Androgen- and Estrogen-Dependent Regulation of Insulin-Degrading Enzyme in Subcellular Fractions of Rat Prostate and Uterus

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Innumerous data support the fact that insulin-degrading enzyme (IDE) is the primary enzymatic mechanism for initiating and controlling cellular insulin degradation. Nevertheless, insulin degradation is unlikely to be the only cellular function of IDE, because it appears that some cellular effects of insulin are mediated by IDE as a regulatory protein. Insulin-degrading enzyme shows a significant correlation with various cellular functions, such as cellular growth and differentiation, and the expression of IDE is developmentally regulated. Besides insulin, other substrates are also degraded by IDE, including various growth-promoting peptides. It has also been shown that IDE enhances the binding of androgen to DNA in the nuclear compartment. It is also known that the androgen hormones have a stimulatory effect on prostate growth, and that estradiol stimulates uterine growth. To establish whether IDE is regulated by a cellular prostate/uterine growth stimulus, the present study assessed whether IDE was modified in quantity and activity during proliferative conditions (castration + testosterone in the male rat, or castration + estradiol or the proestrus phase of the estrous cycle in the female rat) and autolysis (castration or the metestrus phase of the estrous cycle) using cytosolic and nuclear fractions of rat prostate and cytosolic fractions of rat uterus. The activity and amount of IDE decreased in the cytosolic fraction with castration and during metestrus, and increased with testosterone or estradiol treatment and during proestrus. In the nuclear fraction, the quantity of the IDE followed the same pattern observed in the cytosolic fraction, although without degradative activity. The data presented here suggest that IDE may participate in prostatic and uterine growth and that the testosterone or estradiol and/or prostate and uterus insulin-like growth factors may be important factors for the expression and regulation of IDE in the prostate and uterus. Exp Biol Med 230:479–487, 2005

Key words: insulin-degrading enzyme; testosterone; estradiol; castration; prostate; uterus; estrous cycle

Introduction

Insulin-degrading enzyme (IDE) is an intracellular protein that degrades insulin and other peptides and hormones (1). Insulin degradation is a regulated process and is considered an integral part of the hormone interaction with its target tissues (2, 3). Insulin-degrading enzyme also degrades insulin-like growth factor-I and -II (IGF-I and -II; Ref. 4) and transforming growth factor-α (TGF-α; Refs. 5, 6) that, together with insulin, produce biological effects that induce growth and differentiation (7). In addition to degradation, IDE has multiple cellular functions, including binding and regulatory functions, evidencing a more direct role of this enzyme in generating insulin effects (2). For example, it has been demonstrated that IDE interacts with and enhances the DNA binding of androgen receptor (AR) and glucocorticoid receptor (GR), suggesting that IDE may be important for the transcriptional activity of steroid receptors (8). Further, Kuo *et al.* (9) have demonstrated that IDE is differentially expressed and regulated during development and growth in various tissues, particularly those of the male reproductive system. All of these observations suggest that IDE is involved in the regulation of cell growth and development. However, the physiological role of IDE is yet to be established. To evaluate the regulation of IDE by stimulus of cell proliferation and apoptosis, we used the androgenic regulation of rat prostate gland and the estrogenic regulation of rat uterus as experimental models. The objective of the present study was to examine whether IDE is modified in quantity and

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activity in the cytosolic and nuclear fractions of a rat prostate homogenate and in the cytosolic fraction of a rat uterus homogenate in conditions of cell proliferation (castration + testosterone for male rats, and castration + estradiol or during the proestrus phase of the estrous cycle for female rats) or apoptosis (castration or the metestrus phase of the estrous cycle). The results demonstrated that the level and activity of IDE increased in the cytosolic fraction during the induction of cell proliferation and decreased in conditions of apoptosis in both tissues. In the nuclear fraction of the prostate homogenate, the quantity of the IDE followed the same pattern observed in the cytosolic fraction, although, interestingly, without degradative activity.

Materials and Methods

Materials. Crystalline pork insulin was kindly provided by Bio-Bras (Belo Horizonte, Brazil). Carrier-free ^{125}I was obtained from Amersham International (Buckinghamshire, UK). Sephadex G-50 Fine was obtained from Pharmacia (Uppsala, Sweden). Peptide synthesis was performed by the Instituto de Quimica, USP (São Paulo, Brazil). Hemocyanin and activated agarose (Sulfolink Coupling Gel) were purchased from Pierce Chemical Co. (Rockford, IL). Reagents for polyacrylamide gel electrophoresis (SDS-PAGE), DEAE-Bio-Gel A Agarose and Affi-Gel Hz were obtained from Bio-Rad (Hercules, CA). Testosterone propionate was obtained from Sigma Chemical Co. (St. Louis, MO). Estradiol benzoate was obtained from Hoechst Marion Roussel S/A (São Paulo, Brazil). All drugs were of analytical grade.

Animals, Treatments, and Preparation of Tissue Extracts. Adult male and female Wistar rats weighing 250–300 g, bred in our animal facilities, were housed under a controlled environment (temperature, 25°–29°C; lights on from 0600 to 1800 hrs) with free access to standard laboratory chow and tap water. Castration in the male rat was performed via a scrotal incision under ether anesthesia. After castration, the rats were divided into two groups. Three days after castration, one group (Castrated + T; $n = 5$) received 300 μg/100 g body weight of testosterone propionate in corn oil injected subcutaneously daily for 3 days. The other group (Castrated) received vehicle instead of testosterone propionate. All female rats used in the experiments showed regular 4–5 day estrous cycles monitored by vaginal cytology for 2 weeks before the initiation of the experiment. The animals were ovariectomized and, 3 days later, treatment was begun with 2 μg/100 g body weight of estradiol benzoate (OVX + EB; $n = 5$) in corn oil injected subcutaneously daily for 3 days. One group of ovariectomized (OVX; $n = 5$) rats was used as a control and received vehicle instead of estradiol benzoate. Rats were sacrificed 24 hrs after the final injection and 6 days after castration. Additionally, intact female rats were sacrificed on the day of proestrus ($n = 5$) or during metestrus ($n = 5$). After the treatment period previously described, the animals were sacrificed using ether anesthesia, and their ventral prostates or uteri were collected quickly, weighed and placed immediately, and either stored in liquid nitrogen for posterior Western blot analysis and insulin degradation study or were used for determination of dry weight. Our institution's animal care committee approved all treatments.

Preparation of Cytosolic and Nuclear Fractions From Rat Ventral Prostates. All operations were performed at 0°–4°C. Ventral prostates or uteri (500 mg, pooled from three to four rats for each experiment) were obtained from intact Wistar rats, from castrated rats, and from castrated plus testosterone- or estradiol-treated rats; cut into small pieces, and homogenized in 2 ml of buffer A (1.5 m*M* EDTA, 2 m*M* mercaptoethanol, 15% glycerol [vol/vol], 3 m*M* $MgCl_2$, 3 m*M* $CaCl_2$, and 10 m*M* Tris-HCl, pH 7.4), by 10 strokes of a motor-driven Teflon-glass homogenizer (7 ml; Kontes, Vineland, NJ). The homogenate was filtered through nylon gauze and centrifuged at 700 *g* for 10 mins. The supernatant was centrifuged at 100,000 *g* for 60 mins. The resultant supernatant was considered a cytosolic fraction. The nuclear pellets were resuspended once in 3 ml of buffer B (buffer A plus 1% Triton X-100 and 10% ethanol; Ref. 10), stirred, and resedimented. This washing procedure was repeated twice with buffer A. After centrifugation (2000 *g* for 3 mins), the pellet was resuspended in buffer C (buffer A plus 0.5% Triton X-100 and 400 m*M* NaCl) and kept under agitation (every 15 mins) for 2 hrs at 4°C, before centrifugation at 10,000 *g* for 5 mins. The supernatant was kept as the nuclear fraction. Proteins from nuclear and cytosolic fractions were precipitated with ammonium sulfate (0.42 g/ml of supernatant, 60% saturation; Ref. 11). The suspension was centrifuged at 17,000 *g* for 20 mins. The supernatant was discarded and the precipitate was dissolved in buffer D (1.5 m*M* EDTA, 10% glycerol [vol/vol], 10 m*M* $MgCl_2$, and 10 m*M* Tris-HCl, pH 7.4). This material was used for Western blot and ^{125}I-insulin degradation studies.

SDS-PAGE and Immunoblotting. Electrophoresis and immunoblotting were carried out with minigels (7 cm × 8 cm × 1 mm) containing a 10% gradient of polyacrylamide, following established procedures (12, 13). Samples (25–30 μg of protein) were subjected to electrophoresis for 45 mins at 200 V. After electrophoresis, the gels were soaked in transfer buffer (25 m*M* Tris-HCl, 192 m*M* glycine, and 20% [vol/vol] methanol, pH 8.3) for 10 mins, sandwiched between a sheet of nitrocellulose paper and two sheets of blotting paper, and assembled in an immunoblotting apparatus (Bio-Rad), in which proteins were electrotransferred to nitrocellulose paper at 70 V (350 mA) in transfer buffer. The membrane was blocked for 2 hrs at room temperature with 5% of albumin in 20 m*M* Tris-HCl, pH 7.5, and 0.154 *M* NaCl. Then, the nitrocellulose was incubated overnight at 4°C with anti-p15 (1:500 dilution), washed, and the antibody was bound and detected with alkaline phosphatase–

conjugated goat anti-rabbit IgG (Bio-Rad), according to manufacturers' protocols.

Preparation of IDE Antibody. The synthetic peptide, YKEMLAVDAPRRHK (p15), corresponding to residues 940 to 953 of rat IDE sequence (14) plus a cysteine added to the N-terminal, was prepared by the Instituto de Quimica, USP. The peptide was coupled to keyhole limpet hemocyanin (KLH) using maleimide (inject-activated immunogen conjugation kit; Pierce), mixed with complete Freund's adjuvant, and injected into two Norfolk-Californian female rabbits (LD or LM). The coupled-peptide preparation was 175 μg of p15 per 1 mg of KLH. The immunization scheme was as follows: 150 μg of coupled p15 was injected into rabbits in the first and the second injection (first booster). The second and the third boosters were 50 μg of coupled p15. All boosters were performed monthly using incomplete Freund's adjuvant. Blood was collected between 15 and 20 days after each booster, and the antibody was tested for specific reactivity with the labeled peptide (p15). Antibody titer was controlled by radioimmunoassay (RIA; 5 pg/ml of ^{125}I-p15) and variable serum dilution. When an adequate titer of anti-p15 was reached, specificity and sensitivity was controlled with cytosol from different rat tissues, using a 1:30,000 dilution of anti-p15. The specificity was assumed as the superposition (parallelism) of the competitive curves obtained with 50–1000 pg/ml of p15 pure peptide as standard and different cytosolic protein concentrations (0.33–4 mg/ml). Anti-p15 was immunopurified by affinity chromatography using an agarose-bound peptide (p15) column (Sulfo-link Coupling gel; Pierce). After elution with 0.1 m*M* glycine, pH 2.5, the eluate containing anti-p15 was dialyzed and concentrated. Both IgG antibody and immunopurified IgG antibody were used. Immunoblot comparison of LD antibody (450 μg/ml) suggests a 700 times purification of IgG anti-p15.

Degradation Assay. ^{125}I-insulin was predominantly labeled in tyrosine A-14, with a specific activity between 250 and 300 mCi/mg and conserved biological activity (15). ^{125}I-insulin degradation was measured by trichloroacetic acid (TCA) precipitation (16). Cytosolic and nuclear fractions (protein concentrations ranging from 25 mg/ml to 125 mg/ml) in 500 μl of 20 m*M* Tris-HCl, pH 7.4, and 10 m*M* $MgCl_2$ with ^{125}I-insulin were incubated at 32°C during 15 mins. The reaction was stopped by the addition of 200 μl of 50% (v/v) TCA and 300 μl of 1% (w/v) bovine serum albumin (BSA) at 4°C, kept for 120 mins at room temperature or overnight at 4°C, then centrifuged in a Sorvall RC-2B at 4000 rpm for 15 mins at 4°C, after which the pellets and supernatants were separated. Degradation was calculated as the increase in ^{125}I-insulin fragments (TCA soluble) in the supernatant. This reaction was linear in relation to protein concentrations (25 mg/ml to 125 mg/ml) and time of incubation (5 mins to 60 mins) at 32°C.

Other Analysis. Protein concentrations were determined according to Bradford (17) using bovine serum albumin as standard. ^{125}I-insulin control (precipitation with TCA without cytosolic fraction) contained more than 92% of intact insulin; hence, the experimental results were corrected for this value. Values are given as percent of ^{125}I-insulin degradation per milligram of cytosolic proteins/ml $\times 10^{-1}$ (3). Data are expressed as the mean ± SEM (n = 3 to 9 experimental determinations) of at least two different experiments. Statistical comparisons between groups were performed using the Student's t test.

Results

To study whether IDE is modified in quantity and activity in the cytosolic and nuclear fractions of a rat prostate homogenate in conditions of cell proliferation (castration + testosterone) or apoptosis (castration), we measured the quantity of IDE separately in cytoplasmic and nuclear fractions by Western blot. The same subcellular fractions were also used to study insulin-degrading activity. Supernatant (100,000 g for 60 mins) and nuclear (800 g for 10 mins, pellet) protein extracts from ventral prostate homogenates were considered as cytosolic and nuclear fractions, respectively. These fractions were used to study insulin-degrading activity and relative IDE protein levels from ventral prostates of intact, 6-day castrated, and 3-day castrated rats after 3 days of testosterone administration. Immunoblotting with antisera obtained against the carboxyl-terminal (p15 peptide) region of IDE along with the TCA method were used for detecting the presence and the activity of IDE at a nuclear and cytosolic fraction level in all of the experimental groups. The Western blot analyses reveal (Fig. 1A and B, upper panel) a 110-kDa band corresponding to the IDE protein in all groups and in both subcellular fractions (nuclear and cytosolic). The relative IDE protein level, as determined by densitometric measurement of Western blotting, indicated a 2.4- and 2.7-fold reduction of IDE protein levels from 6-day castrated rats compared with intact rats in nuclear and cytosolic fraction, respectively. Three days after the administration of testosterone to castrated rats, IDE protein levels increased approximately 4.4- and 4.0-fold compared with castrated rats in nuclear and cytosolic fractions, respectively. Figure 1C shows that there is a linear correlation (r^2 = 0.97) between the amount of IDE in nuclear and cytosolic fractions, as determined by comparative densitometric analysis of IDE Western blot (data derived from Fig. 1A and B). These results suggest that the alteration observed in IDE protein level relative to the different androgenic status is similar in both subcellular fractions. To assess whether the alteration observed in the IDE level is associated with IDE activity, we determined the ^{125}I-insulin degradation in the same protein extracts from the three experimental groups. The insulin degradation was not detectable in the nuclear fraction of any group. In the cytosolic fraction, the insulin degradation was linear with the amount of proteins and incubation time at 32°C in the three experimental groups.

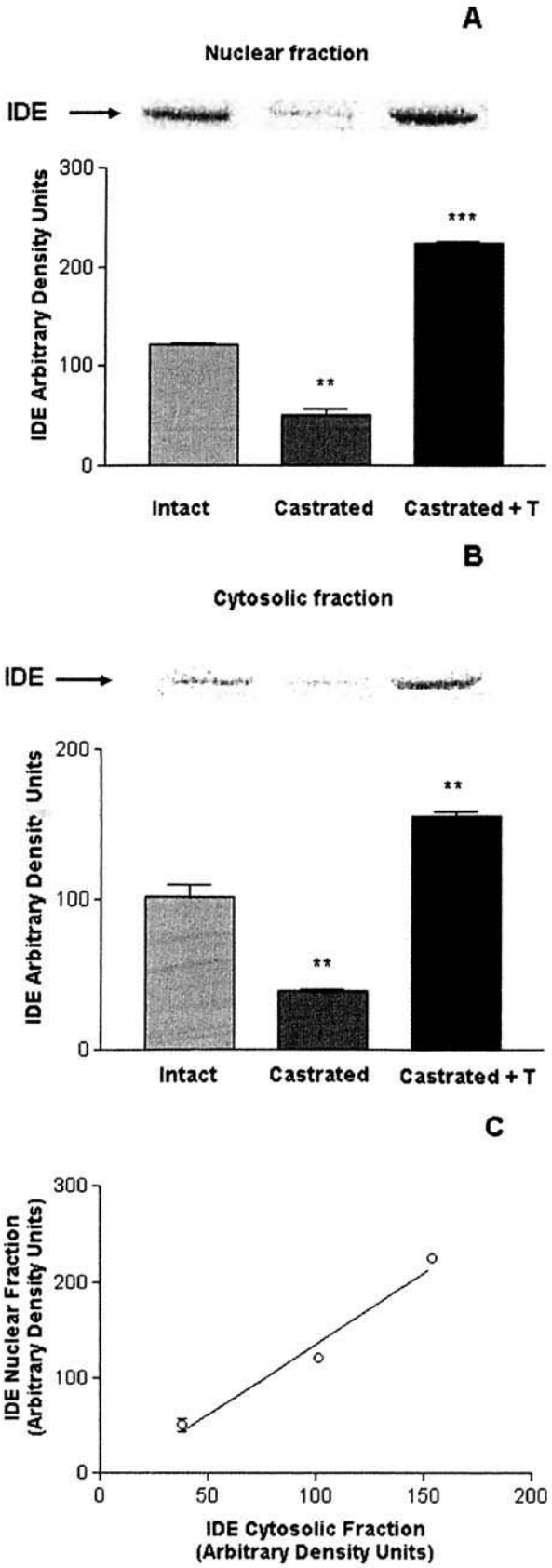

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Figure 1. Insulin-degrading enzyme protein levels as determined by Western blot analysis. Densitometric measurement of relative IDE protein levels in nuclear (A) and cytosolic fractions (B) from ventral prostates of intact rats, 6-day castrated rats, and 3-day castrated rats, after 3 days of testosterone administration. (C) Correlation between IDE protein levels in nuclear and cytosolic fractions; $r^2 = 0.9724$. Data are expressed as means ± SEM; $n = 3$. $^{**}P < 0.005$ vs. intact; $^{***}P < 0.001$ vs. intact; for conditions, see Materials and Methods.

Figure 2 illustrates the effect of castration and of castration following the administration of testosterone on cytosolic IDE activity. The insulin degradation analysis, Figure 2A, indicated a 1.6-fold reduction of IDE activity in 6-day castrated rats compared with intact rats, and a 2.3-fold increase of IDE activity in the testosterone-treated group compared with the castrated group. Insulin degradation plotted against IDE amount in all three groups (Fig. 2B; data derived from Figs. 1B and 2A) results in a linear correlation ($r^2 = 0.995$), suggesting that the altered insulin degradation observed in the cytosolic fraction in all three groups is related to the amount of IDE expressed as a consequence of the different androgenic status. In another series of experiments, we explored whether IDE is also modified in quantity and activity in cytosolic fractions of a rat uterus homogenate in conditions of cell proliferation (OVX + estradiol and proestrus) or apoptosis (OVX and metestrus). Immunoblot and activity analysis were performed on cytoplasmic fractions of rat uterus, as described in Materials and Methods. An immunoreactive band (with antisera obtained against the carboxyl-terminal, p15 peptide) at 110 kDa was observed in cytoplasmic fractions from uteri of OVX and OVX + EB rats and from uteri of rats in proestrus or metestrus, as shown in Figures 3A and 4A (upper panel), respectively. Similarly, in cytosolic fractions from uteri of female rats, the relative IDE protein, insulin degradation, and uterine weight increased by 62.5%, 38.0%, and 50.1%, respectively, concomitant with estradiol-induced cell proliferation (as shown in Fig. 3A and B). These parameters were also studied in the physiological conditions of a high estradiol level (proestrus) and a low estradiol level (metestrus). Figure 4A and B illustrates the increase of 66.9%, 17.4%, and 35.9% of the relative IDE protein, insulin degradation, and uterine weight, respectively, in proestrus as compared with metestrus.

Discussion

It has been shown that IDE is regulated during development and is differentially expressed in various rat tissues, supporting the possibility that IDE plays a physiological role in the regulation of cellular growth and development (9). In the present study, we evaluated the activity and quantity of the IDE during situations of cell proliferation and apoptosis *in vivo* in the adult rat. We used the prostate and the uterus as suitable models of induced

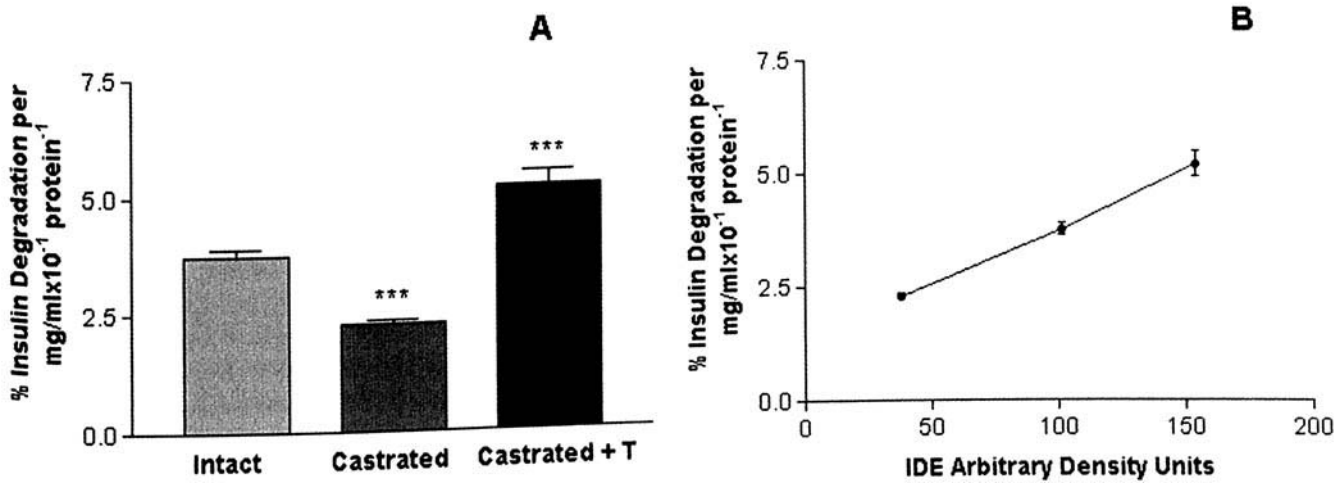

Figure 2. (A) Cytosolic ^{125}I-insulin degradation from ventral prostates of 6-day castrated rats and castrated rats after administrations of testosterone. Different concentrations of cytosolic proteins (60, 90, 120 mg/ml from prostate; 100,000 *g* for 60 mins, supernatant) were incubated with ^{125}I-insulin (20,000 cpm) in 10 m*M* $MgCl_2$ in 20 m*M* Tris-HCl, pH 7.4, for 15 mins at 32°C. The reaction was terminated by addition of 0.4 vol TCA (50% v/v) and 0.6 vol bovine albumin (1% w/v) at 4°C. After centrifugation, pellet and supernatant were separated, and the pellet was washed and radioactivity measured (radioactivity total = cpm pellet + cpm supernatant). The radioactivity in the supernatant was considered degraded ^{125}I-insulin. The degradation was linear in relationship to the incubation time and cytosolic protein concentrations. Values are given as percent of degraded ^{125}I-insulin (see Materials and Methods) per mg/ml $\times 10^{-1}$ of cytosolic proteins. Results are the means ± SEM of nine determinations. ***$P < 0.001$ vs. intact, unpaired *t* test, 2-tailed. (B) Correlation between percent of insulin degradation and IDE protein levels in cytosolic fractions; $r^2 = 0.9950$.

apoptosis by castration or of cell proliferation by testosterone/estradiol treatment of castrated rats. In the present study, we found that, under the condition of cell proliferation, the activity and quantity of the IDE increases in the cytosolic fraction of the prostate homogenate, whereas, under the condition of apoptosis, there is a decrease in these two parameters. We also detected the presence of IDE in the nuclear fraction of the prostate homogenate. In this fraction, the quantity of the IDE followed the same pattern observed in the cytosolic fraction, although no degradative activity of this enzyme was found. Our study demonstrated a positive correlation between ^{125}I-insulin degradation and the relative quantification of IDE in the cytosolic fraction, indicating that the activity of the IDE

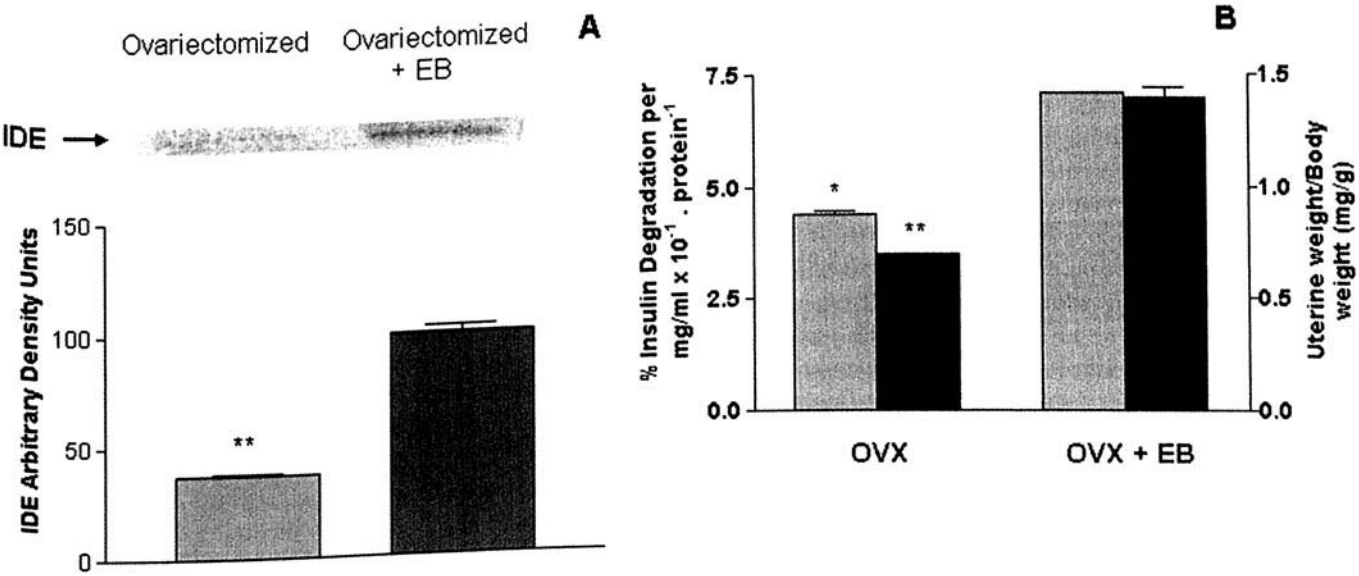

Figure 3. (A) Insulin-degrading enzyme protein levels as determined by Western blot analysis. Densitometric measurement of relative IDE protein levels in cytosolic fractions from uteri of 6-day castrated rats (OVX) and 3-day castrated rats after 3 days of estradiol administration (OVX + EB). Data are expressed as means ± SEM ($n = 5$). **$P < 0.001$ vs. OVX + EB. (B) Cytosolic ^{125}I-insulin degradation (light gray column) and uterine weight/body weight (mid gray column) from uteri of 6-day ovariectomized rats and ovariectomized rats after administrations of estradiol. Values of ^{125}I-insulin degradation are given as percent of degraded ^{125}I-insulin per mg/ml $\times 10^{-1}$ of cytosolic proteins. Results are the means ± SEM of four determinations. Light gray column: *$P < 0.001$ vs. OVX + EB; unpaired *t* test, 2-tailed. Values of uterine weight/body weight are given as mg/g; mid gray column: **$P < 0.001$ vs. OVX + EB, unpaired *t* test, 2-tailed. For experimental conditions, see Materials and Methods and Figure 2A.

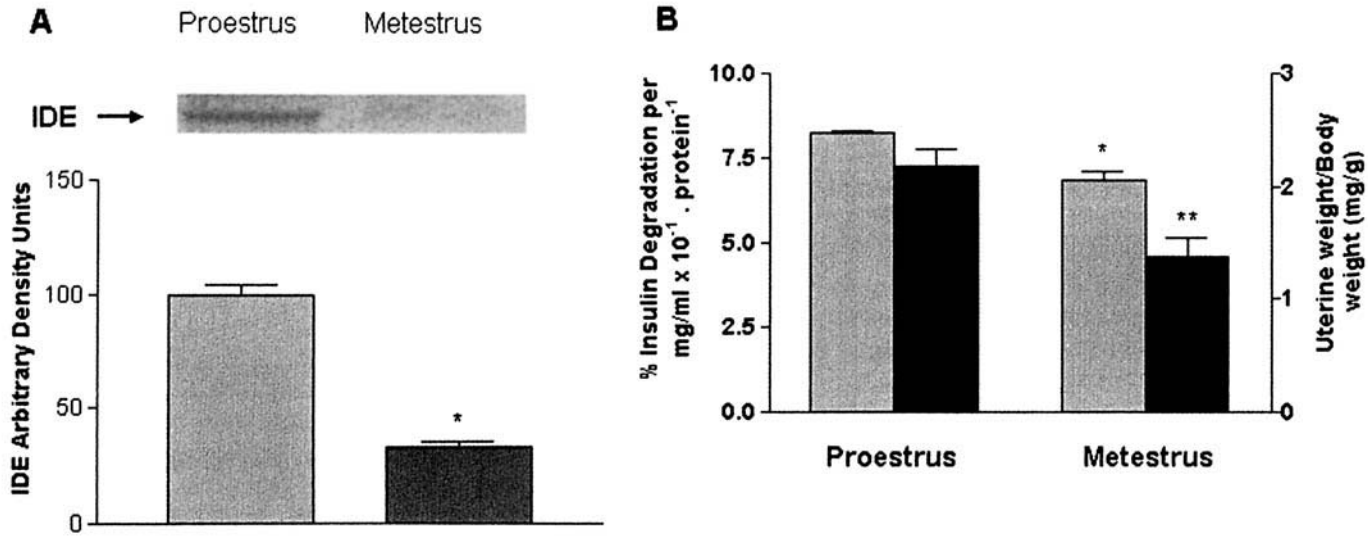

Figure 4. (A) Insulin-degrading enzyme protein levels as determined by Western blot analysis. Densitometric measurement of relative IDE protein levels in cytosolic fractions from uteri of rats in the proestrus and metestrus phases of the estrous cycle. Data are expressed as means ± SEM ($n = 5$), $^*P < 0.001$ vs. proestrus, unpaired t test, 2-tailed. (B) Cytosolic ^{125}I-insulin degradation (light gray column) and uterine weight/body weight (mid gray column) from uteri of rats in the proestrus and metestrus phases of the estrous cycle. Values of ^{125}I-insulin degradation are given as percent of degraded ^{125}I-insulin per mg/ml × 10^{-1} of cytosolic proteins. Results are the means ± SEM of four determinations; light gray column, $^*P < 0.001$ vs. OVX + EB, unpaired t test, 2-tailed. Values of uterine weight/body weight are given as mg/g; mid gray column: $^{**}P < 0.001$ vs. OVX + EB, unpaired t test, 2-tailed. For experimental conditions, see Materials and Methods and Figure 2A.

is dependent on the quantity present, and that both parameters are dependent on the androgenic state of the animal. It is known that the development, growth, and function of the prostate are androgen dependent (18). Androgen action is mainly indirect, through prostatic production of certain growth factors (19–21). These locally produced growth factors are considered autocrine and/or paracrine mediators of the stromal–epithelial interaction (22). Several studies have indicated that insulin-like growth factors (IGFs), epidermal growth factor (EGF), keratinocyte growth factor, TGF-α, and basic fibroblastic growth factor are mitogenic in prostate tumor cells and normal prostate cells (19, 22–24). Some of these growth factors are IDE substrates, such as IGFs and TGF-α, or bind to IDE, such as EGF (6, 25). Additionally, there is evidence that IDE and the receptors for insulin and IGFs share a common anatomic distribution (26). Being a protease that degrades insulin and growth factors, it is feasible to presume that the increase in growth factors that occur in the prostate in situations of cellular proliferation may lead to an increase in the receptors for growth factors and, consequently, to an increase in IDE to terminate the growth signal. On the other hand, a reduction in the expression and/or bioavailability of these growth factors may lead to apoptosis. It is known that castration significantly reduces the local expression and concentration or bioavailability of certain prostate-derived growth factors that are known to affect prostate cell proliferation (27, 28). This reduction in the expression and/or bioavailability of growth factors leading to apoptosis should consequently lead to a reduction in IDE. Indeed, we observed a reduction in both the levels and the activity of IDE in the prostate gland of castrated animals. The results obtained in the nuclear fraction were interesting because they showed the presence of IDE with no degradative activity, demonstrating that the proteolytic activity is not the sole physiological function of the enzyme. The possibility of contamination of the nuclear fraction by the cytosolic IDE was ruled out because no degradative activity was found in the nuclear fraction. The presence of IDE in the nucleus and the absence of its degradative activity suggest an interaction between IDE and AR, as has been previously shown (3, 8, 29). The inhibition of IDE activity was shown to be necessary for the accumulation of insulin or insulin–cytosolic protein complexes in nuclei (30). We have demonstrated that IDE activity is inhibited by a phosphorylation reaction (3). Furthermore, recent studies[1] suggest that inhibition of IDE activity in the cytosolic fraction mediated by a phosphorylation reaction increases the binding of IDE to DNA-cellulose; this could have significant effects on the interaction of IDE with AR, and nuclear translocation. The regulatory protein function of IDE has also been observed in different subcellular fractions, with different intracellular effectors related to intracellular insulin action (29, 31). Insulin acts intracellularly on proteasomes through IDE (31), and variation in the amounts of IDE induced by androgen or estrogen status could be important for the regulation of protein content and turnover. We have also demonstrated that estradiol upregulates IDE in the uteri of rats. The quantity and activity of IDE were increased in cytosolic fractions of a homogenate of rat uterus in the proestrus phase of the

[1] Udrisar DP, Wanderley MI, Cresto JC, unpublished results, 1999.

estrous cycle or when ovariectomized and treated with estradiol, and were decreased in rat uterus in the metaestrus phase of the estrous cycle or when ovariectomized. In the first experimental situation, the endometrium proliferates in response to the cyclic increase in plasma estradiol during proestrus or to the exogenous administration of estradiol. In the second experimental situation, the endometrium atrophies as a consequence of the cyclic decrease in plasma estradiol during metestrus or after ovariectomy. It is well established that the cyclic pattern of cellular proliferation that occurs in the endometrium during the estrous cycle reflects changes in the systemic levels of estradiol and progesterone (32). During the estrous cycle, estradiol levels begin to increase late in metestrus, reaching peak levels at the height of proestrus, and returning to baseline at estrus (33). An elevation of total uterine DNA and increase in cell proliferation also characterize proestrus (34, 35). Our results suggest that IDE may play a significant role in uterine cell proliferation during the estrous cycle of adult female rats and in response to estradiol. These results also suggest that the activity of IDE is dependant on quantity, and that both parameters are dependent on the estrogenic status of the animals. Further, the conditions of cell proliferation and apoptosis may regulate the activity and quantity of IDE, and estradiol may be an important factor for the gene expression of IDE in the uterus. The mechanisms through which estradiol affects the expression of IDE are yet to be determined. Estradiol stimulates uterine proliferation and differentiation by acting through the estrogen receptor (ER). Several studies have demonstrated that the effect of estradiol on uterine growth is mediated by locally produced growth factors (36–38), where IGFs appear to play major roles in the action of estradiol (39, 40). Insulin-degrading enzyme degrades insulin and amyloid β-protein (Aβ), and alterations in the metabolism of these substrates are critically important in the pathogenesis of Type II diabetes mellitus and Alzheimer's disease, respectively (2, 41, 42). Recent studies suggest that testosterone and 17β-estradiol reduce neuronal secretion of Alzheimer's β-amyloid peptides (43, 44). Additionally, it has been demonstrated that ovariectomy and 17β-estradiol modulate the levels of Aβ peptides in the brain (45). Thus, physiological conditions or compounds that upregulate or disinhibit IDE would be expected to lower hyperinsulinism or Aβ levels *in vivo*. The data presented here suggest that IDE may participate in prostatic and uterine growth and that testosterone/estradiol may be an important factor for the expression and regulation of IDE in both tissues. The possibility of a protein–protein interaction (IDE–AR, IDE–ER) for IDE regulation exists (3, 8, 29, 46), and may be important for various biological phenomena, such as insulin resistance and Alzheimer's disease.

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