

# Transforming growth factor- $\beta_1$ is a potent inhibitor of interleukin-1 $\beta$ action in whole ovarian dispersates

S G Derman, S Kol, I Ben-Shlomo, C E Resnick, R M Rohan and E Y Adashi

Division of Reproductive Endocrinology, Department of Obstetrics and Gynecology, University of Maryland School of Medicine, Baltimore, Maryland, USA  
(Requests for offprints should be addressed to E Y Adashi who is now at Division of Reproductive Sciences, Department of Obstetrics and Gynecology, University of Utah Health Sciences Center, 546 Chipeta Way, Mailbox 20, Salt Lake City, Utah 84108, USA)

(S G Derman is now at 3131 Princeton Pike, Building 3, Lawrenceville, New Jersey 08648, USA)

(S Kol is now at Department of Obstetrics and Gynecology, Rambam Medical Center, Haifa, Israel)

(I Ben-Shlomo is now at Department of Obstetrics and Gynecology, Haemek Medical Center, Afula, Israel)

## Abstract

Transforming growth factor  $\beta_1$  (TGF $\beta_1$ ) acts as an inhibitor of the actions of interleukin-1 $\beta$  (IL-1 $\beta$ ) in various organ systems. In order better to understand the inter-actions between these polypeptides in the ovary, we evaluated the effect of TGF $\beta_1$  co-treatment on various IL-1 $\beta$ -mediated actions in cultures of whole ovarian dispersates. Treatment with IL-1 $\beta$  enhanced media accumulation of nitrites (4.8-fold), prostaglandin E<sub>2</sub> (PGE<sub>2</sub>, 3.9-fold) and lactate (2.0-fold), and enhanced glucose consumption (2.1-fold). Treatment with TGF $\beta_1$  alone did not significantly affect any of these parameters. However, the addition of TGF $\beta_1$  inhibited IL-1 $\beta$ -stimulated nitrite (100%), PGE<sub>2</sub> (44%) and lactate (78%)

accumulation and inhibited IL-1 $\beta$ -stimulated glucose consumption (74%) in a dose-dependent manner. The addition of TGF $\beta_1$  also suppressed the steady-state levels of IL-1 $\beta$ -stimulated IL-1 $\beta$ , type I IL-1 receptor and IL-1 receptor antagonist transcripts (98, 67 and 83% inhibition respectively). These data suggest that TGF $\beta_1$  is capable of inhibiting several IL-1 $\beta$ -stimulated endpoints. Since IL-1 has been identified as a possible proinflammatory mediator of ovulation and TGF $\beta$  has been implicated as a promotor of fibrosis and healing, we speculate that IL-1 and TGF $\beta$  might play antagonistic roles in the normal ovulatory sequence.

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## Introduction

Evidence suggests a role for interleukin-1 $\beta$  (IL-1 $\beta$ ) as an intermediary in the ovulatory process (Ben-Shlomo & Adashi 1994). IL-1 $\beta$  synergizes with luteinizing hormone in bringing about ovulation (Brannstrom *et al.* 1993, Takehara *et al.* 1994), and components of the intraovarian IL-1 system (IL-1 $\beta$  and the type I IL-1 receptor (IL-1R)) are only expressed during a narrow periovulatory window (Hurwitz *et al.* 1991, Polan *et al.* 1994, Simon *et al.* 1994, Wang *et al.* 1997). Furthermore, IL-1 $\beta$  is capable of promoting a number of ovulation-associated phenomena in cultured ovarian cells, including an increase in prostaglandin production (Kokia *et al.* 1992, Townson & Pate 1994), stimulation of nitric oxide synthesis (Ben-Shlomo *et al.* 1994b), induction of collagenase activity (Hurwitz *et al.* 1993) and a metabolic shift towards aerobic glycolysis as evidenced by increased lactate accumulation and glucose consumption (Ben-Shlomo *et al.* 1997). Additionally, IL-1 $\beta$  appears to enhance the production of mRNA transcripts encoding for itself (Hurwitz *et al.* 1991). Taken together, these data provide evidence in support of the

notion that ovulation is akin to an inflammatory response, a concept originally put forward by Espey (1994).

The role of transforming growth factor- $\beta$  (TGF $\beta$ ) in ovarian physiology is less clear (Shull & Doetschman 1994). The TGF $\beta$ s are a series of ubiquitous cytokines originally described for their ability to induce the growth of rat kidney fibroblasts (Border & Noble 1994). One of three isoforms of TGF $\beta$  found in mammalian cells, TGF $\beta_1$ , is a 25 kDa homodimer expressed in granulosa cells (human, rat, pig), theca-interstitial cells (human, rat), and in human cumulus cells (Thompson *et al.* 1989, Mulheron & Schomberg 1990) and may be considered a putative intraovarian regulator (Adashi & Rohan 1992). In the ovaries of cyclic rats, levels of TGF $\beta_1$  transcripts are rather low, but rise 12 h following an ovulatory dose of human chorionic gonadotropin, a rise which is sustained throughout the life of the corpus luteum when pregnancy ensues (Gaddy-Kurten *et al.* 1989). In tissue culture systems, TGF $\beta$  has been shown significantly to alter the proliferation and differentiation of granulosa (Adashi & Resnick 1986, Feng *et al.* 1986, Knecht *et al.* 1986, Ying *et al.* 1986, Dodson & Schomberg 1987, Hutchinson *et al.*

1987, Skinner *et al.* 1987, Adashi *et al.* 1989) and theca-interstitial (Gangrade & May 1990, Hernandez *et al.* 1990, Roberts & Skinner 1991) cells. In general, TGFβ<sub>1</sub> inhibits follicle cell growth and facilitates follicle cell differentiation.

Since IL-1β is a putative mediator of inflammation (Dinarello 1994) and TGFβ<sub>1</sub> may be a mediator of fibrosis and repair (Border & Noble 1994), one might suspect that these two polypeptides are antagonistic in action to one another. Evidence for such an antagonism exists in multiple organ systems (Hamilton *et al.* 1993, Scott-Burden *et al.* 1993, Chandrasekhar *et al.* 1994, Dumont & Kastner 1994, Koide *et al.* 1994, Perrella *et al.* 1994). Given the antagonistic action of TGFβ and IL-1β in other systems, we sought to investigate their interactions in the rat ovary. Preliminary data from our laboratory have shown that TGFβ<sub>1</sub> blocks IL-1β-stimulated nitrite accumulation (Ben-Shlomo *et al.* 1994a). In this paper we develop the hypothesis that TGFβ<sub>1</sub> is a potent antagonist of IL-1β action in the rat ovary, by demonstrating that TGFβ<sub>1</sub> is capable of attenuating some, but not all, IL-1β-mediated endpoints.

## Materials and Methods

### Reagents and hormones

McCoy's 5a medium (modified, without serum) and tissue culture reagents were obtained from Gibco BRL Life Sciences (Grand Island, NY, USA). Collagenase (*Clostridium histolyticum*; CLS type I; 144 U/ml) was purchased from Worthington Biochemical Corp. (Freehold, NJ, USA). DNase, (bovine pancreas; 21 U/ml) and prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) were obtained from Sigma Chemical Co. (St Louis, MO, USA). [5,6,8,11,12,14,15-<sup>3</sup>H(N)]PGE<sub>2</sub> was obtained from New England Nuclear (Boston, MA, USA). Recombinant human IL-1β was generously provided by Drs Errol B De Souza and C E Newton of DuPont-Merck Pharmaceutical Co. (Wilmington, DE, USA). Recombinant human TGFβ<sub>1</sub>, inhibin-A and activin-A were generously provided by Dr Jennie Mather, Genentech (South San Francisco, CA, USA). Recombinant human mullerian inhibiting substance (MIS) and plasmin-cleaved recombinant human mullerian inhibiting substance (pcMIS) were generously provided by Dr Richard Cate, BioGen (Cambridge, MA, USA).

### Animals and tissue culture

Immature (25–28 day old) Sprague-Dawley female rats were obtained from Zivic-Miller Laboratories (Zelienopole, PA, USA) and killed by CO<sub>2</sub> asphyxiation and cervical dislocation.

Whole ovarian dispersates were obtained from immature rats, prepared by collagenase/DNase dispersion as

previously described (Hernandez *et al.* 1990) and cultured (37 °C, 5% CO<sub>2</sub>, 95% air) in McCoy's 5a medium (modified, without serum), supplemented with L-glutamine (2 mM), penicillin (100 U/ml), and streptomycin (100 µg/ml). Cell cultures intended for RNA extraction were incubated (1.5 × 10<sup>6</sup> cells/3 ml) in 10 × 50 mm dishes for 48 h. For all other experiments, cells were cultured (5 × 10<sup>5</sup> cells/ml) in 35 × 10 mm dishes for 72 h.

### Nitrite assay

Media nitrite, the stable metabolite of nitric oxide biosynthesis, was detected as previously described (Ben-Shlomo *et al.* 1994b).

### Prostaglandin E<sub>2</sub> assay

PGE<sub>2</sub> content of conditioned media was measured, as described, using a specific radioimmunoassay with PGE<sub>2</sub>-directed antisera, generously provided by Dr Harold R Behrman (Jaffe *et al.* 1973).

### pH assay

In order accurately to determine the pH levels of fresh, cultured media, we developed a rapid colorimetric assay which takes advantage of the pH-dependent color changes in the phenol red indicator contained in the McCoy's 5a medium. Media (200 µl) were removed at 72 h from each culture dish and immediately placed into the individual wells of a 96-well microtiter plate. The microtiter plate was then allowed to re-equilibrate in the incubator for 2 h, after which media absorbance (570 nm) was promptly determined. The optical density values were extrapolated onto a pH standard curve (buffered media) to calculate the pH of unknowns.

### Lactate assay

Lactate concentrations in conditioned media were determined by a modification of a previously described method (Gloster & Harris 1962) in which the NADH product is monitored at 340 nm following the NAD-linked conversion of lactate to pyruvate by lactate dehydrogenase with hydrazine trapping of pyruvate to ensure that the reaction goes to completion. The assay was adapted as follows to a microtiter plate system using premixed reagents from a commercially available kit (Sigma #826-A, Sigma Chemical Co.). Conditioned media (20 µl of 20-fold diluted) or varying volumes (20–100 µl) of L-lactic acid standard (50 µg/ml) were diluted in the microtiter plate wells to a volume of 100 µl after which were added NAD/glycine buffer solution, pH 9.2 (75 µl), and lactate dehydrogenase solution (25 µl). Following a 30-min incubation period at ambient temperature, absorbance

(340 nm) was read in a UVmax plate reader (Molecular Devices, Menlo Park, CA, USA). The standard curve was linear in the range used (1–25  $\mu\text{g}/\text{ml}$ ) and media blanks were negligible. Lactate concentrations were calculated from the standard curve using a software package ( $\Delta$ Soft, BioMetallics, Inc., Princeton, NJ, USA) designed for the plate reader. The within-assay coefficients of variation were 14.5 and 6.9% and the between-assay coefficients of variation were 11.7 and 6.7% for the low and high standards respectively.

#### Glucose assay

Glucose concentrations in conditioned media were determined by the modification of a previously described enzymatic method (Raabo & Terkildsen 1960) wherein the glucose oxidase and peroxidase reactions are coupled with the chromogenic oxygen acceptor *o*-dianisidine. The assay was adapted as follows to a microtiter plate system using premixed reagents from a commercially available kit (Sigma #510-A, Sigma Chemical Co.). Unconditioned or conditioned media (10  $\mu\text{l}$  of 20-fold diluted) or varying volumes (1.5–30  $\mu\text{l}$ ) of standard glucose solution (50  $\mu\text{g}/\text{ml}$ ) were diluted in the microtiter plate wells to a volume of 30  $\mu\text{l}$  to which were added the color reagent/enzyme solution (200  $\mu\text{l}$ ), prepared as described by the manufacturer. Following a 45-min incubation period at ambient temperature, absorbance of oxidized *o*-dianisidine (450 nm) was read in the UVmax plate reader. The standard curve was linear in the range used (2.5–50  $\mu\text{g}/\text{ml}$ ). Glucose concentrations were calculated from the standard curve. The values for glucose consumption reported herein were calculated by subtracting the value determined in conditioned media from the glucose value in unconditioned media.

#### RNA extraction

RNA was extracted from cells using an acid-phenol technique (RNAzol B, Tel-Test, Inc., Friendswood, TX, USA) according to the manufacturer's protocol.

#### Nucleic acid probes

The rat IL-1 $\beta$  cDNA was provided in PUC8 by Dr A Shaw of Glaxo (Geneva, Switzerland). A 222 bp PstI fragment of the original cDNA was sub-cloned into pGEM2. T7-driven transcription of the EcoRI-linearized plasmid yielded a 272 nt riboprobe which upon hybridization was projected to generate a 222 nt protected fragment (Hurwitz *et al.* 1991).

Rat type I IL-1R probe was generated as previously described (Scherzer *et al.* 1995). Briefly, reverse transcription was performed with 1  $\mu\text{g}$  total RNA and the reaction products amplified with trans-species oligonucleotide primer sets. In order to generate a plasmid clone suitable

for riboprobe synthesis, the PCR products were ligated into a pCR1000 vector. The expected lengths for the probe and the resultant protected fragments are 374 and 307 nt respectively.

The rat IL-1 receptor antagonist (IL-1RA) probe was generated by reverse transcription followed by the polymerase chain reaction (PCR). The cDNA was synthesized from macrophage-derived total RNA (1  $\mu\text{g}$ ) using random primers (pdN6) and Moloney Murine Leukemia Virus reverse transcriptase. Polymerase chain reactions were performed with AmpliTaq polymerase in the recommended reaction mixture. Primers were selected from the published sequence (Eisenberg *et al.* 1991) using Primer Designer software (Scientific and Educational Software, State Line, PA, USA). The sense strand primer was 5'-AGCGAATTTCCTTGACACAAGACAGGCACA-3' and the antisense strand primer was 5'-ATAGTCGACCAGTCACCTAATCTCTCTCC-3'. These primers span the putative alternative splice acceptor site in the intracellular IL-1RA isoform (Haskill *et al.* 1991, Cominelli *et al.* 1994). The resultant 286 bp PCR product was cloned into the EcoRI and BamHI sites of a pBS vector using the restriction sites incorporated into the 5' ends of the sense and antisense strand primers respectively. The resultant plasmid was sequenced and found to conform to the previously published rat IL-1RA cDNA sequence (Eisenberg *et al.* 1991). After digestion with HindIII and transcription with T7 RNA polymerase, a 300 nt full-length riboprobe was generated, which was projected to protect 267 nt and 224 nt segments of the secretory and intracellular IL-1RA mRNA respectively. The construct was further verified using tissue RNA with established preference for one of the two subtypes: macrophage and liver for secretory IL-1RA and small bowel and skin for intracellular IL-1RA.

The ribosomal protein large 19 (RPL19) probe was generated as previously described (Scherzer *et al.* 1995). The resultant transcription was projected to generate 283 or 234 nt probes capable of protecting a 194 or 153 nt segment respectively.

#### RNase protection assay

Linearized DNA templates were transcribed with T7 RNA polymerase to specific activities of 800 Ci/mmol [ $\alpha$ - $^{32}\text{P}$ ]UTP (IL-1 $\beta$ , type I IL-1R, and IL-1RA) or 160 Ci/mmol [ $\alpha$ - $^{32}\text{P}$ ]UTP (RPL19). The riboprobes were gel-purified as described (Kol *et al.* 1996) in an effort to eliminate transcribed products that are shorter than the full length probes. The assay was performed as previously described (Lowe *et al.* 1987). Gels were exposed to XAR film (Kodak, Rochester, NY, USA) for varying lengths of time with intensifying screens. To generate quantitative data, gels were also exposed to a phosphor screen (Molecular Dynamics, Sunnyvale, CA, USA). The resultant digitized data were analyzed with Image Quant

Software (Molecular Dynamics). The hormonally-independent RPL19 mRNA signal was used to normalize the IL-1β, type I IL-1R, and IL-1RA mRNA data for possible variation in RNA loads. Specifically, the net protected signal (respective background subtracted) to net RPL19 ratio was calculated for each sample and gene of interest.

#### Data analysis

Data are presented as means ± s.e. of multiple, separate experiments (*n* noted in figures), each with replicate assays. RIA data analysis was carried out using Curve Fit software, a package based on the 4-parameter logistic equation designed to fit the results to a sigmoidal function curve. Percentage inhibition by TGFβ<sub>1</sub> is calculated as:  $1 - ((TI - C) / (I - C)) \times 100\%$ , where C, I, TI are the values for control-, IL-1β-, and IL-1β+TGFβ<sub>1</sub>-treated cells respectively. Statistical significance was determined by ANOVA (Fisher's Protected Least Significant Difference) analysis using Statview 512+ for MacIntosh (Brain Power, Inc., Calabasas, CA, USA).

## Results

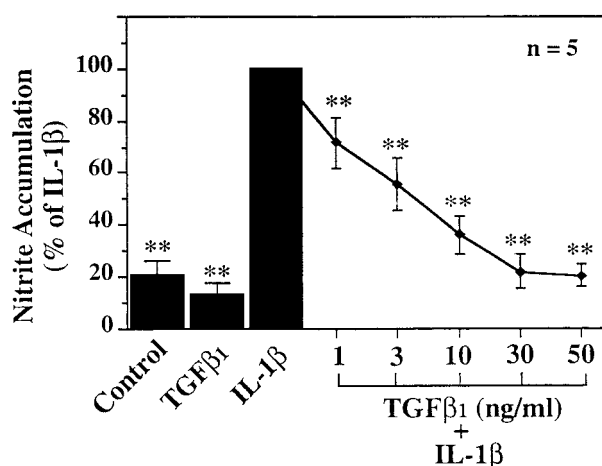
### *TGFβ<sub>1</sub> inhibits IL-1β-stimulated nitrite accumulation: specificity*

We have previously demonstrated (Ben-Shlomo *et al.* 1994a) that TGFβ<sub>1</sub> inhibits IL-1β-stimulated nitrite accumulation in cultured whole ovarian dispersates. These experiments were replicated and are shown here for clarity (Fig. 1). TGFβ<sub>1</sub> suppressed nitrite accumulation in media from IL-1β-treated cells at all doses studied ( $P < 0.01$ ) and in a dose-dependent fashion. Maximum (100%) inhibition was reached with 30–50 ng/ml TGFβ<sub>1</sub>, with an ED<sub>50</sub> of approximately 3 ng/ml.

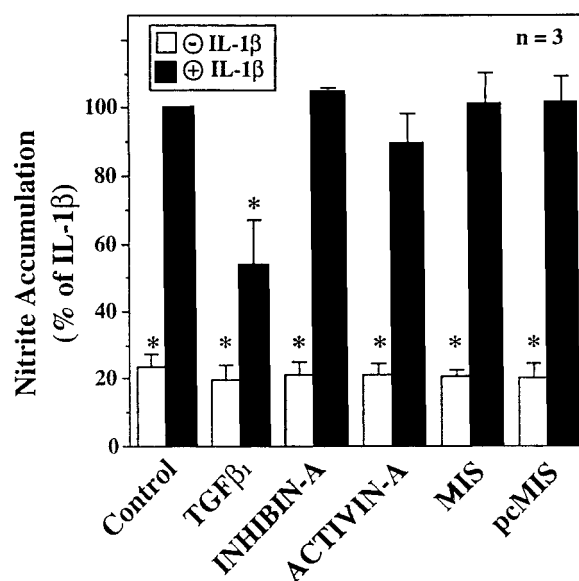
In order to determine whether other members of the TGFβ superfamily were capable of inhibiting IL-1β action, we cultured IL-1β-treated ovarian cells and untreated control cells in the presence or absence of various members of the TGFβ superfamily as shown in Fig. 2. Although TGFβ<sub>1</sub> significantly decreased nitrite production in IL-1β-treated cells (see also Fig. 1), none of the other growth factors tested (inhibin-A, activin-A, MIS, or pcMIS) produced similar results. We also tried to examine whether TGFβ<sub>1</sub> inhibited nitrite accumulation in cells treated with other cytokines. However, none of the other cytokines studied (interferon γ, tumor necrosis factor α, or IL-6, with or without IL-6 soluble receptor) was capable of inducing nitrite accumulation (data not shown).

### *TGFβ<sub>1</sub> suppresses IL-1β-stimulated prostaglandin E<sub>2</sub> accumulation at the highest dose studied*

Since IL-1β stimulates ovarian PGE<sub>2</sub> levels (Kokia *et al.* 1992, Brannstrom *et al.* 1993, Townson & Pate 1994), we examined whether this effect was blocked by TGFβ<sub>1</sub>

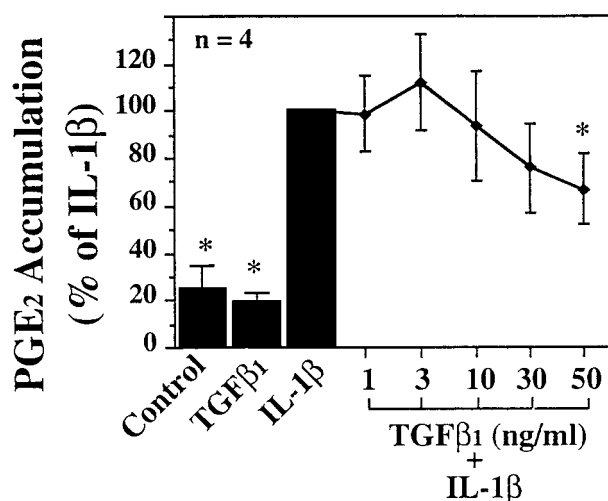


**Figure 1** TGFβ<sub>1</sub> inhibition of IL-1β-stimulated nitrite accumulation: dose dependence. Whole ovarian dispersates ( $5 \times 10^5$  cells/ml) were cultured for 72 h without treatment (Control), or with TGFβ<sub>1</sub> (10 ng/ml), IL-1β (10 ng/ml) or IL-1β (10 ng/ml)+TGFβ<sub>1</sub> (1–50 ng/ml). Media nitrite concentrations were determined and the values normalized as the percentage of the IL-1β response ( $46.191 \pm 4.7 \mu\text{M}$ ). \*\* $P < 0.01$  compared with IL-1β (ANOVA).



**Figure 2** TGFβ<sub>1</sub> inhibition of IL-1β-stimulated nitrite accumulation: specificity. Whole ovarian dispersates ( $5 \times 10^5$  cells/ml) were cultured for 72 h in the absence or presence of IL-1β (10 ng/ml), with or without addition of various substances of the TGFβ superfamily (TGFβ<sub>1</sub>, 10 ng/ml; inhibin-A, 50 ng/ml; activin-A, 50 ng/ml; MIS, 5 μg/ml; pcMIS, 5 μg/ml). Media nitrite concentrations were determined and the values normalized as the percentage of the IL-1β response. \* $P < 0.05$  compared with IL-1β (ANOVA).

(Fig. 3). IL-1β stimulated a 3.9-fold increase in media PGE<sub>2</sub> accumulation, as expected. Following an additional small and insignificant rise in PGE<sub>2</sub> levels with the addition of the lowest (1 ng/ml) dose of TGFβ<sub>1</sub>, there was



**Figure 3** The effect of increasing doses of TGF $\beta_1$  on IL-1 $\beta$ -stimulated PGE<sub>2</sub> accumulation. Whole ovarian dispersates were cultured as described in Fig. 1 and media PGE<sub>2</sub> levels were detected by radioimmunoassay. Data are normalized as the percentage of IL-1 $\beta$  response ( $6766.750 \pm 1302.665$  pg/ml). \* $P < 0.05$  compared with IL-1 $\beta$  (ANOVA).

a linear decrease in PGE<sub>2</sub> levels with increasing doses of TGF $\beta_1$ . However, unlike the effects of TGF $\beta_1$  on nitrite accumulation (Fig. 1), this decline (44% inhibition) achieved statistical significance ( $P < 0.05$ ) only at the maximal dose of TGF $\beta_1$  (50 ng/ml).

#### *TGF $\beta_1$ partially suppresses the IL-1 $\beta$ -stimulated metabolic shift towards glycolysis*

IL-1 $\beta$  mediates a shift toward glycolysis in aerobically-cultured ovarian cells (Ben-Shlomo *et al.* 1997). As shown in Fig. 4, this shift is characterized by an IL-1 $\beta$ -stimulated increase in media lactate accumulation (2.0-fold; left panel) and glucose consumption (2.1-fold; right panel). Increased lactate accumulation is accompanied by a lowering of media pH (pH 7.4) by 0.2 units (Fig. 4, inset). IL-1 $\beta$ -stimulated lactate accumulation was unaffected by TGF $\beta_1$  at doses  $< 10$  ng/ml, but was significantly inhibited at the higher doses of 30 ng/ml (54% inhibition,  $P < 0.05$ ) and 50 ng/ml (68% inhibition,  $P < 0.01$ ). TGF $\beta_1$  (50 ng/ml) also partially reversed (26%) the IL-1 $\beta$ -induced decline in pH ( $P < 0.05$ ). In addition, TGF $\beta_1$  partially reversed IL-1 $\beta$ -stimulated glucose consumption in a dose-dependent fashion, with a maximum effect at a dose of 10 ng/ml (74% inhibition,  $P < 0.05$ ). No further decreases, and variable inhibition were seen at the higher doses.

#### *TGF $\beta_1$ suppresses IL-1 $\beta$ -stimulated increases in mRNA transcripts for IL- $\beta$ , type I IL-1R and IL-1RA*

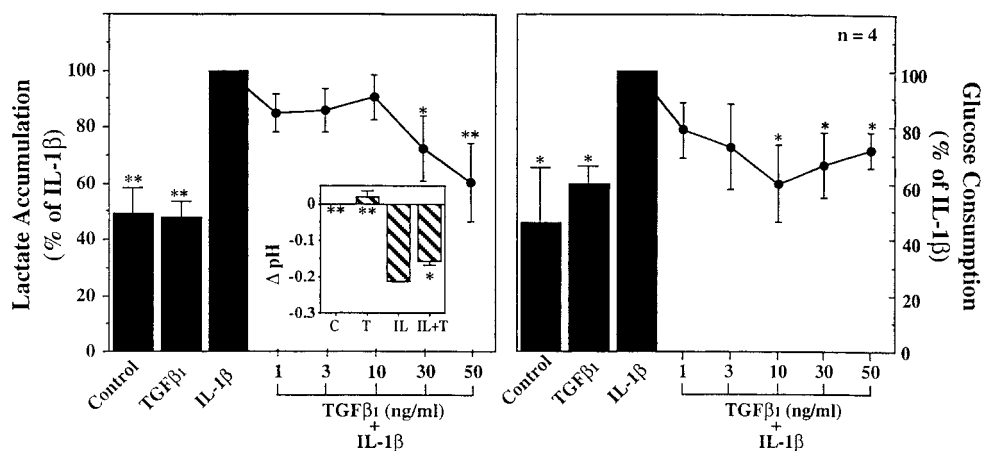
In order further to assess the ability of TGF $\beta_1$  to inhibit IL-1 action, we cultured ovarian cells in the absence

or presence of IL-1 $\beta$ , with and without TGF $\beta_1$  and examined mRNA transcripts for type I IL-1R, IL-1 $\beta$ , and IL-1RA. IL-1 $\beta$  stimulated the levels of type I IL-1R and IL-1 $\beta$  transcripts (Fig. 5) as well as of secretory IL-1RA transcripts (Fig. 6) (2.3-, 10.2-, and 6.5-fold increase respectively). Intracellular IL-1RA signals were too faint to generate meaningful data. Simultaneous treatment with TGF $\beta_1$  (10 ng/ml) returned mRNA levels for all three transcripts to near baseline levels (67, 98, and 83% inhibition respectively). These data demonstrate that TGF $\beta_1$  can dramatically block the IL-1 $\beta$ -mediated rise in mRNA levels for components of the IL-1 $\beta$  system.

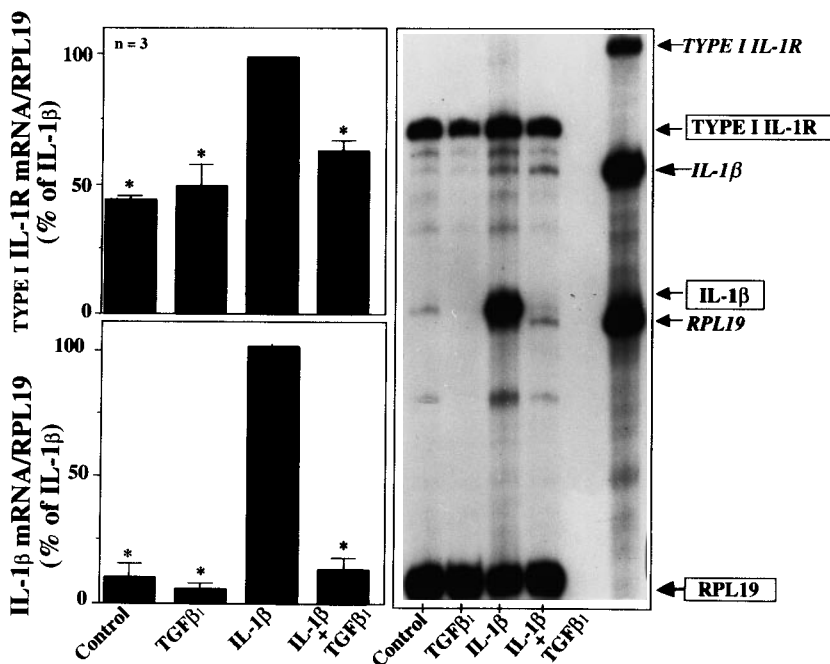
## Discussion

Herein we show that TGF $\beta_1$  antagonizes several of the actions of IL-1 $\beta$  in whole ovarian dispersates, but with differing sensitivity. IL-1 $\beta$  stimulation of nitrite accumulation, and of IL-1 $\beta$ , type I IL-1R, and secretory IL-1RA mRNAs are all dramatically inhibited (100, 98, 67, and 83% respectively) by the concurrent addition of 10–50 ng/ml TGF $\beta_1$  (Figs 1, 5 and 6). Lactate accumulation and glucose consumption are also inhibited (78 and 74% respectively) at higher doses of TGF $\beta_1$  (Fig. 4). IL-1 $\beta$ -stimulated PGE<sub>2</sub> accumulation is reversed (44% inhibition) at the highest dose of TGF $\beta_1$  (Fig. 3), albeit to a lesser degree than other endpoints we examined. In addition, we have shown previously (Ben-Shlomo *et al.* 1994a) that TGF $\beta_1$  has no effect on IL-1 $\beta$ -stimulated media lactate dehydrogenase levels. These data suggest that TGF $\beta_1$  selectively inhibits IL-1 $\beta$ -stimulated endpoints in the order: nitrite  $\sim$  IL-1 $\beta$  mRNA  $\sim$  IL-1RA mRNA  $>$  lactate  $\sim$  glucose consumption  $\sim$  IL-1R mRNA  $>$  PGE<sub>2</sub>  $>$  media lactate dehydrogenase activity. The antagonistic action of TGF $\beta_1$  appears to be specific, since other members of the TGF $\beta$  superfamily were incapable of inhibiting IL-1 $\beta$  action (Fig. 2).

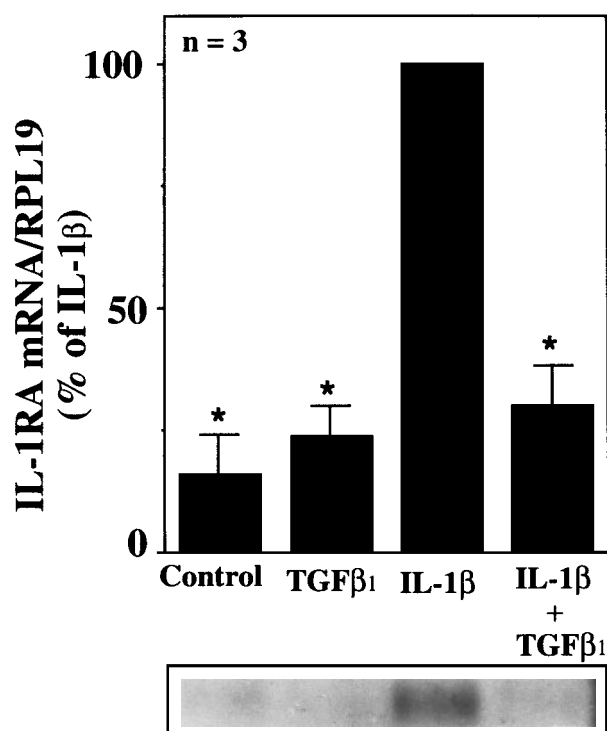
The precise reason(s) underlying the apparent differential ability of TGF $\beta_1$  to inhibit distinct IL-1 $\beta$ -supported endpoints remains uncertain. Conceivably, higher dosages of TGF $\beta_1$  might have proven effective with respect to endpoints such as PGE<sub>2</sub>, lactate generation, or glucose consumption. However, use of higher dosages of TGF $\beta_1$  would have meant dosages beyond the 'physiological' range. Consequently, no such experiments were carried out. Alternatively, it may be that the apparent differential effectiveness of TGF $\beta_1$  may reflect the degree of responsiveness of various endpoints to IL-1 $\beta$  stimulation. Given the desire to fix the IL-1 $\beta$  dose and given the need to avoid the pharmacological range, detailed dose–response studies for each and every endpoint were not undertaken. It is possible, therefore, that the higher dose of IL-1 $\beta$  may have rendered the inhibitory TGF $\beta$  potential more apparent.



**Figure 4** The effect of increasing doses of TGFβ<sub>1</sub> on the IL-1β-stimulated metabolic shift towards glycolysis. Whole ovarian dispersates were cultured as described in Fig. 1, except that media were assayed for lactate and glucose concentration and pH. Data are normalized as the percentage of IL-1β response (1016.341 ± 134.106 μg/ml for lactate; 1116.580 ± 198.305 μg/ml for glucose). The insert shows the change in media pH for control (C), TGFβ<sub>1</sub>- (10 ng/ml; T), IL-1β- (50 ng/ml; IL) and TGFβ<sub>1</sub> (10 ng/ml)+IL-1β (50 ng/ml)-treated cells. \*P<0.05, \*\*P<0.01 compared with IL-1β (ANOVA).



**Figure 5** The effect of TGFβ<sub>1</sub> on IL-1β-stimulated accumulation of type 1 IL-1R and IL-1β transcripts. Whole ovarian dispersates (1.5 × 10<sup>5</sup> cells/3 ml) were cultured for 48 h without treatment (Control), or with TGFβ<sub>1</sub> (10 ng/ml), IL-1β (10 ng/ml) or IL-1β (10 ng/ml) TGFβ<sub>1</sub> (10 ng/ml). Extracted RNA was probed by RNase protection assay using RPL19 as a normalization probe. The bar graphs represent RPL19-normalized data, expressed as a percentage of the IL-1 response. An autoradiograph of a complete representative gel is also shown (right panel). Full length RNA fragments are identified by italics and protected fragments are enclosed in a box.



**Figure 6** The effect of TGF $\beta_1$  on IL-1 $\beta$ -stimulated accumulation of mRNA for the IL-1RA. Whole ovarian dispersates were cultured and RNA was extracted and probed for IL-1RA mRNA as described in Fig. 4. The bar graph represents RPL19-normalized data, expressed as a percentage of the IL-1 response. \* $P < 0.05$  compared with IL-1 $\beta$  (ANOVA). The secretory IL-1RA protected fragment band from a representative gel is also shown. Intracellular IL-1RA signals were too faint to generate meaningful data.

Antagonism of IL-1 $\beta$  by TGF $\beta$  in the ovary is consistent with its action in other tissues. In cartilage, TGF $\beta_1$  attenuates the IL-1-stimulated rise in plasmin activator-1 and -2 levels and their transcripts (Hamilton *et al.* 1993), and the production of the osteonectin/SPARC protein (Chandrasekhar *et al.* 1994). With respect to the immune system, TGF $\beta$  suppresses IL-1-stimulated interferon  $\gamma$  production (Dumont & Kastner 1994). In the cardiovascular system, TGF $\beta$  attenuates IL-1-supported accumulation of nitrites in vascular endothelium (Scott-Burden *et al.* 1993, Koide *et al.* 1994) as well as the expression of mRNA for the inducible isoform of nitric oxide synthase (Perrella *et al.* 1994). Additionally, TGF $\beta$  suppresses IL-1-stimulated nitrite accumulation in retinal epithelial pigment cells (Goureau *et al.* 1994). In contrast, TGF $\beta$  enhances IL-1 $\beta$ -stimulated accumulation of leukemia inhibitory factor (Elias *et al.* 1994a) and IL-11 (Elias *et al.* 1994b) in lung fibroblasts and IL-1 $\beta$ -stimulated accumulation of IL-6 in intestinal epithelial cells (McGee *et al.* 1993). However, TGF $\beta$  can generally be considered an inhibitor of IL-1 $\beta$  action in most organ systems, as noted above.

The mechanism(s) underlying the antagonistic relationship between IL-1 $\beta$  and TGF $\beta$  in the ovary and other tissues remains unclear at this time. Given the ability of TGF $\beta_1$  to block IL-1 $\beta$ -mediated increases in mRNA for components of the IL-1 system (Figs 5 and 6), one might speculate that this inhibition is operational at a pretranscriptional or transcriptional level.

The significance of TGF $\beta_1$  antagonism of IL-1 action as it relates to ovarian physiology is a matter of speculation. It is possible that IL-1 $\beta$ , a promoter of inflammation, and TGF $\beta$ , a promoter of fibrosis and healing, might play complementary roles in the ovulatory process. IL-1 induces a number of inflammatory-like phenomena which have been shown to be associated with ovulation (Kokia *et al.* 1992, Hurwitz *et al.* 1993, Ben-Shlomo *et al.* 1994b, Townson & Pate 1994). In contrast, the function of TGF $\beta$  in ovarian physiology is uncertain (Shull & Doetschman 1994), but evidence suggests that TGF $\beta$  may act as a general regulator of follicle cell differentiation (Dodson & Schomberg 1987). Given the pattern of expression of IL-1 mRNAs during a narrow periovulatory window (Hurwitz *et al.* 1991, Polan *et al.* 1994, Simon *et al.* 1994) and the periovulatory rise and sustained elevation of TGF $\beta$  mRNA levels throughout the luteal phase (Gaddy-Kurten *et al.* 1989), we hypothesize that IL-1 mediates inflammatory-like phenomena at midcycle, while TGF $\beta$  may play a role in the healing process and in the development of the corpus luteum.

We have shown that TGF $\beta_1$  inhibits multiple endpoints of IL-1 $\beta$  action in whole ovarian dispersates, and that this action may be unique among the members of the TGF $\beta$  superfamily which we studied. Furthermore, TGF $\beta_1$  appears to affect these various endpoints at different concentrations, suggesting a selective action on IL-1 endpoints. We speculate that IL-1 $\beta$  and TGF $\beta$  might play antagonistic, yet complementary roles in the normal ovulatory sequence.

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