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DISTINCT RECEPTORS FOR IGF-I, IGF-II, AND INSULIN ARE PRESENT ON BOVINE CAPILLARY ENDOTHELIAL CELLS AND LARGE VESSEL ENDOTHELIAL CELLS

Robert S. Bar and Mary Boes

Veterans Administration Hospital, Dept. of Internal Medicine
and Diabetes-Endocrinology Research Center,
U. of Iowa, Iowa City, IA 52240

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Endothelial cells were cultured from bovine fat capillaries, aortae and pulmonary arteries and their interactions with ^{125}I-IGF-I, ^{125}I-MSA (an IGF-II), ^{125}I-insulin and the corresponding unlabeled hormones were evaluated. Each endothelial culture showed similar binding parameters. With ^{125}I-insulin, unlabeled insulin competed with high affinity while IGF-I and MSA were ≈1% as potent. With ^{125}I-MSA, MSA was ≥ IGF-I in potency and insulin did not compete for binding. Using ^{125}I-IGF-I, IGF-I was ≥ MSA whereas insulin decreased ^{125}I-IGF-I binding by up to 72%. Exposing cells to anti-insulin receptor antibodies inhibited ^{125}I-insulin binding by > 90%, did not change ^{125}I-MSA binding, while ^{125}I-IGF-I binding was decreased by 30-44%, suggesting overlapping antigenic determinants between IGF-I and insulin receptors that were not present on MSA receptors. We conclude that cultured capillary and large vessel endothelial cells have distinct receptors for insulin, IGF-I and MSA (IGF-II).

Insulin receptors are present on most endothelial cells (1-6). However, the cross reactivities among insulin and the insulin-like growth factors (IGFs) with vascular endothelium, especially at the capillary level, are not clear. We have previously demonstrated that endothelial cells cultured from large blood vessels have receptors for insulin and multiplication stimulating activity (MSA) (7), an insulin-like growth factor of the IGF-II class. In the present study, endothelial cells were cultured from bovine fat capillaries as well as from bovine pulmonary arteries and aortae, and their interactions with insulin, IGF-I, IGF-II (MSA) and anti-insulin receptor antibodies were determined.

MATERIALS AND METHODS

1. Cell cultures.

Cultures of endothelial cells from bovine pulmonary arteries and aortae were initiated from vessels obtained from freshly slaughtered steers and heifers as previously described (7). Cells had a uniform appearance, demonstrated an epithelioid growth pattern, were uniformly positive for Factor VIII antigen, synthesized and released prostacyclin, angiotensin-converting enzyme and plasminogen activator. Cultures of capillary endothelial cells were initiated from adipose tissue from the periaortic region and processed as pre-

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viously described by Kern et al. (8). The diameter of vessels contained in the original adipose tissue averaged 4.8 ± 1.6 μ (range 3.5-8.0 μ). Capillary cells grew in an epithelioid pattern, were uniform in appearance, maintained strict density dependence and demonstrated uniform factor VIII immunofluorescence. For experiments, large vessel endothelial cultures were used at passages 6-12 while capillary cells were used at passage 1-3.

2. Binding studies.

A_{14}-[^{125}I]-iodoinsulin was prepared by the method of Lioubin et al. at specific activities of ~300 μCi/μg insulin (9). MSA was purified and labeled with Na^{125}I at a specific activity of ~220 μCi/μg MSA by methods previously described (10). IGF-I was iodinated by the method of Van Obberghen-Schilling (11) with the modification of a lowered concentration of chloramine T, at specific activites of 228-236 μCi/μg IGF-I. MSA was a kind gift of Drs. M. Rechler and S.P. Nissley, Bethesda, MD. IGF-I and the unlabeled IGF preparation were kind gifts of Dr. Rene Humbel, Zurich, Switzerland. The unlabeled IGF contained a partially purified mixture of IGF-I and IGF-II with an activity of 36 mU/mg protein (12).

Binding studies were performed with adherent cells in 6 well (^{125}I-insulin) or 12-well (^{125}I-MSA, ^{125}I-IGF-I) trays as previously described (1,7). Adherent cells were incubated with labeled and unlabeled peptides for 90 minutes, 22°C in Hepes buffer (100 mM, pH 7.8), the supernatant fluid removed, the cells washed extensively with cold buffer then removed from the dish with 0.5N NaOH and counted. Nonspecific binding, defined as the amount of cell-associated ^{125}I-hormone in the presence of maximal unlabeled hormone (10^5 ng/ml insulin, 10^3 ng/ml MSA, 5×10^3 IGF) was subtracted from each binding point to yield specific binding. The NaOH treatment removed all cells and radioactivity from the dish.

3. Anti-insulin receptor antibody studies.

Confluent, monolayer cultures were incubated with serum containing antibodies against the insulin receptor (serum B-2) (13) at serum dilutions of 1:250 and 1:100. Cells were incubated with anti-receptor serum or control serum from normal subjects for 60 minutes at 22°C, washed extensively then evaluated for total and "nonspecific" binding as previously described.

RESULTS

The isotherms for competition studies measuring the binding of ^{125}I-MSA, ^{125}I-IGF-I and ^{125}I-insulin in the presence of their respective unlabeled hormones are given in Figure 1 for capillary, pulmonary arterial and aortic endothelial cells. To facilitate comparison among the 3 cultures, the binding data have been expressed as a percent of maximal ^{125}I-hormone bound. Similar patterns of binding were observed for each endothelial cell. For ^{125}I-MSA (figure 1, left panels), MSA and IGF were equipotent in their ability to compete for receptor binding. Insulin did not alter maximal ^{125}I-MSA binding, even when present at concentrations of 100,000 ng/ml. Maximal specific ^{125}I-MSA binding (tracer alone) averaged 43%, 20% and 17%/10^6 cells for capillary, pulmonary arterial and aortic cells, respectively.

With ^{125}I-IGF-I (figure 1, middle panels) unlabeled MSA and IGF again competed for binding with equal potency. However, in contrast to studies with

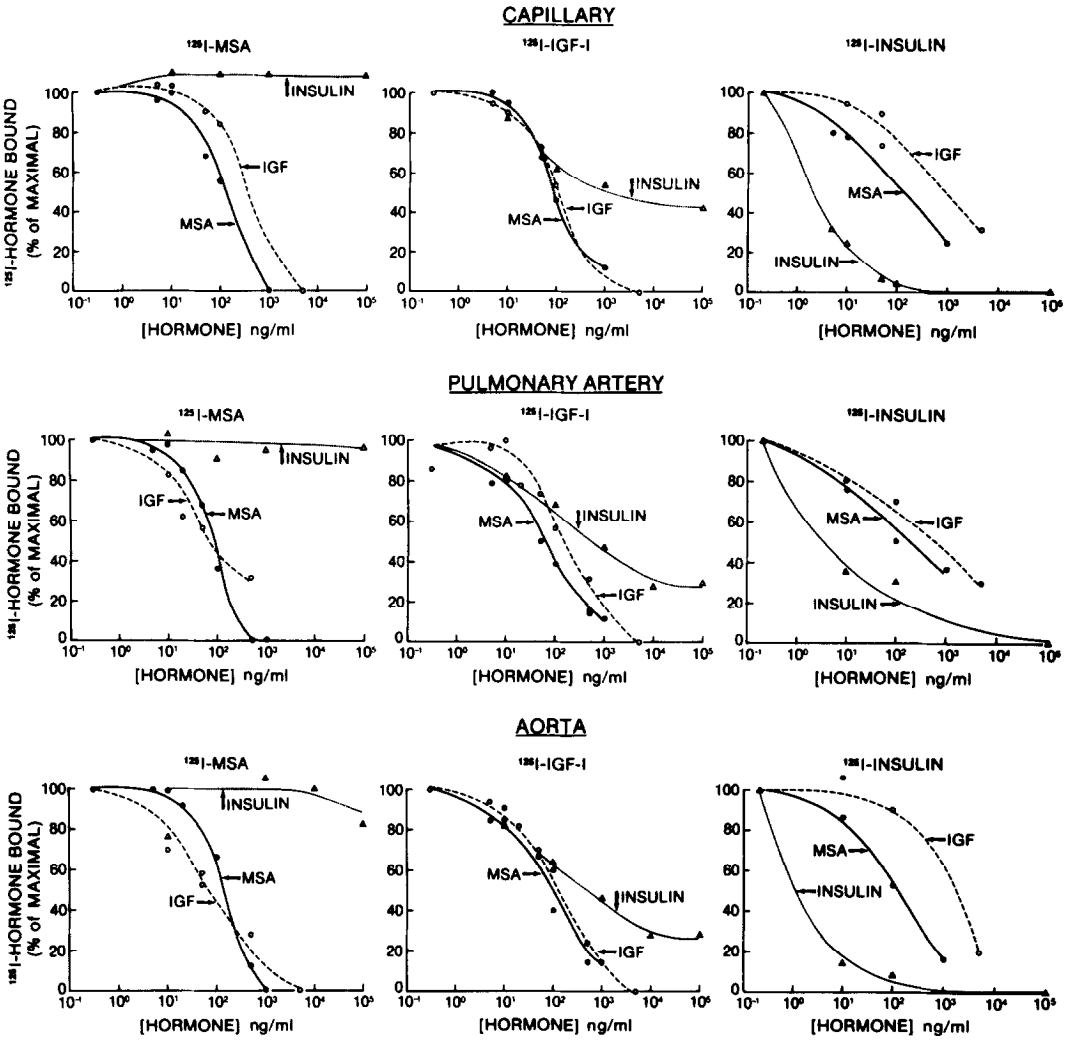

Figure 1. The interactions of ^{125}I-MSA (left panels), ^{125}I-IGF-I (middle panels) and ^{125}I-insulin (right panels) with unlabeled MSA, IGF and insulin in endothelial cells cultured from bovine fat capillaries (top), bovine pulmonary arteries (middle) and bovine aortae (bottom). Data are expressed as % maximal specific binding of the ^{125}I-hormone. Data for capillary and pulmonary arterial cells represent the mean of 2 separate studies, while for aorta reflect a single study.

^{125}I-MSA, unlabeled insulin decreased ^{125}I-IGF-I binding in all three cell cultures. At insulin concentrations of 100 ng/ml, maximal ^{125}I-IGF-I binding was decreased by 30-38%, at 1000 ng/ml binding was decreased by 46-54% and at insulin concentrations of 100,000 ng/ml binding was decreased by 58-72%. Maximal ^{125}I-IGF-I binding averaged 21, 6.3 and 7.8 for capillary, pulmonary arterial cells and aortic cells.

When using ^{125}I-insulin as the labeled ligand (figure 1, right panels) unlabeled insulin competed with high affinity having an ID_{50} [1] averaging 2 ng/ml. Both MSA and the unlabeled IGF preparation also competed for binding but were ~1% as potent as unlabeled insulin. In these studies, the IGF was equal to or slightly less potent than MSA in competing for ^{125}I-insulin binding. Maximal specific ^{125}I-insulin (tracer alone) averaged 6%/10^6 cells for capillary cells, 1.8%/10^6 cells for pulmonary arterial cells and 3.5%/10^6 cells for aortic cells.

Results of exposing capillary endothelial cells to serum containing anti-insulin receptor antibodies are shown in figure 2, with data expressed as a percent of control values. Serum B-2 at a dilution of 1:250 caused an 85% decrease in ^{125}I-insulin binding ($p < .0001$ vs. control[2]) and a 97% decrease at a 1:100 dilution. Similar exposure did not significantly alter ^{125}I-MSA binding but, for ^{125}I-IGF-I binding, caused a 30% decrease at 1:250 dilution ($p < .0001$ vs. control[2]) and a 44% decrease at a 1:100 dilution.

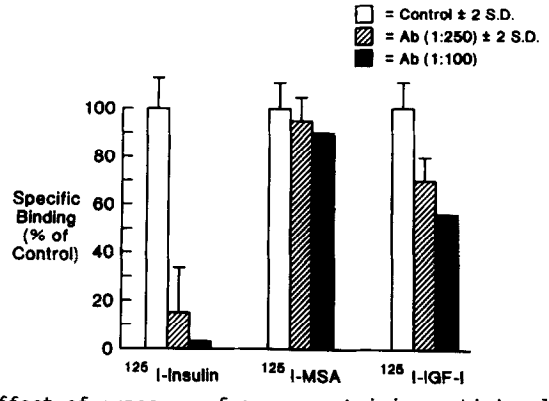

Figure 2. The effect of exposure of serum containing anti-insulin receptor antibodies on the binding of ^{125}I-insulin (left), ^{125}I-MSA (middle) and ^{125}I-IGF-I (right). Data are given for control (open bars), serum B-2 at 1:250 dilution (hatched bars) which represent the mean ± 2 standard deviations of 4 studies and for serum B-2 at 1:100 dilution (closed bars), which represents a single study.

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1 ID_{50} = Concentration of unlabeled ligand required to decrease maximal ^{125}I-ligand binding by 50%.

2 Student's t-test

DISCUSSION

In the present study, we present evidence that cultured bovine endothelial cells from both capillaries and larger vessels have distinct receptors for insulin, IGF-I and MSA (IGF-II). That endothelial cell receptors for IGF-I and IGF-II were indeed distinct was supported by 2 types of data. Insulin competed for IGF-I binding but did not compete for IGF-II binding. Furthermore, serum containing anti-insulin receptor antibodies partially inhibited IGF-I binding but had no effect on IGF-II binding, suggesting overlapping antigenic determinants between receptors for insulin and IGF-I that are not present on the IGF-II binding sites.

These distinct cellular receptors are likely to have relevance in 2 general areas of endothelial function. First, it is becoming clear that insulin can alter several cellular functions of endothelial cells, particularly those derived from capillary sources. Thus, King et al. (2) demonstrated that insulin, at physiologic concentrations, stimulates glucose incorporation into glycogen in retinal capillary endothelial cells but not in large vessel endothelial cells. Several investigators have also shown clear effects of insulin on glucose and lipid metabolism in isolated microvessel preparations from cerebral and retinal sources, with each microvessel preparation containing a predominance of endothelial cells (3,4). Second, the endothelial cells may also function in the transport of intact insulin out of the vascular compartment. After binding to the endothelial cell receptor, both our group and King et al. have shown that a portion of the receptor-bound insulin then moves to other cellular compartment(s) and is rapidly extruded from the cell as intact insulin (14,15). That the endothelial cell is capable of appropriate vectorial movement of insulin has been indicated by 2 types of studies. In one (15), insulin was shown to be transported across endothelial cells grown on nucleopore filters that form a limiting cellular membrane. Blocking insulin binding with anti-receptor antibodies or excess unlabeled insulin significantly inhibited this transport process. In the other experimental model (16), the migration of insulin out of the capillary space to its tissue site of action was evaluated in perfused hearts, a

system demonstrated to have several insulin sensitive cardiac muscle functions and specific binding sites for insulin on the capillary endothelium (6). Blocking the capillary insulin binding sites by pretreatment with unlabelled insulin inhibited the appearance of intact insulin in cardiac muscle by > 75% (16).

Interactions of IGFs with vascular endothelium could result in similar effects, i.e. alteration of endothelial cell function and transport of IGF by the endothelial cell. Indeed, we have already shown that MSA is a potent stimulator of proteoglycan synthesis in cultured pulmonary arterial endothelial cells (17). Insulin had no effect on proteoglycan synthesis in these same cells indicating that the MSA stimulation of proteoglycan synthesis represented a specific effect mediated through an IGF receptor. Although no direct data are available for IGF processing and transport by endothelium, a recent study by Mottola et al. (18) indicated that placenta, a tissue rich in vascular endothelium and possessing receptors for IGF-I and IGF-II similar to those reported in the present study (L. Harrison, personal communication), can transport ^{125}I-IGF-II from the maternal circulation to the fetal circulation in an intact form. The exact roles of the specific endothelial receptors in each of these processes, i.e. mediating alterations of endothelial cell function and transport of IGFs by endothelium, are areas of obvious importance and interest requiring further study.

ACKNOWLEDGEMENTS

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