

*Originals***Insulin Binding, Insulin Degradation and Glucose Metabolism in Human Monocytes**

H. Beck-Nielsen and O. Pedersen

Medical Department III and Department of Clinical Chemistry, County Hospital, Aarhus, Denmark

Summary. Insulin binding, insulin degradation and glucose metabolism were studied in highly purified preparations of monocytes. Steady state specific insulin binding was found at 15 °C, whereas no plateau was reached at 37 °C because of considerable insulin degradation at this temperature. ¹²⁵I-insulin non-specifically bound to monocytes at 15 °C remained constant for 120 min. In contrast non-specifically bound ¹²⁵I-insulin increased during incubation at 37 °C. About one third dissociated slowly to a wash-out medium suggesting an intracellular uptake of this fraction of non-specifically monocyte bound insulin. Monocytes did not degrade insulin at 15 °C. At 37 °C insulin was degraded partly by “proteases” released from the cells and partly by the specific insulin receptor. We found that about 35% of the total monocyte receptor bound iodoinsulin dissociated to a washout medium as degraded insulin. Furthermore, the degradation velocity of receptor bound insulin was proportional to the receptor occupancy. Thus, at 37 °C receptor bound insulin is the substrate for insulin degradation in monocytes and the reaction between the insulin molecule and the insulin receptor is conceivably considered not to be bimolecular at 37 °C unlike at 15 °C. Previously, no biological effect of insulin on monocytes has been demonstrated. In this study we found that insulin increased glucose uptake (25%, $p < 0.01$) and lactate release (12%, $p < 0.05$) in monocytes with ED₅₀-values within the physiological range. To obtain 50% of maximal biological effect it was necessary to activate only a few percent of the receptors.

Key words: Insulin receptors, insulin degradation, glucose and lactate metabolism, monocytes, lymphocytes and mononuclear leucocytes.

Mononuclear leucocytes have been used extensively for insulin binding studies in man. The major part of insulin binding to mononuclear cells is accounted for by the monocytes [1, 2]. Previously, it has not been technically possible to isolate pure preparations of monocytes. In this study we have measured insulin binding to almost pure preparations of monocytes and lymphocytes, respectively, in order to estimate the ratio between insulin binding to monocytes and lymphocytes; it has previously been assumed that this ratio is constant [2]. In monocytes the time course of total and non-specific cell binding and the nature of non-specific cell binding at 15 °C and 37 °C have been studied.

Degradation of insulin has generally been assumed to be independent of insulin binding to the specific membrane receptor [3]. Terris and Steiner [4] found, however, that insulin binding and insulin degradation in hepatocytes were closely related. We have therefore examined the relationship between insulin binding and insulin degradation in monocytes.

Despite a high insulin binding capacity of monocytes no biological effect of insulin has been previously observed in these cells. Using purified preparations of monocytes we have also studied the effect of insulin on monocyte glucose metabolism.

Material and Methods

Young (20–28 years old) healthy persons with normal body weight (90–110% of ideal weight) were examined. Sixty per cent of the volunteers were women. None of them took any drugs during the study period. Informed consent was obtained from all subjects.

Preparations of Cells

Mononuclear leucocytes were isolated from 100–150 ml blood drawn from a antecubital vein at 0800 h after an overnight fast. The blood was collected in tubes containing EDTA (dipotassium

salt) 30 g/100 ml and the cells were fractionated on Lymphoprep (Na-metrizoate from Nyegård, Oslo) gradients according to Boyum [5]. Isolates contained 15–25% monocytes, 75–80% lymphocytes, about 1% granulocytes and a variable number of platelets and erythrocytes [2].

Monocytes were isolated by resuspending the mononuclear leucocytes in RPMI-1640 medium containing 50 g/l bovine serum albumin (BSA), penicillin 5 U/l, streptomycin 0.5 µg/l, L-glutamine 200 mmol/l, and Hepes (N-2-hydroxy-ethylpiperazine-N-2-ethane-sulphonic acid) 25 mmol/l. Suspensions of 4×10^6 cells per ml were incubated for 2 hours at 37°C in petri dishes. During incubation monocytes adhere to the bottom of the dishes.

After removal of non-adherent cells, dishes were washed at 37°C with Hanks balanced salt solution (HBSS) containing 50 g/l BSA. Chilled HBSS containing 5 g/l BSA was then added to the dishes and the adherent cells were gently removed with a gum stick. The released cells were washed twice at 20°C and resuspended in HBSS containing 5 g/l BSA at a concentration of about 10^6 cells/ml. Twenty ml of the suspension was layered over 10 ml of a suspension of one part 14% ficoll/isopaque and one part Lymphoprep in a centrifuge tube. By centrifugation at 2000 g for 20 min dead cells were spun to the bottom. Cells layered in the interphase were harvested and after washing resuspended in the incubation buffer [6].

Monocytes were identified by morphological and cytochemical criteria in cytocentrifuged smears using an alpha naphthyl acetate esterase method [7]. Monocytes comprised $79\% \pm 9\%$ (mean \pm SD) of total mononuclear leucocytes in the suspension ($n = 27$). The contamination with granulocytes and erythrocytes was insignificant, but a variable number of platelets was present. Using the trypan blue exclusion test monocyte viability was about 95% [8].

Lymphocytes were isolated among the non-adherent cells of the petri dishes; 0.2 g carbonyl iron powder (Grade SF, GAF comp., New York) was added to 10 ml cell suspension. After incubation at 37°C for 30 min phagocytic monocytes and granulocytes were removed with a magnetic stick [2]. The final lymphocyte suspension was contaminated with less than 1% monocytes. The ratio between erythrocytes and lymphocytes was 1:3, whereas the number of granulocytes and platelets was insignificant. The insulin binding capacity of erythrocytes is only 10% of that of lymphocytes [2]. Thus contamination of the lymphocyte suspension with erythrocytes is probably unimportant.

Incubation Buffer

A Hepes buffer was used in all incubation studies. The buffer contained in mmol/l: Hepes, 100; KCl, 4.7; NaCl, 80; MgSO₄, 1.2; CaCl₂, 2.5; NaH₂PO₄, 0.2; Na₂HPO₄, 1.0; and glucose, 5. Human albumin was present at 10–30 g/l. pH was 7.4 at 37°C and 7.8 at 15°C. During incubation for 180 min at 37°C pH of the medium dropped by 0.2 from the original pH.

Insulin Binding Studies

¹²⁵I-insulin iodinated and purified [9] by ion exchange chromatography to a specific activity of about 150 µCi/µg was a gift from Novo Research Institute. About 90% of the iodinsulin contained only one iodine atom per insulin molecule. The iodine atoms were attached to the tyrosine in the A₁₉ position.

All cell types were incubated in duplicate with ¹²⁵I-insulin at a concentration of 35 pmol/l (0.2 ng/ml) in 100 mmol/l Hepes buffer. Incubation time and cell number concentration are given in the legends to the figures. For competition studies native insulin in increasing concentrations was added to the incubation medium. At

the end of incubation 250 µl cell suspension was added to a 1.5 ml microtube containing 1.0 ml icecold buffer. Cell bound and free insulin were separated by centrifugation in a Beckman microfuge for 1 min. The cell-free supernatant (containing free ¹²⁵I-insulin) was carefully aspirated and the cell pellet was washed twice with cold buffer. The tip of the microtube, which contained the cells with bound ¹²⁵I-insulin, was excised and both free and cell-bound radioactivity was counted. Total cell binding indicates the radioactivity of the cell pellet. Non-specific cell binding is the radioactivity of the pellet in the presence of an excess of unlabelled insulin (10^{-5} mol/l). Specific cell binding is defined as the difference between total and non-specific binding. Specific cell binding is expressed as a fraction of the total radioactivity.

Binding Analysis. The results of the binding studies are presented in 3 ways:

- 1) The specific cell bound fraction of insulin at the tracer concentration (35 pmol/l).
- 2) The specific cell bound fraction plotted as a function of total insulin concentration (competition curve).
- 3) The concentration of native insulin necessary to reduce the specific cell bound fraction of ¹²⁵I-insulin at insulin tracer concentration 50% (50% inhibition) is for comparative studies taken as an estimate of apparent receptor affinity.

Determination of Extracellular Trapped ¹²⁵I-Insulin

The fraction of iodinsulin trapped extracellularly after separation of cell bound and free insulin was determined by ¹⁴C-L-glucose (0.55 µCi/ml) incubation, specific activity: 61 Ci/mol, for 1 min at 20°C. The separation procedure was as described above. Radioactivity in the cell pellet was counted in a liquid scintillation counter.

Degradation of ¹²⁵I-Insulin

The degradation of iodinsulin was estimated by its solubility in 10% (w/v) trichloroacetic acid (TCA). The amount degraded was expressed as the percentage contribution to the total amount of ¹²⁵I-insulin.

Degradation of Labelled Insulin Dissociated from Monocytes

Monocytes (7×10^6 /ml) were incubated in duplicate with ¹²⁵I-insulin (140 pmol/l) for 1 hour at 37°C. Cells were recovered and resuspended in 1 ml washout buffer. ¹²⁵I-insulin was allowed to dissociate into the washout medium for 1 hour at 37°C. Then TCA was added to the medium after removing of cells. Using this method total degradation in the washout medium was determined.

Control experiments were carried out under the same conditions but without the presence of labelled insulin for the first hour of the incubation period, but after resuspension of monocytes in the washout medium labelled insulin was added in a concentration similar to the amount of insulin bound to cells in the parallel study. The incubation was continued for 1 hour and the fraction of ¹²⁵I-insulin degraded was determined. ¹²⁵I-insulin degraded at the receptor site is the difference between the fractions degraded in the two washout media.

In the time course studies for dissociation of degraded and non-degraded insulin the media degradation was inhibited by adding native insulin (10^{-5} mmol/l) to the washout medium. Hence, no control experiments were necessary in the latter experiments.

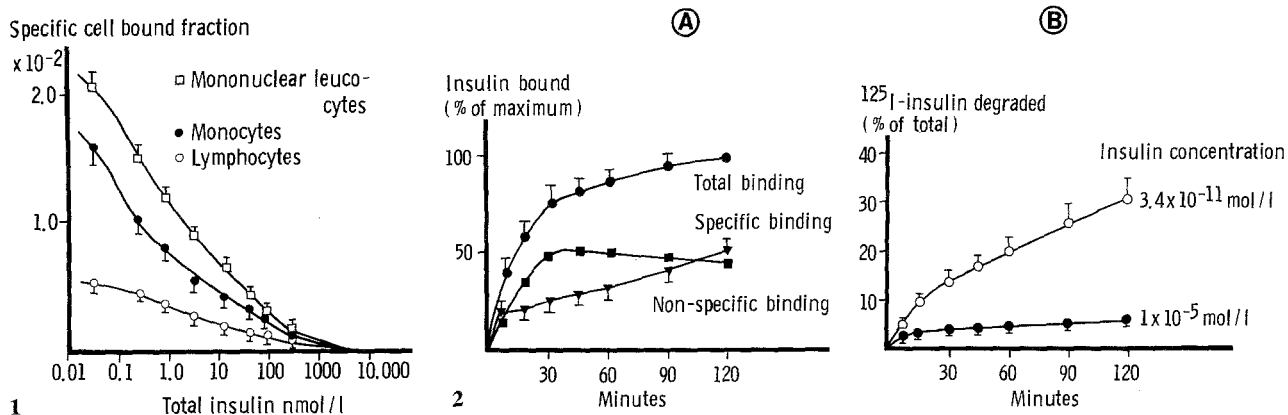


Fig. 1. Insulin binding to mononuclear leucocytes and highly purified preparations of monocytes and lymphocytes in normal subjects (mean \pm SEM, $n = 10$). Insulin binding to cells in purified preparations of monocytes and lymphocytes from each person was adjusted to the concentration of these cells in the suspension of mononuclear leucocytes. The concentration of monocytes was $6.5 \times 10^6/\text{ml}$ and of lymphocytes $35 \times 10^6/\text{ml}$

Fig. 2A and B. Binding and degradation of ^{125}I -insulin. In panel A total cell binding and non-specific cell binding at 37°C are plotted as functions of the incubation time (mean \pm SEM, $n = 6$). Specific cell bound fraction is the difference between the two binding variables. Panel B shows ^{125}I -insulin degraded, in percent of total radioactivity of the supernatant. Degradation of labelled insulin was tested by the precipitability of iodoinsulin in a 10% TCA solution

Degradation of ^{125}I -Insulin Taken up by the Cells

The degradation of iodoinsulin taken up by monocytes was studied using TCA precipitability after insulin had been extracted by a solution of acetic acid (3 mol/l) and urea (6 mol/l) for 1 hour at 15°C .

Metabolic Effects of Insulin

Glucose Uptake. Glucose uptake by monocytes was estimated comparing the glucose concentration in the medium at time zero with that at the end of the incubation. Medium glucose concentration was measured by the method of Feteris [10]. Cells were incubated in the HEPES buffer at 37°C for 90 min without and with increasing concentrations of insulin. A buffer glucose concentration of 2.5 mmol/l was used, because the optimal insulin effect was achieved at this concentration. The standard deviation (of replicates) for determination of glucose uptake was found to be $0.020 \mu\text{mol}/3 \times 10^6$ monocytes/90 min.

Lactate Release. Subsequent to glucose uptake monocytes release lactate. The lactate release was measured comparing the lactate concentration in the medium at the end of incubation to that at zero time. Incubation conditions were identical to those described for glucose uptake. Lactate in the medium was measured enzymatically [12]. The standard deviation for determination of lactate release was found to be $0.033 \mu\text{mol}/3 \times 10^6$ monocytes/90 min.

Determination of Water Space in Monocytes. Intracellular water space of monocytes was measured by the ^{14}C -3-0-methyl-d-glucose uptake. Monocytes (about $5 \times 10^6/\text{ml}$) were incubated with the tracer (0.87 $\mu\text{Ci}/\text{ml}$), specific activity: 59 Ci/mol, for 30 min at 37°C with and without insulin (10^{-8} mol/l). Cells were separated from free ^{14}C -3-0-methyl-d-glucose by centrifugation through silicone oil [2]. Radioactivity in the cell pellet was counted in a liquid scintillation counter.

Statistical Methods

Wilcoxon's test for paired differences was employed for comparison of glucose uptake and lactate release in the cells treated with and without insulin. The precision of the methods for determination of glucose uptake and lactate release was evaluated calculating the standard deviations of duplicates.

Spearman's coefficient of rank was applied in correlation studies.

Results

Insulin Binding to Suspensions of Mononuclear Leucocytes, Monocytes and Lymphocytes

Figure 1 shows that insulin binding to the mononuclear leucocytes of a heterogeneous cell suspension was similar to the total of insulin binding to monocytes and lymphocytes of the purified cell preparations. Monocytes bound significantly ($p < 0.01$) more insulin than lymphocytes at each insulin concentration tested (Fig. 1). The amount of insulin necessary to reduce the specific ^{125}I -insulin binding 50% was for monocytes about 1 nmol/l and for lymphocytes about 3 nmol/l, suggesting a higher receptor affinity in monocytes. Scatchard plots [11] (not shown) were curvilinear and estimated from the intercept on the x-axis the number of insulin receptors on monocytes was about 10 times that of lymphocytes (about 19,000 versus 1,900).

In cell preparations from 10 normal persons the ratio between insulin binding to lymphocytes and

monocytes was studied. We found that this ratio was fairly constant from person to person. At insulin tracer concentration the ratio (k) was 0.063 ± 0.012 (mean \pm SD). The value of k increased with increasing receptor occupancy ($k = 0.078 \pm 0.031$ at the highest insulin concentration tested, Fig. 1).

Specific Insulin Binding, Non-Specific Insulin Binding and Insulin Degradation

A maximum of specific insulin binding to monocytes in purified suspensions was reached after 30 min at 37°C (Fig. 2) and after 80 min at 15°C (Table 1). At 15°C the binding level was maintained for further 40 min (Table 1), whereas the binding decreased in time at 37°C (Fig. 2). Non-specifically bound insulin

Table 1. Time course for ^{125}I -insulin binding to monocytes at 15°C. Total cell binding and non-specific binding are plotted as functions of incubation time. Specific cell binding fraction is the difference between the two binding variables. Binding values are given as percentage of maximal binding ($t = 120$ min, for total binding). Mean values of 3 experiments are shown

	Minutes					
	2	15	30	60	90	120
Total binding	20%	55%	75%	90%	100%	100%
Non-specific binding	10%	12%	10%	12%	14%	13%
Specific binding	10%	43%	65%	78%	86%	87%

averaged 12% of maximally bound insulin at 15°C and was constant (Table 1). At 37°C the non-specifically bound fraction increased as a function of time (Fig. 2) from 18% at 2 min to 50% after 2 hours. At 37°C enhanced degradation of ^{125}I -insulin in the incubation medium was also found (Fig. 2B). Addition of excess insulin reduced degradation of the labelled insulin (Fig. 2B), but the degradation still increased slightly with time. Degradation was constant and insignificant (5%) at 15°C (not shown).

Figure 3A shows how non-specifically bound ^{125}I -insulin at 15°C and 37°C, respectively, dissociated into a washout medium. Iodinsulin non-specifically bound at 15°C dissociated immediately to the washout medium. Only about 5% was still "bound" after 5 min. At 37°C, however, only $2/3$ of non-specifically bound iodinsulin was released after 5 min. Figure 3B shows that about 20% of non-specifically bound iodinsulin dissociated to the washout medium was degraded after 1 hour. To determine the contribution of extracellularly trapped ^{125}I -insulin to the amount of non-specifically bound insulin, we measured the ^{14}C -L-glucose distribution "space". Using this method we found at 15°C that about 10% of non-specifically bound ^{125}I -insulin was attributable to extracellularly trapped radioactivity.

Mechanism of Insulin Degradation

Comparison of Degradation of Prebound and Not Prebound Insulin. The degradation of prebound and not prebound ^{125}I -insulin at 37°C was studied. Forty

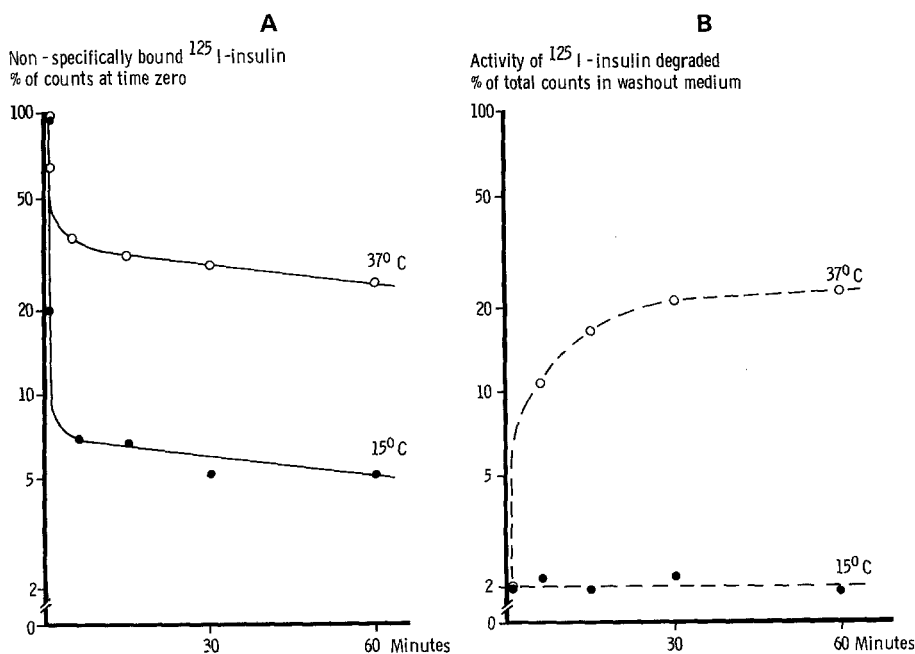


Fig. 3A and B. Release of non-specifically bound ^{125}I -insulin to a washout medium. Panel A shows the release of non-specifically bound ^{125}I -insulin to a washout medium (at 15°C) containing 10^{-5} mol/l native insulin (mean \pm SEM, $n = 5$). Monocytes concentration was 5×10^6 /ml. Panel B shows the degradation of released ^{125}I -insulin as a function of time

two percent of receptor bound ^{125}I -insulin dissociated to the washout medium was TCA soluble after 1 hour. In control experiments (no prebound iodoinulin) monocytes were incubated with labelled insulin at a concentration similar to that of the washout medium following dissociation of ^{125}I -insulin bound to the receptors. Under such conditions insulin binding to its receptor is insignificant. Only 12% of this labelled insulin was degraded. An identical fraction was degraded when ^{125}I -insulin was incubated with the cell free supernatant from the control experiment.

Time Course for Dissociation of Receptor Bound Insulin

^{125}I -insulin bound to monocytes was allowed to dissociate to a washout medium containing excess unlabelled insulin (10^{-5} mol/l), which should totally inhibit insulin degradation in the medium. Insulin was released into the medium in two components, one soluble in TCA and one precipitable in TCA (Fig. 4). The half-time for release was similar for the two components. After dissociation for 1 hour the TCA soluble component averaged 35% of total radioactivity.

Degradation of Receptor Bound Insulin as a Function of Receptor Occupancy. Corrected for non-specifically bound ^{125}I -insulin, the percentage of radioactivity released in a TCA soluble form (degraded ^{125}I -insulin) was independent of the occupancy in a concentration range of 0.2–66.0 nmol/l insulin. The fraction of degraded insulin was about 36%. Thus, binding and degradation of ^{125}I -insulin were proportional.

Acid Extraction of Iodoinulin Taken up by the Cells. By acid extraction of ^{125}I -insulin taken up by monocytes at 37°C we found that the recovery of radioactivity was near to 100% and about 10% of ^{125}I -insulin extracted was degraded. On the other hand less than 5% of iodoinulin extracted from cells incubated with tracer plus native insulin (10^{-5} mol/l) was degraded. At 15°C all insulin extracted was non-degraded.

Insulin Effects on Monocytes

Glucose uptake and lactate release were measured in suspensions of purified monocytes isolated from 17 subjects. Positive correlations were found between the two components measured and the monocyte concentration ($R = 0.84$, $p < 0.001$ and $R = 0.83$, $p < 0.001$, respectively).

Figure 5 shows a linear glucose uptake and lactate

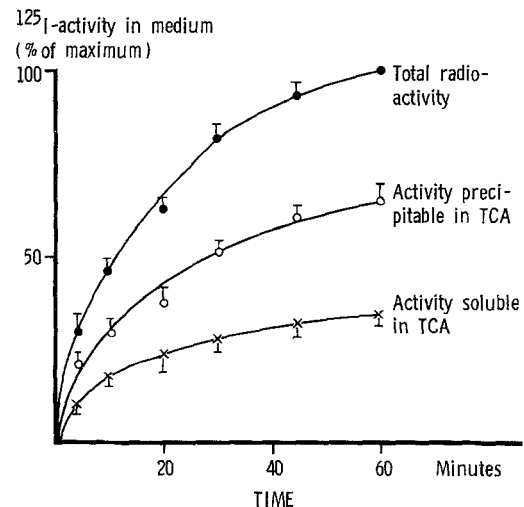


Fig. 4. Dissociation of receptor bound ^{125}I -insulin to a washout medium. Dissociation of degraded and non-degraded ^{125}I -insulin from monocytes ($5 \times 10^6/\text{ml}$) was studied (mean \pm SEM, $n = 3$). The values were corrected for non-specifically bound iodoinulin, which averaged 45% of total binding. At time 60 min more than 90% of receptor bound ^{125}I -insulin was dissociated

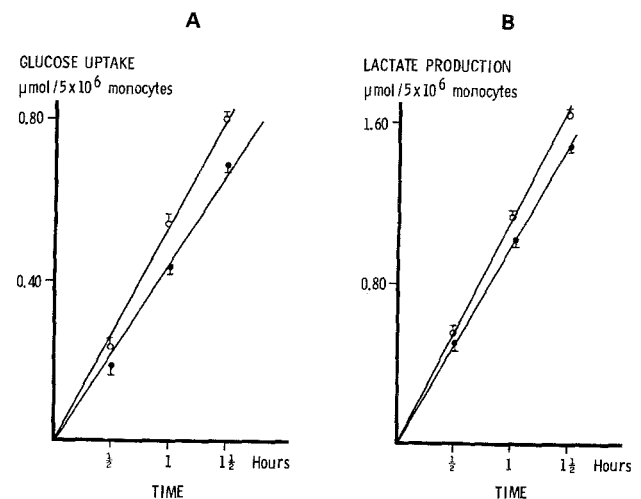


Fig. 5 A and B. Time course for glucose uptake and lactate release in monocytes. Monocyte suspensions ($5 \times 10^6/\text{ml}$) were incubated at 37°C without (●-●) and with (○-○) insulin, 10^{-8} mol/l. Initial glucose concentration was 2.5 mmol/l. Glucose uptake **A** and lactate release **B** are shown

release in isolated monocytes during 90 min. An enhancement both of glucose uptake and lactate release was induced by insulin. This effect was not caused by an increase in the monocyte water space as the total 3-O-methyl-D-glucose equilibrium space of monocytes was not influenced by insulin [mean \pm SD of 6 replicates: 2717 ± 224 cpm/ 5×10^6 monocytes (basal) and 2793 ± 350 cpm/ 5×10^6 monocytes (plus insulin, 10^{-8} mol/l)].

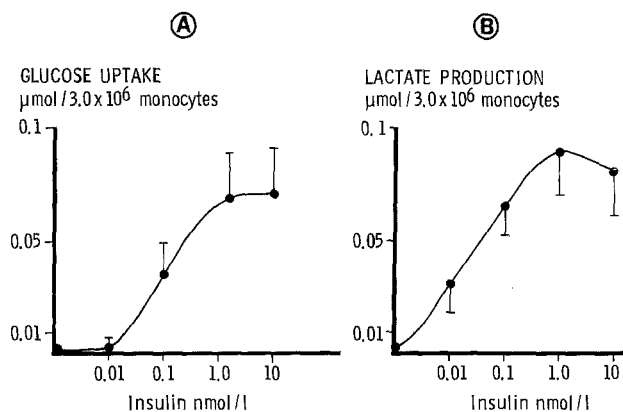


Fig. 6 A and B. Insulin dose-response curves for glucose uptake and lactate release by isolated monocytes. Monocyte suspensions were incubated at 37°C for 90 min (mean \pm SEM, $n = 17$). Initial medium glucose concentration was 2.5 mmol/l. Glucose uptake **A** and lactate release **B** are shown. The insulin induced increase in glucose uptake was statistically significant for insulin concentrations higher than 0.01 nmol/l ($p < 0.01$) whereas the increase in lactate release was statistically significant for all insulin concentrations tested ($p < 0.05$)

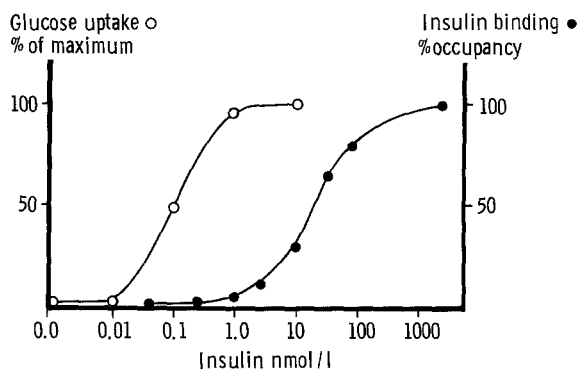


Fig. 7. Dose-response curves for the effect of insulin on glucose uptake and for the insulin binding to monocytes. Glucose uptake ($n = 17$) and insulin binding ($n = 5$) were expressed as percent of maximal effect and percentage of receptor occupancy, respectively. Both variables were determined at 37°C. For binding studies the monocytes were incubated for 40 min. Only about 10% of ^{125}I -insulin in the medium was degraded at that time

Table 2. The effect of cytochalasin B on the insulin mediated glucose uptake in monocytes. Glucose uptake was measured in monocytes from 3 normal persons (mean \pm SD). Insulin concentration was 10^{-8} mol/l and the cytochalasin B concentration was 5 $\mu\text{g/ml}$

	Glucose uptake $\mu\text{mol}/11 \times 10^6$ cells/hour	
	Basal	+ insulin
- cytochalasin B	0.47 ± 0.03	0.60 ± 0.05
+ cytochalasin B	0.15 ± 0.03	0.21 ± 0.05

Insulin stimulation caused a maximal 25% rise in glucose uptake and a 12% rise in lactate release (Fig. 6). The increase in both glucose uptake and lactate release was statistically significant. The insulin effect on both variables was dose-related (Fig. 6A and B).

To investigate the insulin effect on the facilitated diffusion of glucose we blocked this process with cytochalasin B. Table 2 shows that cytochalasin B reduced the basal glucose uptake, whereas the insulin stimulated uptake was unchanged (increased about 30%, Table 2).

Figure 7 depicts the relationship between the dose-response curves for insulin binding and glucose uptake in monocytes at 37°C.

Discussion

The Monocyte Insulin Receptor Assay

Because of technical difficulties in obtaining purified preparations of monocytes a mixed suspension of mononuclear leucocytes is commonly used. Using mononuclear leucocytes it is desirable to correct for insulin binding to lymphocytes before adjustment of monocyte binding to a mean concentration of monocytes. In this study we found that the ratio between insulin binding to monocytes and lymphocytes in normals is fairly constant from person to person. Therefore, it is possible with high precision to estimate the insulin binding to monocytes alone from the binding data obtained from a mixed suspension of mononuclear leucocytes [2].

In studies of insulin binding to purified preparations of monocytes we found that a steady state for the specific insulin binding could be obtained at 15°C, whereas no plateau was reached at 37°C because of a considerable insulin degradation at this temperature. The fall of the binding level at 37°C was not as high as expected, which can be explained by intracellular uptake of degraded ^{125}I -insulin. Degradation in the medium depends on the insulin concentration. Hence, the insulin degradation is greatest corresponding to the first points on the competition curve and lowest corresponding to the last points. The non-specific binding determined from the last point on the competition curve, therefore, is underestimated. So, at 37°C some part of the insulin binding at tracer concentration may be explained by intracellular uptake of ^{125}I -insulin. This phenomenon was first demonstrated by Gliemann and Sonne [13] in studies of rat adipocytes.

At 15°C the non-specifically monocyte bound fraction was constant during 0–120 min incubation.

In contrast, non-specific cell binding increased during the incubation period at 37°C in parallel to the increase in insulin degradation. Non-specifically bound ¹²⁵I-insulin at 15°C was immediately released to a washout medium. Therefore, this binding fraction can be explained by physical adsorption to the cell membrane and by trapping of labelled insulin in the extracellular water space. So, the non-specific cell binding can be reduced by washing the cells in 4°C NaCl before separation of bound and free insulin.

At 37°C two thirds of non-specifically cell bound iodinsulin was released immediately as non-degraded insulin, whereas the remaining one third was released more slowly. After one hour about 20% of the released insulin was found to be TCA soluble. We suggest that the non-specifically bound iodinsulin which can be released within a few minutes may be caused by physical adsorption to the cells plus a small amount of ¹²⁵I-insulin trapped in the buffer among cells. This fraction corresponds to the non-specific cell binding at 15°C. On the other hand the fraction of non-specifically cell bound iodinsulin which was released slowly appears not to be due to a simple cell adsorption. We think that this fraction of insulin might have been taken up into the cells. Gliemann and Sonne's observation that rat adipocytes took up degraded insulin when they were incubated with these products at 37°C is consistent with this interpretation.

Insulin Degradation

In this study we found only an insignificant degradation both of receptor bound insulin and insulin present in the incubation medium at 15°C, whereas insulin degradation was considerable at 37°C. At this temperature insulin was degraded in the medium by "proteases" released from the cells, but also receptor bound insulin was a substrate for degradation. We found that prebound iodinsulin was degraded to a higher degree than the same amount of free iodinsulin. Furthermore, insulin dissociated from the receptor to a washout medium was degraded even though the activity of insulin degrading "proteases" in the medium was inhibited. Finally, we found that the degradation velocity was proportional to the receptor occupancy. About 35% of monocyte receptor bound iodinsulin was released as degraded insulin. When only about 10% of the iodinsulin extracted from the monocytes by acid was degraded most insulin must be degraded by dissociation from the receptor. So, at 37°C 35% of monocyte receptor bound insulin is released as degraded insulin. In rat hepatocytes Terris and Steiner [4] found that about 42% of total bound insulin (without correction for

non specific binding) was released as degraded insulin, while Gliemann and Sonne [13] in rat adipocytes showed that about 50% of the released insulin was degraded. The finding that receptor bound insulin can be degraded was confirmed by Dial et al. [14] using isolated hepatocyte membranes from mice. Therefore, we can conclude that receptor bound insulin is the substrate for insulin degradation at 37°C. Thus, in contrast to insulin binding to the monocyte receptor at 15°C, the binding at 37°C is not a simple bimolecular reaction.

We found that about 10% of iodinsulin taken up by monocytes at 37°C was degraded. Whether this fraction is bound to the receptors or translocated to the inside of the cells, as suggested by Carpentier [15] and Goldfine [16], is a question which cannot be answered from this study.

Insulin Binding and Insulin Effect

Glucose uptake and lactate release were highly correlated with the monocyte concentration in the suspension studied. Therefore it is unlikely that the poor contamination with platelets and lymphocytes has any significant influence on the metabolic variables measured. Furthermore, we found the basal glucose uptake in monocytes to be in the same order of magnitude as reported by King et al. [17].

Previously, no insulin effect on monocytes has been reported. In the present study we found that insulin increases both the glucose uptake and the lactate release in highly purified preparations of monocytes. The effect was dose related with ED₅₀-values within the physiological range. In human granulocytes Leroux et al. [18] have demonstrated a similar effect of insulin on glucose uptake (increased 40%) and lactate release (increased 15%), while there was no effect of insulin on 3-O-methyl glucose influx or glycogenogenesis. They concluded that insulin increases the glucose consumption in granulocytes by an increase of glycolysis. In monocytes we found that cytochalasin B could not eliminate the insulin effect on glucose consumption, indicating that insulin did not primarily stimulate the glucose carrier. On the other hand lactate production increased after insulin stimulation, but only about 50% of the glucose taken up was converted to lactate. Therefore, insulin seems to activate other intracellular pathways, too.

From Figure 6 it can be seen that the half maximal biological effect of insulin was obtained by activation of only a few percent of the receptors, whereas maximal effect was obtained by activation of about 25% of the receptors. These results are in accordance with the findings in adipocytes from rat and mice [19, 20].

Conclusion

Monocytes possess insulin receptors with an affinity constant and a number of sites per surface area in the same order of magnitude as that described in human adipocytes [19]. Furthermore, the glucose metabolism in monocytes is sensitive to insulin. Similar to what has been demonstrated in adipocytes [19, 20] and hepatocytes [4] we found that the monocyte receptor bound insulin is substrate for degradation. So, the insulin receptors on monocytes seem to be fairly similar to the insulin receptors on major target cells for insulin action.

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Dr. H. Beck-Nielsen
Medical Department III
County Hospital
DK-8000 Aarhus, Denmark