Insulin-Receptor Interaction in Isolated Fat Cells

I. THE INSULIN-LIKE PROPERTIES OF p-CHLOROMERCURIBENZENE SULFONIC ACID*

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T. MINEMURA AND OSCAR B. CROFFORD

From the Departments of Medicine and Physiology, Vanderbilt University, Nashville, Tennessee 37203

SUMMARY

Suspensions of fat cells were prepared from rat epididymal adipose tissue by digestion of the tissue with crude bacterial collagenase. One portion of each suspension was exposed to p-chloromercuribenzensulfonic acid (CMS), washed, and then incubated in Krebs-Ringer-bicarbonate buffer. A second portion, the control, was not exposed to CMS, but it was otherwise treated identically. Acceleration of glucose transport was estimated from the rate of conversion of glucose-$^{14}$C to $^{14}$CO$_2$ and $^{14}$C-labeled lipids. Inhibition of the lipolytic action of adrenocorticotropic hormone (ACTH) was evaluated by measuring (a) the cyclic 3',5'-AMP concentration of the cell suspensions and (b) the rate of release of glycerol into the incubation medium.

The optimal concentration of CMS was 10$^{-3}$ M and the optimal exposure time was 30 min. Glucose utilization by cells exposed to CMS was 5 to 20 times that of the control, but only 50 to 80% that of cells maximally stimulated by insulin. Most of the increased glucose utilization was due to increased glucose entry via a specific transport system since glucose utilization, when stimulated by CMS, was inhibited by 3-O-methyl glucose. Cells exposed to CMS showed a slight increase in nonspecific permeability, however, since (a) there was a slight increase in the amount of malic dehydrogenase appearing in the incubation medium, and (b) stimulation by CMS plus insulin exceeded that of insulin alone when the glucose concentration of the incubation medium was <20 mM. Incubation of CMS exposed cells in 2,3-dimercaptopropanol resulted in partial reversal of the stimulatory effect of CMS.

Exposure of cells to CMS inhibited lipolysis both in the basal state and when lipolysis was stimulated by ACTH. This inhibition was overcome either by a 10-fold increase in the concentration of ACTH or by the addition of theophylline, showing that the CMS exposure had not irreversibly inhibited the lipolytic enzymes of the cell. The antilipolytic action of CMS was probably mediated through an effect on the adenyl cyclase system since the increase in cyclic 3',5'-AMP produced by ACTH was inhibited by the CMS exposure. This inhibitory effect of CMS was likewise overcome by a 10-fold increase in the ACTH concentration.

We concluded that the metabolism of CMS-treated fat cells mimicked that of cells incubated with insulin in that glucose transport was stimulated and lipolysis was reversibly inhibited. Since the reaction of CMS with isolated fat cells seemed analogous to its reaction with the sulfhydryl groups of other proteins, we postulated that the insulin-like actions of CMS were mediated through a combination between CMS and certain sulfhydryl containing elements located on or near the cell surface.

The binding of insulin to a specific receptor on the membrane of isolated fat cells is presumed to be the initial step in a sequence of molecular events leading to acceleration of glucose transport and inhibition of lipolysis. The nature of the receptor and the chemistry of the hormone-cell interaction are unknown (3-5).

One approach to the study of insulin receptor interaction has been the investigation of substances having biological activities which mimic those of insulin. A variety of substances exhibit such insulin-like activities when incubated in vitro with rat adipose tissue (6-13) or the cells isolated from this tissue (14-22). In most reports, however, there is no precise biochemical description of either the identity or reactivity of the test substances themselves. Thus, it is not yet possible to identify a biochemical reaction which is common to all such substances, and, therefore, likely to be shared by insulin itself.

The reaction of p-chloromercuribenzensulfonic acid with the sulfhydryl groups of both simple and complex proteins has been previously reported (23-26). In the present studies we have investigated certain of the biological actions of CMS. Our objectives were first, to determine whether the reaction of CMS with the membranes of isolated fat cells was analogous to its reaction with the sulfhydryl groups of simpler structures, and second, to determine how closely the biological actions of insulin on isolated fat cells were mimicked by CMS.

EXPERIMENTAL PROCEDURE

Materials—p-Chloromercuribenzensulfonic acid and glutathione (reduced form) were obtained from Sigma. 3-O-Methyl-
d-glucose and L-glucose were obtained from Calbiochem. The enzymes and cofactors used for the glycerol and malic dehydrogenase assays were obtained from Boehringer Mannheim. Uniformly labeled d-glucose-6-\(^{14}C\) (6.42 \(\mu\)Ci per \(\mu\)mol) was obtained from New England Nuclear Corporation and used without further purification. The adrenocorticotropic hormone used for determining the dose-response relationship (Fig. 6) was a highly purified preparation (Parke, Davis and Company) with a potency of 80 units per mg. The ACTH used for the routine incubations was commercially available ACTH (Parke, Davis and Company), 40 units per vial.

Incubation Techniques—The incubation procedure was essentially the same as that originally described by Rodbell (27). Briefly, isolated fat cells were prepared from the epididymal adipose tissue of fed rats by digestion of the tissue with crude bacterial collagenase (Worthington). The isolated cells were then incubated for 1 hour in Krebs-Ringer-bicarbonate buffer (pH 7.4), equilibrated with \(\text{O}_2\)-\(\text{CO}_2\) (95:5). The metabolic activity of replicate samples taken from a single pool of cells was then measured by the rate of conversion of \(^{14}C\)-labeled glucose to \(^{14}C\)-labeled lipids. The results were expressed in terms of fat cell lipid, determined from the weight of a washed chloroform-methanol (2:1) extract of an aliquot of the cell suspension. Since fat cells isolated from the epididymal adipose tissue of fed rats are approximately 90% lipid, the fresh weight, dry weight, and fat cell lipid weight are all about the same.

Exposure of Fat Cells to CMS—The cell suspension was divided into two portions. One portion was made to a volume of 5 ml and exposed to CMS by adding a volume (usually 50 \(\mu\)l) of \(10^{-3}\) M CMS. The cells were then incubated at 37° for the stated time (usually 30 min) and the exposure was terminated by washing the cell suspension three times in fresh buffer. The other portion was not exposed to CMS, but it was otherwise treated identically. Aliquots of the CMS-exposed cells and the control cells were then incubated at 37° for 1 hour, with or without insulin (usually 100 units per ml).

Release of Malic Dehydrogenase—The cell suspension was divided into three portions; one was exposed to CMS as described above and the second served as the control. The third portion was homogenized, centrifuged, and the fat-free supernatant used to determine the malic dehydrogenase content per unit weight of fat cells (28). Aliquots of the CMS-exposed and control cells were incubated for the times indicated, and preliminary separation of the incubation medium from the cells was achieved by centrifugation. The incubation medium was then passed through a Millipore filter (5.0-\(\mu\)m pore size) to remove the few remaining cells before using the filtrate for the assay of malic dehydrogenase content. Using the malic dehydrogenase content of the homogenate of fresh cells as 100%, the malic dehydrogenase released from the incubated cells was expressed as percentage of the total malic dehydrogenase per 100 mg of cells released per interval of time.

Measurements of CMS Concentration—Since the Krebs-Ringer-bicarbonate used for the incubation medium contained 3.5% bovine serum albumin, and since the CMS was added to the buffer prior to the addition of the fat cells, it seemed probable that some of the CMS was bound to the albumin and that the concentration of CMS to which the cells were actually exposed was less than the total CMS concentration of the incubation medium. This was investigated by measuring the CMS concentration in the albumin-free ultrafiltrates of the incubation media. A Diaflo ultrafilter (Amicon Corporation) with a membrane intended to prevent the passage of molecules larger than 10,000 molecular weight was used to prepare the ultrafiltrates. The effectiveness of the filter was verified by finding less than 0.01% of the albumin in the ultrafiltrate. CMS concentration, before and after filtration, was determined spectrophotometrically (23). When no albumin was present in the incubation medium, the CMS concentration was unchanged by the filtration process. When the incubation medium contained 3.5% albumin, however, the approximate CMS concentration before filtration was \(10^{-2}\) M and after filtration was \(10^{-4}\) M. This indicated that the CMS free to combine with the cells was approximately 1 order of magnitude below the total CMS concentration of the incubation medium.

Lipolysis—The rate of lipolysis was estimated from the rate of release of glycerol into the incubation medium. The buffer used for preparation of the cell suspension and for incubation of the cells was the same as that used for the routine incubations except that it did not contain glucose. At the end of 1 hour, the reaction was stopped by the addition of 0.25 ml of 30% perchloric acid, and the protein-free centrifugate was used for the enzymatic assay of glycerol (29).

Measurements of Cyclic AMP Concentration—In this series of experiments the standard incubation procedures were altered slightly so as to duplicate the conditions used previously for measuring the cyclic AMP concentration of isolated fat cells (30). Briefly, the initial cell suspension was divided into two portions; one was exposed to \(10^{-3}\) M CMS for 30 min while the other served as the control. After the CMS exposure, both portions were washed three times in fresh buffer and then dispensed into incubation vials. Each vial was incubated for 20 min at 37° and then received additions of caffeine and ACTH. Following the additions, the cells were incubated for 10 min at 37°, the reaction was stopped by the addition of perchloric acid and boiling, and the filtrate was subsequently assayed for cyclic AMP.

Evaluation of Significance of Data—The data are presented as the means of three observations with the range of observed values used as the index of dispersion. In most comparisons, the difference between the means was large and was considered significant if there was no overlap of the ranges. This simplified statistical treatment resulted from the increased reproducibility achieved by taking multiple samples from a single pool of cells. Each experiment has been repeated and confirmed at least twice.

RESULTS AND COMMENTS

Effect of CMS Concentration of Glucose Utilization—With an exposure time of 30 min, a CMS concentration of \(10^{-3}\) M produced maximum stimulation of glucose utilization (Fig. 1). With the control cells, the addition of 100 units of insulin per ml resulted in a 20- to 30-fold stimulation of glucose utilization. As shown previously, this insulin concentration is about four times that required for maximum stimulation (31, 32). It is apparent that glucose utilization by cells maximally stimulated by CMS was only 80%, that of the cells maximally stimulated by insulin. Nevertheless, the CMS-exposed cells utilized glucose at a rate 5 to 20 times that of the nonstimulated controls.

CMS concentrations greater than \(10^{-2}\) M not only failed to stimulate, but actually inhibited the basal glucose metabolism. To in-
vestigate this point further, experiments were performed to test the effect of high concentrations of CMS on the oxidation of \( ^{14} \text{C} \)-labeled intracellular substrates formed by prior incubation of the cells with glucose-{\(^{14}\)}C and insulin (Table I). The absence of a stimulatory effect of insulin on the cells not exposed to CMS confirms the absence of glucose-{\(^{14}\)}C in the final incubation medium and supports the view that insulin does not accelerate the formation of CO\(_2\) from various intracellular precursors. Since 10\(^{-3}\) M CMS inhibited strongly the production of CO\(_2\), at least part of the inhibition of basal glucose utilization observed in Fig. 1 could be due to inhibition of glucose metabolism at a step subsequent to glucose transport.

Effect of CMS Exposure Time on Stimulation of Glucose Utilization—With a CMS concentration of 10\(^{-3}\) M, an exposure time between 20 and 45 min resulted in maximum stimulation (Fig. 2). The insulin response of the control cells was less than that shown in Fig. 1. The difference was attributed to the fact that incubation of the control cells was delayed for 60 min to correspond to the longest of the CMS exposure times. From the data in Figs. 1 and 2, we selected a 30-min exposure to 10\(^{-3}\) M CMS as the standard exposure to be used in all subsequent experiments.

Effect of CMS and Insulin on Ratio of CO\(_2\) to Total Lipid—
In Figs. 1 and 2, glucose utilization refers to the sum of microgram atoms of glucose carbon oxidized to CO\(_2\) plus microgram atoms of glucose carbon incorporated into total lipid. This sum accounts for approximately 90\% of the total quantity of glucose metabolized. In a series of six experiments, the ratios (glucose oxidized to CO\(_2\)/glucose incorporated into total lipid) were (mean \( \pm \) S.E.): control cells, 0.39 \( \pm \) 0.038; cells stimulated by insulin (100 \( \mu \)units per ml), 0.59 \( \pm \) 0.017; and cells stimulated by CMS (10\(^{-3}\) M for 30 min), 0.57 \( \pm \) 0.022. Thus, both the insulin- and CMS-stimulated cells showed a slight, but significant (\( p < 0.01 \)), increase in the ratio, but there was no significant difference in this ratio when the insulin-stimulated cells were compared to the CMS-stimulated cells.

Effect of 3-O-Methyl-D-Glucose and L-Glucose on Metabolism of D-Glucose—The data in Table II show that the presence of 15 mM 3-O-methyl-D-glucose, which is transported by the D-glucose transport system, did not affect glucose metabolism, compared to the CMS-stimulated cells. The difference in this ratio when the insulin-stimulated cells were compared to the CMS-stimulated cells was significant (\( p < 0.05 \)).

TABLE I

<table>
<thead>
<tr>
<th>Treatment of cells</th>
<th>Mean (^{14})CO(_2) production</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>488</td>
<td>377-310</td>
</tr>
<tr>
<td>Control + insulin</td>
<td>476</td>
<td>361-398</td>
</tr>
<tr>
<td>CMS exposed</td>
<td>4</td>
<td>0-12</td>
</tr>
<tr>
<td>CMS exposed + insulin</td>
<td>5</td>
<td>0-15</td>
</tr>
</tbody>
</table>

\* From three observations.
transport system but not metabolized (14, 33, 34), inhibited the basal, insulin-stimulated, and CMS-stimulated metabolism of 1 mM D-glucose. The fact that the CO₂ to total lipid ratio decreased when glucose metabolism was inhibited by the addition of 3-O-methyl glucose does not indicate that the site of 3-O-methyl glucose inhibition is something other than an inhibition of glucose transport since previous data have shown the CO₂ to total lipid ratio to decrease when glucose transport is decreased by such simple measures as lowering the glucose concentration of the incubation medium (31). In contrast to the results obtained with 3-O-methyl glucose, L-glucose, which has virtually no affinity for the D-glucose transport system of adipose tissue (33), had no inhibitory effects (data not shown). It was concluded that most of the acceleration of glucose metabolism produced by CMS, like that produced by insulin, was due to an acceleration of glucose transport, and not due to a nonspecific increase in the permeability of the cell membrane.

**Additive Effects of CMS and Insulin**—When the glucose concentration of the incubation medium was 0.1 mM, the glucose metabolism of fat cells treated with CMS and incubated with insulin was slightly, but consistently, greater than that of control cells incubated with insulin (Fig. 3). When the glucose concentration was 20 mM (a) the control rate of glucose utilization was approximately 10-fold greater, (b) the stimulatory effect of insulin, relative to the control, was less marked, and (c) glucose metabolism by CMS-treated cells incubated with insulin did not exceed glucose metabolism of control cells incubated with insulin. Our interpretation of these results will be discussed subsequently.

**Release of Malic Dehydrogenase**—The purpose of these experiments was to see if the plasma membrane had been damaged by exposure of the cells to CMS. Loss of integrity of the plasma membrane can be assessed from the leakage of malic dehydrogenase into the incubation medium (35). The rate of release of malic dehydrogenase from CMS-treated cells and from control cells is shown in Fig. 4. The data are expressed as percentage of total malic dehydrogenase in the homogenate of an equal weight of cells. During the course of a 2-hour incubation, there was a slight increase in the amount of malic dehydrogenase released by the CMS-treated cells. Even after 2 hours of incubation, however, the total amount of malic dehydrogenase released was less than 10% of the total malic dehydrogenase in the system. There was no significant difference in the release of malic dehydrogenase from control cells incubated with or without insulin.

**Reversal of CMS Effect by Thiol Compounds**—The data shown in Fig. 5 indicate that the acceleration of glucose metabolism, induced by exposure of the cells to CMS, could be partially reversed by incubation of the cells with 2,3-dimercaptopropanol.
The interpretation of the data was complicated by the fact that 2,3-dimercaptopropanol had a slight stimulatory effect when used alone. Other compounds tested were: glutathione (reduced form) \((10^{-4}, 5 \times 10^{-3}, 10^{-3}, \text{and } 10^{-4} \text{ M})\); 1,4-dithiothreitol (Cleland’s reagent) \((2 \times 10^{-4} \text{ M})\); 2,2-dithiodipyridine \((2 \times 10^{-4} \text{ M})\); and 4,4-dithiodipyridine \((2 \times 10^{-4} \text{ and } 10^{-2} \text{ M})\). Under the conditions tested, the dithiodipyridine compounds, when used alone, had no significant effects on glucose utilization, and did not reverse the stimulatory effect of CMS. Dithiothreitol alone stimulated the rate of glucose utilization almost as much.

**Figure 5.** Partial reversal of the stimulatory effect of \(p\)-chloromercuribenzenesulfonic acid (PCMBS) by 2,3-dimercaptopropanol (BAL). The cell suspension was divided into two portions: one served as the control while the other was exposed to \(10^{-4} \text{ M CMS}\) for 30 min (see “Experimental Procedure”). Aliquots of the control suspension were then incubated with no additions, 100 units of insulin per ml, or \(10^{-4} \text{ M 2,3-dimercaptopropanol}\). Aliquots of the CMS-exposed suspension were incubated with no additions or \(10^{-4} \text{ M 2,3-dimercaptopropanol}\). The conditions were otherwise the same as given in the legend of Fig. 1.

**Figure 6.** Relationship between lipolysis and ACTH concentration. Aliquots of a single pool of fat cells were incubated either without insulin (○--○) or with 1 munit of insulin per ml (●--●). Lipolysis was measured by the release of glycerol into the incubation medium (see “Experimental Procedure”). The ACTH used for these experiments had a potency of 80 units per mg. Each point represents the mean of three observations.

**Figure 7.** Inhibition of basal and ACTH-stimulated lipolysis by \(p\)-chloromercuribenzenesulfonic (PCMBS) and by insulin. The cell suspension was divided into two portions: one served as the control while the other was exposed to \(10^{-4} \text{ M CMS}\) for 30 min (see “Experimental Procedure”). The final incubation consisted of six groups of incubation vials. The first three groups contained control cells, CMS-exposed cells, and control cells plus 1 munit of insulin per ml. The second three groups of incubation vials contained, in addition to the above, 1 munit of ACTH per ml. All vials were incubated at 37°C for 1 hour. Lipolysis was measured by the release of glycerol into the incubation medium and is expressed as micromoles of glycerol per 100 mg of fat cell lipid per hour.

**Figure 8.** Ability of higher concentrations of ACTH plus theophylline to overcome the inhibitory effect of insulin and of \(p\)-chloromercuribenzenesulfonic acid (PCMBS). The experimental conditions were the same as described for Fig. 7 except that the final incubation consisted of nine groups of incubation vials. The first three groups contained 0.1 munit of ACTH per ml. The second three groups contained 1.0 munit of ACTH per ml. The third three groups contained 0.1 munit of ACTH per ml and 1.5 mM theophylline.
as CMS. Glutathione alone, in concentrations of $10^{-2}$ and $5 \times 10^{-2}$, had a slight stimulatory effect.

Inhibition of ACTH-stimulated Lipolysis—Lipolysis was measured by the release of glycerol into the incubation medium. The dose-response relationships for ACTH and for ACTH plus insulin are shown in Fig. 6. An ACTH concentration of 1.25 munits per ml (0.1 munit per ml) was selected as the dose of ACTH to be used as the standard lipolytic stimulus in subsequent experiments. This was the dose which produced near maximum stimulation, but whose effect could be inhibited by 1.0 munits of insulin per ml. As shown in Figs. 7 and 8, insulin inhibited the capacity of ACTH (0.1 munit per ml) to stimulate lipolysis. This inhibition was overcome by the addition of theophylline (1.5 mM) or by a 10-fold increase in ACTH concentration. CMS exposure also inhibited the capacity of ACTH to stimulate lipolysis. The lipolytic enzymes were not irreversibly inactivated, however, since the inhibition was overcome either by theophylline or by a 10-fold increase in ACTH concentration.

Cyclic AMP Concentration—Since the antilipolytic action of insulin is probably mediated through a decreased concentration of cyclic AMP, we extended our investigation of the antilipolytic action of CMS by measuring the cyclic AMP concentration of CMS-exposed and control cells (Table III). In agreement with previous results (30), an ACTH concentration of 0.20 munit per ml produced cyclic AMP levels which were approximately half-maximal. Exposure of the cells to CMS inhibited the expected rise in cyclic AMP. With a 10-fold increase in the ACTH concentration (to 2.0 munits per ml), the inhibitory effect of CMS was overcome and the cyclic AMP levels of the CMS-exposed and control cells were equal. With an ACTH concentration of 20 munits per ml, the cyclic AMP levels in the control cells were slightly submaximal, confirming the previous report (30). Somewhat surprisingly, however, the cyclic AMP level in the CMS-exposed cells were higher than in the control cells.

**Table III**

**Effect of CMS on cyclic AMP concentration**

<table>
<thead>
<tr>
<th>ACTH concentration</th>
<th>CMS exposed</th>
<th>Cyclic AMP concentration</th>
<th>Mean</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>munits/ml</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>0.2</td>
<td>ND-0.4</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>+</td>
<td>0.2</td>
<td>ND-0.5</td>
<td></td>
</tr>
<tr>
<td>0.2</td>
<td>0</td>
<td>28.1</td>
<td>24.7-28.8</td>
<td></td>
</tr>
<tr>
<td>0.2</td>
<td>+</td>
<td>3.5</td>
<td>3.2-3.8</td>
<td></td>
</tr>
<tr>
<td>2.0</td>
<td>0</td>
<td>41.6</td>
<td>41.2-42.0</td>
<td></td>
</tr>
<tr>
<td>2.0</td>
<td>+</td>
<td>43.8</td>
<td>42.6-45.1</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>0</td>
<td>36.6</td>
<td>35.5-38.3</td>
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<tr>
<td>20</td>
<td>+</td>
<td>62.5</td>
<td>59.5-66.2</td>
<td></td>
</tr>
</tbody>
</table>

* From three observations.

ND, not detectable.

DISCUSSION

The objectives of this investigation were (a) to determine how closely the biological actions of CMS mimicked those of insulin, and (b) to determine whether the interactions between CMS and fat cells were analogous to the known interactions between CMS and the sulfhydryl groups of other proteins.

The use of CMS was based on the reports of Velick (24) and of Vanstonevink, Weed, and Rothstein (25). Velick used CMS to react with the sulfhydryl groups of glyceroldehyde 3-phosphate dehydrogenase. He reported that CMS was more water soluble than p-chloromercuribenzoate, yet had the same degree of specificity for sulfhydryl groups. Vanstonevink et al. used CMS to measure the number of sulfhydryl groups on the plasma membrane of human erythrocytes. They found that CMS penetrated the cells less rapidly than CMB and attributed this to the increased water solubility of CMS.

We observed the membrane of isolated fat cells to be relatively impermeable to CMS. Overexposure, either in terms of time or concentration, resulted in inhibition of certain enzyme systems within the cell (Table I). One can only speculate that glyceroldehyde 3-phosphate dehydrogenase, which is sensitive to low concentrations of CMS (24), was one of the enzymes so inhibited. The observation that the stimulation of CMS was partially reversed by providing an excess of free sulfhydryl groups in the incubation medium (Fig. 5) is consistent with the view that CMS, when used so to stimulate glucose metabolism, reacts with the sulfhydryl groups of the cell membrane in a manner analogous to the reaction of CMS with the sulfhydryl groups of the simpler systems cited above.

The actions of CMS mimicked those of insulin in that both agents stimulate glucose metabolism. Since it is known from previous reports (14, 31, 32) that glucose transport is the principal step limiting the over-all rate of glucose metabolism when fat cells are incubated without insulin at low glucose concentrations, any agent which substantially stimulates glucose metabolism will necessarily accelerate glucose transport. Nevertheless, for an agent to be called "insulin-like," data supporting this view should be provided. In isolated fat cells, it has not been possible to measure glucose transport directly. Indirect evidence was provided by showing that glucose metabolism, when stimulated either by CMS or by insulin, was inhibited by the addition of a 15:1 molar excess of 3-O-methyl glucose. It has been shown previously that this sugar analogue has transport characteristics similar to those of glucose (34) but has virtually no affinity for adipose tissue hexokinase (14, 33). Consequently, the inhibition produced by 3 O methyl glucose was interpreted to be an inhibition of glucose transport. That the same CO$_2$ to lipid ratio was observed when glucose metabolism was stimulated either by CMS or by insulin is also consistent with the interpretation that both agents stimulate glucose metabolism by acceleration of the glucose transport step.

There were other features of the action of CMS on glucose metabolism which were not insulin like. First, as discussed previously, an overexposure to CMS was inhibitory. We have never observed this with insulin and incubated adipose tissue or isolated fat cells. Second, the effective concentration of CMS, although probably much lower than that added to the incubation medium, was certainly greater than the $10^{-6}$ m insulin which produces a comparable degree of stimulation (31). Third, the effect of CMS was not reversed by washing.
unexpected, is in contrast to the ease with which the stimulation produced by low concentrations of insulin can be reversed (31). Fourth, the CMS exposure was slightly injurious to the cell membrane. This was a minor effect, but one which was suggested by (a) the increased leak of malic dehydrogenase into the incubation medium (Fig. 4), and (b) the additive effect of CMS and insulin at low, but not high, glucose concentrations (Fig. 3). The interpretation of this last observation was based on previous experiments which have established the conditions under which the over-all rate of glucose metabolism is determined by the rate of glucose transport (31). Thus, when the glucose concentration of the incubation medium was 0.1 mM (Fig. 3), the absolute quantity of glucose utilized, even in the presence of optimum concentrations of insulin, was far below the maximum capacity of the cell to metabolize glucose. This indicated that transport of glucose into the cell continued to limit the rate of glucose metabolism even in the presence of insulin, and a significant transmembrane glucose gradient still existed. Under these circumstances, an injury which resulted in an increased permeability of the membrane (i.e. a "leak") could result in an increased rate of glucose entry. This would explain the increased rate of glucose metabolism observed even in the presence of optimal concentrations of insulin. When the glucose concentration of the incubation medium was 20 mM, the cells stimulated by insulin transported glucose faster than glucose could be metabolized. Under these conditions there would be a lesser transmembrane glucose gradient and a small leak in the cell membrane would not be expected to increase the rate of glucose metabolism. If CMS were to accelerate glucose utilization by means other than an increase in the rate at which glucose entered the cell (such as by increasing the phosphorylating capacity of the cell), the additive effect of CMS and insulin should be observed when the glucose concentration was high as well as low.

Although there were a few atypical features about the effects of CMS on glucose metabolism, the predominant action was a stimulation of glucose transport. In this respect it behaved in a manner which was quite insulin-like.

A second insulin-like action of CMS was its ability to inhibit lipolysis both in the basal state and when lipolysis was stimulated by ACTH (Fig. 7). Since the incubation medium did not contain glucose, and since ACTH-stimulated lipolysis is probably mediated through a cyclic AMP system (19,30), this was an insulin-like action of CMS which was independent of its action in accelerating glucose transport. An alternative interpretation would be that CMS inhibited lipolysis simply by entering the cells and irreversibly inactivating the lipolytic enzymes, which would not be an insulin-like effect. Since the inhibition produced by CMS, like that produced by insulin, was overcome either by a 10-fold increase in the concentration of ACTH or by the addition of 1.5 mM theophylline, this latter interpretation was considered unlikely.

The analogy between the antilipolytic actions of insulin and CMS was extended further by showing that the ability of ACTH to produce higher cyclic AMP concentrations was reversibly inhibited by exposure of the cells to CMS (Table III). There are two points about the cyclic AMP measurements for which we have no clear explanation but which deserve comment. First, the present experiments do not explain why the cyclic AMP levels achieved with suprathermal concentrations of ACTH were higher in CMS exposed cells than in control cells. Perhaps CMS also inhibits the cyclic nucleotide phosphodiesterase system, but the inhibition of the adenyl cyclase system predominates at the lower ACTH concentrations. Second, the maximum levels of cyclic AMP (approximately 60 nmoles per g of cells, dry weight) were about 6 times higher than those reported previously for isolated fat cells (30). Since the intracellular water of isolated fat cells is about 5 μl/100 mg, dry weight (36), the present data indicate that the intracellular cyclic AMP concentration can be driven to levels of about 1 mM. This exceedingly high concentration is approximately the same as that observed in rat adrenal glands following the intravenous injection of ACTH (37). In both instances, however, the cyclic AMP concentrations are far above those which exert a maximum effect on any known physiological system.

In conclusion, we have interpreted the data as suggesting that CMS reacts with the sulfhydryl groups on the cell surface and initiates the same sequence of events as those initiated by insulin. By analogy, the data support the view that membrane sulfhydryl groups may be involved in the interaction between insulin itself and some specific receptor on the cell membrane. Other data, however, suggest that this may be an oversimplified interpretation. Thus, maleimide (38) and N-ethylmaleimide (39) which are also thought to react with membrane sulfhydryl groups do not mimic the actions of insulin. Rather, a brief exposure of cells to maleimide blocks the ability of the cells to respond to insulin (38) while the N-ethylmaleimide-treated cells have, in addition to an impaired insulin responsiveness, an impaired ability to transport glucose (39). The interpretation is further complicated by our observation that certain thiol compounds (2,3-dimercaptopropanol, 1,4-dithiothreitol, and reduced glutathione) stimulate glucose utilization. Although we believe that membrane sulfhydryl groups play an important role in the action of insulin and many insulin-like agents, a precise biochemical description of this role is not yet possible.

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REFERENCES

Insulin-Receptor Interaction in Isolated Fat Cells. I

Insulin-Receptor Interaction in Isolated Fat Cells: I. THE INSULIN-LIKE PROPERTIES OF p-CHLOROMERCURIBENZENE SULFONIC ACID

T. Minemura and Oscar B. Crofford

J. Biol. Chem. 1969, 244:5181-5188.

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