Insulin-mimetic Effect of Trypsin on the Insulin Receptor Tyrosine Kinase in Intact Adipocytes*

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It has previously been demonstrated that the insulin-mimetic agent trypsin stimulates autophosphorylation of purified insulin receptors and activates the insulin receptor tyrosine kinase in vitro. We now report the effects of trypsin on whole cell tyrosine kinase activation and insulin receptor autophosphorylation. Trypsin treatment of intact adipocytes produces a time-dependent stimulation of tyrosine kinase activity as measured in lectin extracts containing the insulin receptor, or specifically immunoprecipitated insulin receptor samples. Trypsin treatment of adipocytes also results in a loss of insulin binding capacity, and a linear correlation exists between loss of binding and stimulation of tyrosine kinase activity. Exposure of adipocytes to trypsin is known to result in a time- and dose-dependent cleavage of the insulin receptor α-subunit. Examination of the time courses of stimulation of tyrosine kinase and glycogen synthase activation in our system indicates that the stimulation of tyrosine kinase activity by trypsin occurs with sufficient rapidity and magnitude to be consistent with a role of phosphorylation in the activation of glycogen synthase. Trypsin has further been demonstrated to stimulate autophosphorylation of the β-subunit of the insulin receptor in intact adipocytes. Cells prelabeled with 

\[ ^{32}P\]Pi or for 2 h were exposed to trypsin, and receptors were partially purified over wheat germ agglutinin-agarose columns. Receptors were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and the β-subunit was identified by autoradiography. The protein was extracted and hydrolyzed, and the phosphoamino acids were separated by electrophoresis and quantitated. Two- and five-fold increases in phosphotyrosine were observed with 3 and 10 min of trypsin treatment, respectively. We conclude that trypsin-induced cleavage of the insulin receptor α-subunit is relevant to the ability of trypsin to activate the insulin receptor tyrosine kinase in intact adipocytes. We further conclude that autophosphorylation of the insulin receptor and activation of its tyrosine kinase by trypsin may be important to the insulin-mimetic anabolic effects of trypsin.

The insulin receptor is one of a number of receptors and intracellular proto-oncogenes which are known to be tyrosine kinases (1). In the presence of insulin, the insulin receptor can be shown to autophosphorylate, become activated, and phosphorylate exogenous substrates on tyrosine (2). Furthermore, several endogenous proteins have recently been demonstrated to contain phosphorytrosine after insulin treatment of intact cells (3-5). Receptor autophosphorylation occurs rapidly (6, 7), suggesting that it may be an early event in the signalling of insulin's actions within the cell. However, despite recent evidence suggesting that tyrosine phosphorylation may be important to the insulin-sensitive stimulation of glucose uptake (8, 9), the precise role of phosphorylation in this or other anabolic intracellular actions of insulin remains unclear.

The serine protease trypsin is an insulin-mimetic agent which stimulates glucose uptake (10) and activates pyruvate dehydrogenase (10) and glycogen synthase (10), two regulatory enzymes of lipid and glycogen synthesis. Trypsin has also been demonstrated to evoke autophosphorylation of partially and highly purified insulin receptors (11) and to stimulate insulin receptor-associated tyrosine kinase activity toward exogenous substrates (11). These data, along with reports of selective cleavage of the insulin receptor by trypsin (12, 13), suggest that trypsin may be exerting its insulin-mimetic effects through its action on the insulin receptor. Trypsin therefore appears to be a useful probe in studying the importance of receptor phosphorylation in coupling receptor tyrosine kinase activation with the anabolic effects characteristic of insulin and insulin-mimetic agents. However, the experiments demonstrating trypsin's stimulation of receptor phosphorylation were in vitro studies using solubilized insulin receptors (11). For phosphorylation to be relevant to the insulin-like anabolic effects of trypsin, stimulation of insulin receptor autophosphorylation and tyrosine kinase activation must also occur in intact cells. The present study was therefore undertaken to determine whether trypsin causes in situ phosphorylation of the insulin receptor and activation of the insulin receptor tyrosine kinase.

EXPERIMENTAL PROCEDURES

Materials

\[ ^{1-32}P\]ATP was prepared by Dr. G. E. Vandenhoff (Diabetes Center, University of Virginia, Charlottesville, VA). \(^{14}C\)-I-A14-insulin was from New England Nuclear. \(^{125}I\)-B26-insulin was from Amersham. UDP-[\(^{14}C\)]glucose was synthesized in our laboratory according to published methods (14). \(^{1-3}C\)Pyruvic acid was from Amer- sham. Human serum containing autoantibodies against the insulin receptor was a gift from Dr. G. H. Boden (Health Sciences Center, Temple University, Philadelphia, PA). Rabbit serum containing autoantibodies against amino acids 1313-1325 of the insulin receptor β-subunit was a gift from Lynn Kozma (University of Virginia, Charlottesville, VA). Wheat germ agglutinin coupled to agarose was from Vector Laboratories. Collagenase was from Worthington. Pansorbin

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Trypsin Activates Insulin Receptor Tyrosine Kinase in Situ

was from Behring Diagnostics. BSA (fraction V), phenylmethylsulfonyl fluoride, bacitracin, benzamidine, TLCK, soybean trypsin inhibitor, TPCK-treated trypsin, phosphoserine, phosphothreonine, phosphotyrosine, poly(Glu-Tyr) (4:1), 2-NAD+, thiamin pyrophosphate, and coenzyme A were from Sigma. All reagents for SDS-PAGE were from Bio-Rad.

Methods

Preparation of Intact Adipocytes

Adipocytes were prepared by standard methods (18) from epididymal fat pads of 125-175-g male Sprague-Dawley rats in Krebs-Ringer phosphate buffer (128 mM NaCl, 5.2 mM KCl, 1.4 mM CaCl2, 1.4 mM MgSO4, 10 mM NaH2PO4, pH 7.4) containing 3% BSA. After filtering through a polyester mesh, adipocytes were washed three times, then diluted 1:1 in Krebs-Ringer Tris buffer (124 mM NaCl, 4.8 mM KCl, 1.0 mM CaCl2, 1.0 mM MgSO4, 24.6 mM Trizma (Tris base), 0.18 mM KH2PO4, pH 7.4) containing 1% BSA and treated as described in the figure legends. Reactions were terminated by addition of a 2-fold excess of trypsin inhibitor. Cells were washed with Krebs-Ringer Tris containing 1% BSA and 0.025% trypsin inhibitor and processed as described for the various assays.

Assay of Glycogen Synthase and Pyruvate Dehydrogenase

Washed cells were immediately frozen in liquid N2. For measurement of pyruvate dehydrogenase activity, adipocytes were homogenized and assayed as previously described (16). For measurement of glycogen synthase activity, extracts were prepared as described by Lawrence et al. (17) and assayed as described by Guinovart et al. (18).

Solubilization of Cells

Washed adipocytes were mixed with an equal volume of solubilization buffer containing final concentrations of 50 mM Hepes, pH 7.5, 1% Triton X-100, 10 mM sodium pyrophosphate, 160 mM NaF, 8 mM EDTA, 0.2 mM Na2VO4, 2.5 mM phenylmethylsulfonyl fluoride, 8 mg/ml bacitracin, 1 mg/ml benzamidine, 0.04% soybean trypsin inhibitor as modified from Klein et al. (19) and Yu and Czech (20). The mixture was incubated at 4°C for 45 min, then centrifuged at 150,000 X g for 30 min at 4°C. The resultant infranatants were further purified by affinity chromatography or specific immunoprecipitation as described below.

Antibody Precipitation of Receptors for Assay of Autophosphorylation

Aliquots (0.25 ml) of above infranatants were incubated overnight at 4°C with rabbit serum containing anti-β-peptide antibody (1:50 dilution). Pansorbin was added and, following a 45-min incubation at 4°C, pansorbin-antibody complexes were pelleted in a Beckman Microfuge. Pellets were washed five times with 1.5 ml of buffer containing 50 mM NaF, 1 mM EDTA, 1% Triton X-100, 0.5% deoxycholate followed by one wash with 50 mM Hepes, pH 7.5.

Assay of Receptor Autophosphorylation

Pansorbin-antibody complexes were resuspended in 70 μl containing 50 mM Hepes, pH 7.5, 5 mM MgCl2, 2 mM MnCl2, and 50 μM [32P]ATP (2.5 μCi/μmol) for 30 min at 24°C. The reaction was terminated by the addition of 20 μl of ice cold 0.5% BSA and 0.1 M EDTA. Fifty-μ1 aliquots were mixed with an equal volume of sample buffer (40 μl of Hepes, pH 7.5, 2% SDS, 10% glycerol, 0.1 M dithiothreitol, and 5% β-mercaptoethanol), and samples were boiled for 5 min prior to electrophoresis.

Partial Purification of Insulin Receptors and Protein Determination

Infranatants (as described above) were applied to wheat germ agglutinin-agarose columns pre-equilibrated with column buffer containing 50 mM Hepes, pH 7.5, 0.1% Triton X-100, 0.15 M NaCl, 100 mM NaF, and 50 μM TLCK. Columns were washed with 50 ml of column buffer, then bound glycoproteins were eluted with 1.5-2.5 ml of 0.3 M N-acetyl-d-glucosamine in column buffer. Protein concentrations of eluates were determined by the method of Bradford (21) using BSA as standard.

Assay of Phosphorylation of Exogenous Substrate

Lectin-purified insulin receptor fractions (50 μl) from trypsin-treated or control cells were incubated in a final volume of 70 μl containing 50 μM Hepes, pH 7.5, 5 mM MgCl2, 2 mM MnCl2, and 50 μM [γ-32P]ATP (1.3 μCi/μmol) in the presence or absence of 10 μg of poly(Glu-Tyr) (4:1) for 20 min at 24°C. Reactions were terminated by the addition of 20 μl of ice cold 0.5% BSA and 0.1 M EDTA. Thirty-five-μ1 aliquots were immediately pipetted onto squares (2 x 2 cm) of filter paper (3MM, Whatman) and washed in 10% trichloroacetic acid containing 20 mM sodium pyrophosphate (4 x 30 min). The filters were then washed in acetone, dried, and counted for radioactivity. Nonspecifically bound radioactivity was defined as that bound to the filter in the absence of poly(Glu-Tyr) and was subtracted from that bound in the presence of poly(Glu-Tyr).

Assay of Insulin Binding to Lectin-purified Insulin Receptors

Fifty-μ1 aliquots of lectin extracts were assayed for insulin binding according to the cellulose acetate filtration method of Cuatrecasas (22). Samples were incubated for 30 min at room temperature in the presence or absence of excess unlabeled insulin. Nonspecific binding was defined as that bound to the filter in the presence of excess unlabeled insulin and was subtracted from that bound in the absence of unlabeled insulin.

Affinity Cross-linking of Insulin Receptors

Lectin-purified insulin receptors were incubated for 90 min at room temperature with 1.65 nM [125I]-insulin (1519 Ci/mmol) in the presence or absence of excess unlabeled insulin. Disuccinimidyl suberate in dimethyl sulfoxide was added to a final concentration of 0.1 M, and samples were incubated for an additional 45 min at 4°C. Reaction was terminated by the addition of 100 μM Tris, pH 7.5. Nonimmune or anti-β-peptide antibody-containing serum was added (1:100), and samples were processed as above and subjected to SDS-PAGE under reducing conditions followed by autoradiography.

Preparation of Samples for SDS-PAGE

Antibody Precipitation—1.5-ml aliquots of lectin extracts were incubated overnight at 4°C with human serum containing anti-insulin receptor antibody (1:100 dilution) and 2 mM phenylmethylsulfonyl fluoride. Protein A (Pansorbin) was added and, following a 45-min incubation at 4°C, protein A-antibody complexes were pelleted in a Beckman Microfuge. Pellets were washed two times with 1.5 ml of 50 mM Hepes, pH 7.5, containing 0.1% Triton X-100 followed by three washes with 50 mM Hepes, pH 7.5, without detergent. Sample buffer was added, and samples were boiled for 5 min prior to electrophoresis.

Trichloroacetic Acid Precipitation—0.75-ml aliquots of lectin extracts were precipitated with trichloroacetic acid (10% final concentration) for 10 min at 4°C. Samples were centrifuged for 10 min in a Beckman Microfuge to pellet protein. Protein pellets were washed 4 times with 10% trichloroacetic acid containing 50 mM sodium phosphate, pH 7.5, followed by one wash each with 100% ice cold ethanol and ethanol/ethyl ether (1:1). Sample buffer was added to all precipitates, and the tubes were boiled for 5 min as above.

SDS-PAGE

SDS-PAGE was performed by the method of Laemmli (23) using 3% stacking and 6% resolving gels. The gels were stained with Coomassie Blue, destained, dried, and subjected to autoradiography.

Analysis of Phosphoamino Acids

The radioactive band containing the insulin receptor β-subunit was identified in the trichloroacetic acid-precipitated samples by its known molecular weight and by comparison of its location with a phosphoamino acid standard, and applied over NaOH, then mixed with nonradioactive phosphoamino acid standards, and applied to phosphocellulose TLC plates (Merck). TLC plates were subjected to electrophoresis at pH 3.5 (50 mM pyridine acid+H2O) (25), at 1 kV for 1 h. Plates were dried, phosphoamino acid standards were visualized by ninhydrin staining, and plates were subjected to autoradiography using Kodak XAR-5 film at -70°C.
Trypsin Activates Insulin Receptor Tyrosine Kinase in Situ

Statistical Methods

Results were analyzed for significance by the Student's t test.

RESULTS AND DISCUSSION

In agreement with a previous report from this laboratory (10), exposure of adipocytes to trypsin resulted in a time- and dose-dependent activation of glycogen synthase in the absence of exogenous glucose. To establish a dose-response relationship between trypsin treatment and glycogen synthase activation, adipocytes were incubated with increasing concentrations of trypsin (0.001-0.1%) for 5 min at room temperature (Fig. 1). Under these conditions, maximal stimulation of glycogen synthase activation occurred at a trypsin concentration of approximately 0.01%. In the presence of 0.01% trypsin, Tamura et al. (11) previously observed a time-dependent enhancement of the phosphorylation of solubilized, partially purified rat adipocyte and highly purified human placental insulin receptors (11). Fig. 2 demonstrates that trypsin also causes a rapid activation of glycogen synthase. When adipocytes were incubated at room temperature in the presence of 0.01% trypsin, stimulation of glycogen synthase activation was significant by 0.5 min and was maximal by 10 min of treatment. This stimulation was maintained over the 30-min time period examined. In addition to stimulating glycogen synthase, a 10-min exposure of adipocytes to 0.01% trypsin resulted in a 1.5–2.5-fold stimulation of pyruvate dehydrogenase activity, similar in magnitude to that which is observed with maximal insulin treatment (data not shown). This is also in agreement with an earlier report of pyruvate dehydrogenase activation by trypsin treatment of intact adipocytes from this laboratory (10).

Recently, two laboratories demonstrated that, following insulin treatment of cells, receptor-associated tyrosine kinase activity could be recovered and demonstrated in vitro (19,20). In order to determine whether trypsin treatment of intact adipocytes stimulates trypsin kinase activity, we exposed adipocytes to trypsin or to insulin, then partially purified the glycoproteins containing the insulin receptor over wheat germ aglutinin-agarose and assayed these eluates for their ability to stimulate the incorporation of 32P from ATP into the exogenous substrate poly(Glu:Tyr). As shown in Fig. 3, a 10-min exposure of adipocytes to 0.01% trypsin resulted in a stimulation of tyrosine kinase activity which was similar in magnitude to that produced by maximal insulin treatment. This activation of tyrosine kinase by trypsin is time-dependent, with effects observed by 0.5–1 min and maximal or near maximal stimulation observed by 10 min of trypsin treatment (Fig. 4). Furthermore, comparison of Figs. 2 and 4 reveal that the time course of activation of the tyrosine kinase by trypsin is of sufficient magnitude and occurs sufficiently rapidly to suggest a relationship between it and the activation of glycogen synthase.

Tamura et al. (11) have reported that trypsin treatment of solubilized receptors results in proteolysis of the β-subunit, resulting in a shift of the apparent molecular weight of the β-subunit from 95,000 to 90,000 as determined by PAGE. In intact cells, however, trypsin treatment has no observable effect on the size of the β-subunit (Fig. 5). This difference is probably due to the transmembrane nature of the native β-subunit which makes it less accessible to proteolysis. The
of trypsin inhibitor and rapid solubilization of washed cells. Lectin
Adipocytes were exposed to 0.01% trypsin at room temperature
for the times indicated. Incubations were terminated by the addition
of trypsin inhibitor and rapid solubilization of washed cells. Lectin
extracts were assayed for tyrosine kinase activity as described under
"Experimental Procedures." These data represent one of four similar
independent experiments. Each point represents the mean of four
samples ± S.E. Stimulation of tyrosine kinase activity was significant
by 0.5 min (p < 0.01 for 0.5 min; p < 0.001 for subsequent time
points).

FIG. 4. Time course of activation of tyrosine kinase by trypsin.
Adipocytes were exposed to 0.01% trypsin at room temperature
for the times indicated, and reactions were terminated by the addition
of trypsin inhibitor and rapid solubilization of washed cells. Lectin
extracts were immunoprecipitated from solubilized cells with control serum
(lanes A and P) or anti-β-peptide antibody-containing serum (lanes
B–E). Immunoprecipitates were incubated in the presence of [γ-32P]
ATP as described under "Experimental Procedures," subjected to
SDS-PAGE followed by autoradiography. The band at 95,000 daltons
represents the α-subunit of the insulin receptor. The lighter band
above the α-subunit (130,000 daltons) represents the β-subunit
of the insulin receptor.

apparent intactness of the β-subunit is important in receptor
purification since the intact subunit is sialated and binds to
wheat germ agglutinin columns, thus allowing purification of
trypsinated insulin receptors despite proteolysis of the α-
subunit. It should be further noted, however, that any loss of
receptors due to trypsination would be expected to decrease
the kinase activation response observed. Thus, our data rep-
resent a minimum response with an actual response which
is probably greater than that reported. To address the issue
of receptor recovery, we examined the ability of maximal insulin
concentrations to further stimulate kinase activity in lectin
extracts from control and trypsinated-treated cells. The effect
of insulin was additive to that of trypsin, and, in 10-min trypsin-
pretreated samples, resulted in a slight increase in kinase
activity to a level which was 80% of insulin-treated control
kinase activity levels (data not shown). The 20% loss of
activity may be due to destruction of receptors or to loss of
receptors during purification. However, the 80% recovery of
functional receptors which we observed indicates that suffi-
cient glycosylated residues remain after trypsin treatment of
whole cells to allow the purification of trypsinated insulin
receptors over lectin columns.

To further address the issue of receptor recovery in a
manner independent of lectin purification, we investigated
the time-dependent stimulation of tyrosine kinase activity by
trypsin using immunoprecipitation to purify receptors and
receptors autophosphorylation to assess kinase activity.
When β-subunits from phosphorylated antibody-receptor
complexes were identified on autoradiograms, a time-depen-
dent enhancement of autophosphorylation was observed (Fig.
5). The time course and magnitude of this response were
nearly identical with those which were observed for phosho-
rylation of an exogenous substrate by lectin extracts, with
significant effects observed as early as 1 min and an approxi-
matel 7-fold increase in kinase activity observed by 10 min
of trypsin treatment.

In contrast to what we observed for the β-subunit, trypsin
is known to catalyze rapid cleavage of the insulin receptor α-
subunit in both solubilized receptor preparations (11) and in
intact cells (12, 13). To determine whether a temporal rela-
tionship exists between degradation of the α-subunit of the
insulin receptor and stimulation of tyrosine kinase activity in
the β-subunit, we examined the time course of trypsin action
on the insulin binding and kinase activation of lectin extracts
from trypsin-treated cells. As shown in Fig. 6A, a time-
dependent loss of insulin binding due to trypsin treatment is
associated with increasing activation of the tyrosine kinase.
In four separate experiments, an average of 59% of control
insulin binding remained after a 3-min exposure of adipocytes
to 0.01% trypsin, while less than 10% of insulin binding could
be detected after a 10-min exposure. Although there appears
to be no effect on binding by 1 min of treatment, the increase
in kinase activity is already significant by this time (p <
0.001). However, data from studies in which 125I-insulin was
cross-linked to insulin receptors from trypsinated cells dem-
onstrate that significant proteolysis of the receptors has al-
ready occurred by 1 min of treatment (data not shown). Our
inability to detect this change by insulin binding indicates
that this initial destruction of the receptor does not signifi-
cantly impair the ability of insulin to bind to it, an observation
which has been reported by others (12, 26) as well. As dem-
onstrated in Fig. 6B, a linear correlation (r = 0.92) exists
between trypsin-stimulated tyrosine kinase activity and cleav-
age of the insulin receptor α-subunit as measured by changes
in insulin binding. This is in agreement with data obtained
by Pilch et al. (12) who examined the relationship between
insulin binding and glucose oxidation following trypsin treat-
ment of adipocytes. These investigators concluded that frag-
mentation of the insulin receptor by trypsin was related to its
insulin-mimetic effect to stimulate glucose oxidation. Our
data indicate that trypsin action stimulates the insulin recep-
tor kinase coincident with proteolytic cleavage of the α-
subunit of the receptor. The apparent lack of proteolysis of
the β-subunit suggests that it is proteolysis of the α-subunit
rather than the β-subunit which is responsible for kinase
activation. However, we cannot rule out the possibility that
a small clip of the β-subunit occurs which is undetectable by
conventional methods but which might be related to kinase
activation.

It is now accepted that autophosphorylation of the insulin
receptor activates its associated tyrosine kinase (19, 20, 27,
28). We therefore investigated whether trypsin stimulates
insulin receptor autophosphorylation in whole cells, since a
trypsin-induced increase in receptor phosphotyrosine content
would be further evidence that trypsin activates the insulin receptor tyrosine kinase in situ. When the insulin receptor $\beta$-subunit from trypsin-treated cells which had been prelabeled with $^{32}$PPO$_{4}$ in situ was identified on polyacrylamide gels and examined for radioactivity, a time-dependent increase in the total radioactivity of the $\beta$-subunit was observed. Although the $\beta$-subunit of the insulin-like growth factor 1 receptor is a tyrosine kinase which is activated by autophosphorylation (29) and is similar in size to the insulin receptor $\beta$-subunit (30, 31), it is important to note that rat adipocytes lack insulin-like growth factor 1 receptors (30, 32). Densitometry of the autoradiograms showed an increase in total radioactivity of the $\beta$-subunit of approximately 1.5-fold over control after 3 min of exposure, with approximately 2-fold and 5-fold increases observed after 3 and 10 min of treatment, respectively.

In summary, we have demonstrated that in situ trypsin treatment of rat adipocytes results in stimulation of tyrosine kinase activity. This tyrosine kinase co-purifies with the insulin receptor and several lines of evidence from previous and present work suggest that it is the insulin receptor tyrosine kinase. First, Tamura et al. (11) demonstrated trypsin-induced activation of the insulin receptor tyrosine kinase in highly purified, solubilized insulin receptor preparations. Secondly, we have now demonstrated that trypsin action in situ causes cleavage of the insulin receptor concomitant with its ability to stimulate tyrosine kinase activity in adipocytes. Thirdly, we have demonstrated a linear relationship between destruction of the insulin receptor $\alpha$-subunit as measured by insulin binding and activation of the tyrosine kinase suggesting that the $\alpha$-subunit may be inhibitory with respect to kinase activation. Further, we have demonstrated that exposure of isolated adipocytes to trypsin results in an increase in the phosphotyrosine content of the insulin receptor $\beta$-subunit, an event which is known to be required for activation of the receptor-associated tyrosine kinase. Although our data di-
rectly demonstrate a linear relationship between loss of insulin binding and stimulation of tyrosine kinase activity (Fig. 6B), it is important to note that this relationship is probably not one-to-one. It is possible that after the initial activation of some receptors by trypsin, other nontrypsinized receptors are subsequently activated by transphosphorylation and further contribute to the observed increase in kinase activity. However, if transphosphorylation does occur, it may be a valid and relevant method of propagating the insulin signal as well as that of trypsin.

Finally, our data indicate that trypsin-stimulated insulin receptor autophosphorylation and kinase activation occur under conditions where trypsin is known to have insulin-mimetic metabolic effects on adipocytes, including activation of glycogen synthase and pyruvate dehydrogenase. These data are in keeping with previous results from this laboratory with vanadate and other nontrypsinized receptors as well as those previously reported for vanadate (33, 34), therefore suggest that insulin receptor tyrosine phosphorylation may be an important event in the anabolic effects of insulin-mimetic agents.

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REFERENCES