nM oxytocin. The *vertical bars* through the data points represent the standard error of the mean ($n = 3$). Curves were fitted by eye.

a 3-fold stimulation: $t_{0.5}$ (insulin) = 12.5 sec, $t_{0.5}$ (oxytocin, basal) = 35 sec ($t_{0.5}$ values were obtained from first-order kinetic plots). If oxytocin did have a slight transport-stimulatory activity, then by increasing the specific activity of 3-*O*-methyl-D-glucose, subtle differences between basal and oxytocin rates would become apparent. Figure 4 illustrates that increasing the specific activity 9-fold had no statistically significant effect on the basal rate of 3-*O*-methyl-D-glucose uptake. The uptake of the sugar was measured after 5 sec of incubation, a time point demonstrating nearly optimal insulin responsiveness (Fig. 3).

Adipocyte oxidation of [1-^{14}C]glucose in the presence of phenazine methosulfate, an independent indicator of glucose transport, was not stimulated by oxytocin. The response, in terms of nanomoles of glucose oxidized per hour per 10^5 cells, was as follows: 25.2 ± 0.5, basal; 25.4 ± 0.8, 30 nM oxytocin; and 38.5 ± 0.5, 1.7 nM insulin. A 30 nM concentration of oxytocin has metabolic stimulatory activity equivalent to 0.01 nM insulin (Fig. 2), a concentration of insulin which increased [1-^{14}C]glucose oxidation in the presence of phenazine methosulfate. Thus, the indirect method of measuring glucose transport by following [1-^{14}C]glucose oxidation (18) provides further evidence that oxytocin does not stimulate glucose transport into adipocytes.

Oxytocin and pyruvate dehydrogenase. Pyruvate dehydrogenase activity was measured by the conversion of [1-^{14}C]pyruvate to $^{14}CO_2$ (11). The basal rate of $^{14}CO_2$ production was 52 ± 1 nmoles/2 min/g of tissue, the oxytocin-stimulated rate was 88 ± 1 nmoles/2 min/g of tissue, and the insulin-stimulated rate was 162 ± 5 nmoles/2 min/g of tissue (each value is the mean ±

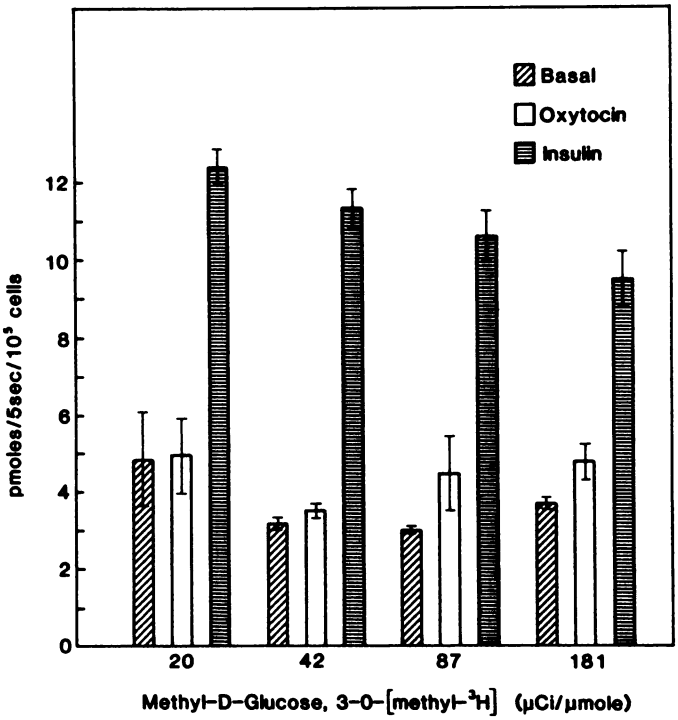

FIG. 4. *Glucose transport in isolated adipocytes*
Uptake of 3-*O*-methyl glucose in isolated adipocytes from a Sprague-Dawley rat was measured as described in the legend to Fig. 3. The results are expressed as picomoles of 3-*O*-methyl glucose uptake per 5 sec/10^5 cells in the absence of hormone or in the presence of 100 nM oxytocin or 1.7 nM insulin at the following specific activities of 3-*O*-[³H] methyl-D-glucose: 20 μCi/mmole, 42 μCi/mmole, 87 μCi/mmole, and 181 μCi/mmole. The *vertical bars* through the data points represent the standard error of the mean ($n = 3$).

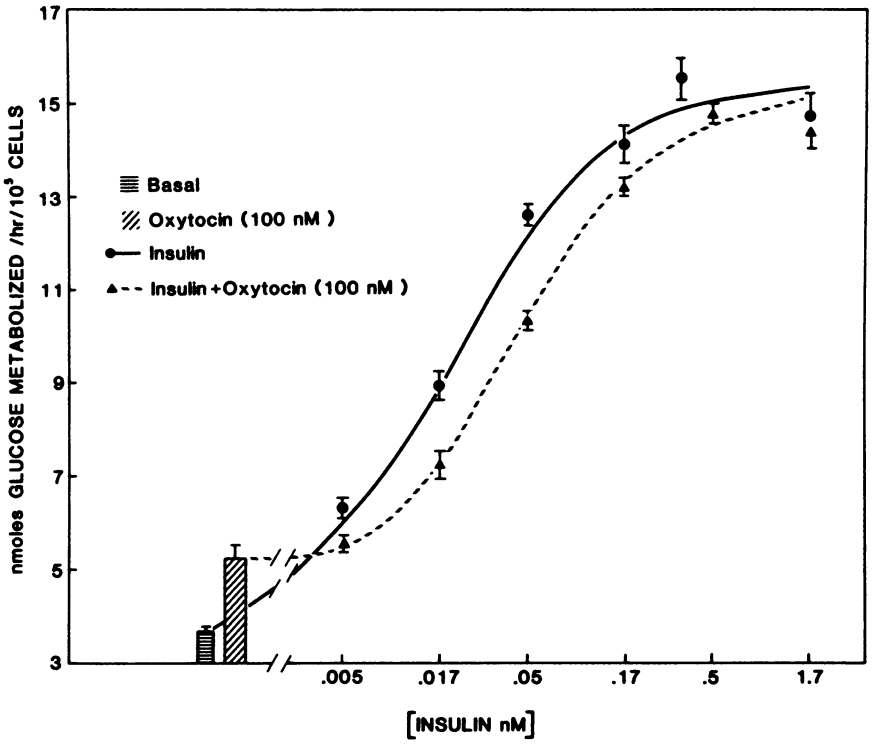

FIG. 5. *Effect of oxytocin (100 nM) on insulin-stimulated glucose oxidation in adipocytes*

The cells were incubated with various concentrations of insulin and various concentrations of insulin plus 100 nM oxytocin, and the conversion of [U-^{14}C]glucose to $^{14}CO_2$ was measured as described under Materials and Methods. The *vertical bars* through the data points represent the standard error of the mean ($n = 3$).

standard error of the mean of four assays). Thus 100 nM oxytocin has 54% of the stimulatory activity of 1.7 nM insulin.

Combined effects of oxytocin and insulin on glucose metabolism and transport. As noted above (Fig. 2), 100 nM oxytocin, the concentration which yields a maximal response for glucose oxidation, has only 24% of the stimulatory activity exhibited by insulin. When the optimal amount of oxytocin was added to incubation media containing submaximal insulin concentrations (0.017–0.17 nM), which alone yielded responses greater than the oxytocin response, the effect of the two hormones in combination was less than the effect of insulin alone. At maximal or near-maximal insulin concentrations (≥0.5 nM), the inhibitory effect of oxytocin on insulin action was no longer apparent (Figs. 5 and 6). The net effect of 100 nM oxytocin on insulin-stimulated glucose oxidation and lipogenesis was a shift in the dose-response curves to the right (Figs. 5 and 6, respectively). In essence, oxytocin renders the adipocyte "insulin-resistant."

As noted above, oxytocin (100 nM) was unable to stimulate 3-*O*-methyl-D-glucose uptake. In the presence of 0.05 nM (0.3 ng/ml) insulin (Fig. 7) and 1.7 nM insulin (data not shown) oxytocin had no effect on the rate of insulin-stimulated hexose uptake. Thus, at optimal and suboptimal levels of insulin, oxytocin is unable to alter insulin-stimulated glucose transport.

DISCUSSION

Oxytocin initiates its effects in adipocytes by binding to a cell surface receptor (1, 2, 4–6). Although previous work (1, 4, 24) did not examine in detail the dose-response relationships for the action of oxytocin and its analogues in adipocytes, it is clear from our data that the specificity of the fat cell receptor is very similar to the specificity of the oxytocin receptor present in uterine tissue (Fig. 1; Table 1). It is possible that a single receptor is responsible for all of the insulin-like actions of oxytocin, since the dose-response curves are so similar. Stimulation of glucose oxidation and lipogenesis are reported here (Fig. 2); inhibition of norepinephrine-stimulated lipolysis over the same concentration range has been previously reported (8). Although the dose-response curves are similar for the different metabolic effects, the maximal oxytocin response relative to the maximal insulin response varies: 24% for glucose oxidation, 37% for lipogenesis, and 60–75% for inhibition of lipolysis (8). Thus, for oxytocin, the efficiency of receptor-effector coupling relative to insulin appears to vary for the different responses.

In adipocytes, one of the earliest events following insulin binding to its receptor is the increased uptake of glucose (25). In the present study, we have used both an indirect and a direct method of measuring glucose transport activity and we have found unequivocally that oxytocin is not capable of stimulating glucose transport over a concentration range where glucose oxidation is fully stimulated. One may conclude that the previously measured effects on glucose transport, observed at comparatively high oxytocin concentrations (2), might be mediated by a receptor other than the oxytocin receptor characterized by our present study. Since in our study the metabolic effects of oxytocin cannot be attributed to the stimulation of glucose transport, one must conclude that the effects may be attributed to the stimulation of one or more enzymes involved in glucose metabolism.

The enzyme we have focused on in the present study,

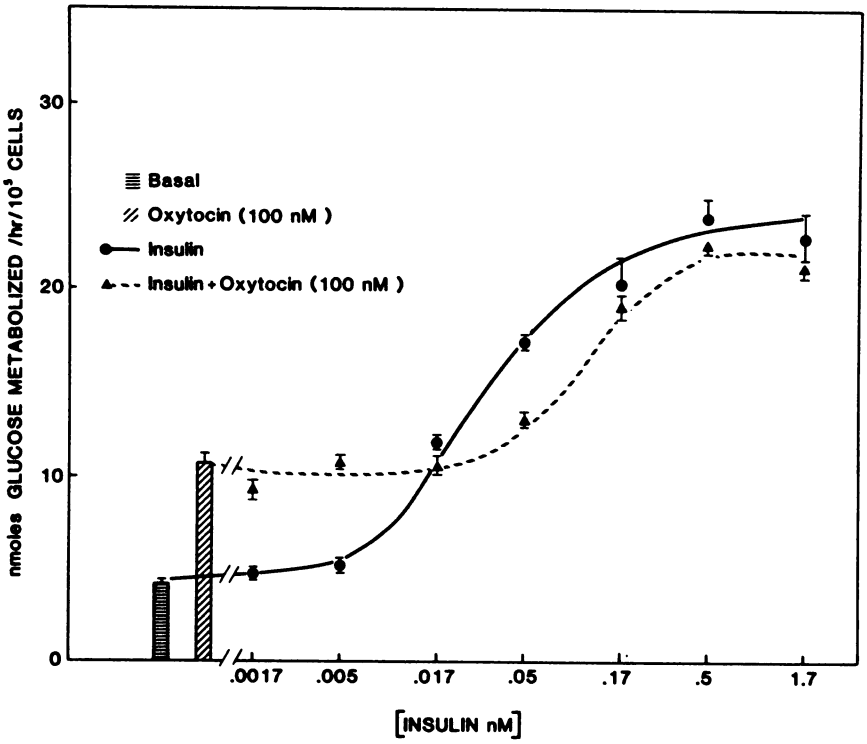

FIG. 6. *Effect of oxytocin (100 nM) on insulin-stimulated lipogenesis in adipocytes*

The adipocytes were incubated with various concentrations of insulin and various concentrations of insulin plus 100 nM oxytocin. The conversion of [U-^{14}C]glucose to ^{14}C-labeled lipids was measured as described under Materials and Methods. The *vertical bars* through the data points represent the standard error of the mean (n = 3).

pyruvate dehydrogenase, is critical for the metabolism of the carbon atoms of glucose, either by the tricarboxylic acid cycle to CO_2 or via the lipogenic pathway to the fatty acyl side chains of triglyceride. The activation by oxytocin of pyruvate dehydrogenase (54% of the maximal insulin response) may alone be sufficient to explain oxytocin's ability to stimulate lipogenesis (37% of a maximal insulin response). Further work is necessary to evaluate the possible role of acetyl CoA carboxylase in the lipogenic response to oxytocin.

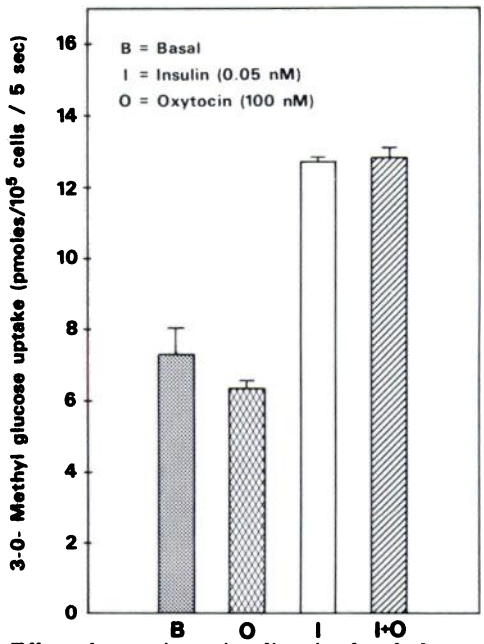

FIG. 7. *Effect of oxytocin on insulin-stimulated glucose transport in rat adipocytes*

Uptake of 3-*O*-methyl glucose was measured as described under Materials and Methods. Results are expressed as picomoles of 3-*O*-methyl-D-glucose uptake/10^5 cells/5 sec in the absence of hormone (basal) and in the presence of 100 nM oxytocin, 0.05 nM insulin, and oxytocin (100 nM) plus insulin (0.05 nM). The *vertical bars* through the data points represent the standard error of the mean (n = 3).

Our results point out a very interesting interaction between the effects of insulin and oxytocin. First, although both polypeptides act via distinct receptors, the maximal cellular response, obtained in the presence of maximally effective concentrations of both agents, is not additive, either for glucose oxidation (Fig. 5) or for lipogenesis (Fig. 6). Second, in the presence of a maximally effective concentration of oxytocin, the insulin dose-response curve is shifted to the right, both for glucose oxidation and for lipogenesis. In essence, oxytocin confers upon the adipocyte a state of insulin resistance. The results thus indicate an interdependence of the effects of insulin and oxytocin and point to the existence of a metabolic event in common (either post-receptor or receptor-related) for the action of both polypeptides. Since oxytocin does not affect insulin-mediated glucose transport at submaximal insulin concentrations (Fig. 7), the results point to an interaction at a post-receptor site.

A number of the metabolic effects of insulin are thought to be mediated by a low molecular weight chemical mediator (26–28). The mediator decreases the activity of cyclic AMP-dependent protein kinase, increases the activity of pyruvate dehydrogenase phosphatase (thereby activating the enzyme), and increases the activity of a low K_m phosphodiesterase (29, 30). Since in the present study we have demonstrated that the metabolic

actions of oxytocin are not mediated via glucose transport stimulation, it will be important in future work with oxytocin to look for mediators that may be similar to the one(s) described for insulin. In this context, it will also be of great interest to evaluate the role of phosphorylation-dephosphorylation reactions (11) in the process of oxytocin-mediated cell activation. In summary, the detailed analysis of the actions of both oxytocin and insulin in a single cell type promises to yield interesting comparative data that will serve to clarify both the common and distinct metabolic processes modulated by these two important hormones.

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