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Physiological and Pharmacological Inhibitors of Luteinizing Hormone-Dependent Steroidogenesis Induce Heat Shock Protein-70 in Rat Luteal Cells*

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ABSTRACT

Heat shock protein (HSP) synthesis increases in cells with a broad range of stress conditions. We recently showed that induction of HSP-70 is associated with inhibition of hormone-sensitive steroidogenesis, but not hormone-sensitive cAMP accumulation, in rat luteal cells by a mechanism associated with interruption of cholesterol translocation in mitochondria. As HSP induction may be an early mediator of luteal regression, we investigated whether physiological and pharmacological inhibitors of luteal function would induce HSP-70 in rat luteal cells. Both [^{35}S]methionine labeling and Western blotting with antibodies against the inducible form of HSP-70 revealed HSP induction in rat luteal cells by 1 μM prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) coincident with inhibition of progesterone synthesis. In contrast, PGE_2 (1 μM) failed to increase HSP-70 synthesis. Phorbol 12-myristate 13 acetate (3 μM), tumor necrosis factor-α (100 ng/ml), and ionomycin (1 μM) also induced HSP synthesis. Induction of HSP-70 was preceded by the rapid activation of heat shock transcription factor, which binds to the heat shock transcriptional control element. Gel retardation assays demonstrated heat shock transcription factor activation within 15 min of $PGF_{2\alpha}$ treatment. Northern analysis with an oligonucleotide probe specific for inducible HSP-70 showed induction at the transcriptional level by the above agents within 30 min. As functional luteal regression is known to display elements of a stress response, the finding that a number of factors that inhibit hormone-sensitive progesterone synthesis rapidly activate the heat shock response further implicates HSPs as possible mediators of luteolysis. (*Endocrinology* **136:** 1775–1781, 1995)

A NUMBER of separate lines of investigation have shown that heat shock proteins (HSPs) may play an important role in luteal regression. We recently proposed a novel function for heat shock protein-70 (HSP-70) in rat luteal cells based on findings that temperatures that induce HSP-70 inhibit hormone-sensitive steroidogenesis without affecting LH-stimulated cAMP levels. Moreover, the transcription inhibitor actinomycin-D blocks HSP-70 synthesis and partially reverses the inhibition of progesterone production, implicating HSP induction as an essential step in heat shock inhibition of steroidogenesis. The mechanism of this inhibition appears to be interference with the translocation of cholesterol to the mitochondria, as 22R-hydroxycholesterol, a cell- and mitochondria-permeant analog of cholesterol, fully reversed heat shock inhibition (1).

HSP-70 has also been recently implicated in prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$)-induced luteal regression. $PGF_{2\alpha}$ administered *in vivo* to the ewe resulted in rapid and sustained accumulation of HSP-70. This occurred before a decrease in serum progesterone levels, leading to the suggestion that HSPs mediate the intracellular protein processing underlying luteal regression (2).

HSP synthesis is activated as a common and ubiquitous response to a wide range of agents in addition to thermal stress (3). In higher organisms, activated heat shock transcription factor (HSF) binds to the heat shock elements (HSEs) found within the 5′-promoter region of the heat shock gene (4), stimulating HSP messenger RNA (mRNA) transcription and HSP synthesis. In some situations, HSPs may appear without HSF activation (5). The precise function of HSPs remains unknown, but it is increasingly clear that they protect cells from irreversible damage during cellular trauma, probably by binding and refolding damaged proteins (6). Other studies suggest that HSPs may serve constitutive functions in nonstressed cells, including intracellular protein processing (7), mitochondrial import of proteins (8), and acting as chaperones for cell surface antigen presentation (9).

In addition to $PGF_{2\alpha}$, a number of other factors abrogate luteal cell function *in vitro*. Phorbol ester, calcium ionophores, and cytokines such as tumor necrosis factor-α (TNFα) inhibit hormone-dependent steroidogenesis (10–12). As these inhibitors of LH-dependent steroidogenesis activate the heat shock response in other cells (13–16), we assessed whether their ability to stimulate HSP production via HSF activation in luteal cells coincided with their blockade of LH-sensitive steroidogenesis. We show that the rapid activation of HSF binding and induction of HSP-70 synthesis by $PGF_{2\alpha}$, phorbol 12-myristate 13 acetate (PMA), and TNFα is concurrent with the inhibition of steroidogenesis by these agents.

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Materials and Methods

Animals and preparation of luteal cells

All animals were housed and cared for in the fully accredited facilities operated by the Division of Animal Care. Treatments and procedures were conducted in accordance with NIH Guidelines for the Care and Use of Laboratory Animals and a protocol approved by the Yale University animal care committee.

Immature (26- to 27-day-old) female rats (Taconic, Germantown, NJ) were injected sc with 50 IU PMSG (Gestyl, Organon Pharmaceuticals, West Orange, NJ). Fifty-four hours later, 25 IU hCG (A.P.L., Ayerst Laboratories, Rouses Point, NY) were injected to induce ovulation and luteinization. Luteal cells were isolated 6–7 days after hCG treatment of the animals, and the luteal cells were enriched in a Percoll gradient, as described previously (17).

Hormones, drugs, reagents, and steroid assays

Ovine LH (NIDDK oLH-24) was a generous gift from NIH (Bethesda, MD). $PGF_{2\alpha}$, PGE_2, PMA, and ionomycin were purchased from Sigma Chemical Co. (St. Louis, MO). TNFα was obtained from R&D Systems (Minneapolis, MN). L-[^{35}S]Methionine and [^{32}P]ATP for labeling were purchased from Amersham (Arlington Heights, IL). The antibody against HSP-72 was obtained from StressGen Biotechnology Corp. (Sidney, Canada). Progesterone levels were determined by RIA, as described previously (17).

Functional studies

In all experiments, 1×10^6 luteal cells were incubated in 1 ml medium (MEM 12360, Gibco-BRL, Grand Island, NY) supplemented with 1% BSA. Isolated cells were allowed to recover for 3 h (37 C) to circumvent any effects of stress caused by the isolation procedure. Cells to be heat stressed were incubated at 45 C for 10 min and allowed to recover for an additional 3 h before treatment for 1 h with 1 μg/ml LH. Other groups were treated for 10 min with $PGF_{2\alpha}$ PGE_2, PMA, ionomycin, or TNFα after the first 3-h recovery period and immediately stimulated with LH for 1 h. For analysis of progesterone, the medium was removed and heat treated at 90 C for 10 min before assay. Medium levels of progesterone were previously shown to reflect total production by rat luteal cells. Cells for other analyses were frozen immediately at −80 C. Details of the experiments are given in the figure legends.

Radioactive labeling of cells and polyacrylamide gel electrophoresis of proteins

Three hours after each treatment, the cells were labeled as previously described (1). Briefly, 3 h after treatment, cells were labeled for 1 h with 20 μCi/ml L-[^{35}S]methionine in 12360 medium. Cellular proteins from equal numbers of cells (1×10^6 cells) were then analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) under reducing conditions. Electrophoresis was performed with a standard running buffer system at a constant voltage of 100 V for 2 h. Gels were then prepared for autoradiography, as described previously (18).

Western analysis

Cells were incubated at 37 C for 4 h and collected by centrifugation ($300 \times g$; 10 min). Before freezing at −80 C, cells were washed in BSA-free medium. Frozen cells were thawed and lysed in a solution containing 2.3% SDS, 20 mM HEPES, 150 mM NaCl, 10% glycerol, 1 mM phenylmethylsulfonylfluoride (PMSF), 5 μg/ml aprotinin, and 0.5 mM sodium orthovanadate. Homogenates were centrifuged ($28,000 \times g$) for 30 min at 4 C, and the supernatant fraction was assayed for protein by the bicinchoninic acid procedure (19), using the Micro BCA Reagent (Pierce Chemical Co., Rockford, IL). Fifty micrograms of protein were mixed with a denaturing loading buffer, heated, and applied to SDS-PAGE gels. Electrophoresis was performed with a standard buffer system at a constant voltage of 100 V for 90 min (1). Detection was carried out as described in the protocol supplied with the Rad-Free system (Schleicher and Schuell, Keene, NH). Briefly, proteins were transferred overnight to membranes at 50 mamp (4 C). Subsequent steps were all performed at room temperature. The membrane was blocked for 3 h with blocking buffer provided by the manufacturer and incubated with primary antibody against HSP-72 (1:5000 dilution) for 1 h. Membranes were then washed and incubated with alkaline phosphatase-secondary antibody conjugate for 30 min, washed, and placed on a chemiluminescent sheet to be visualized by exposure to Kodak XAR film (Eastman Kodak Co., Rochester, NY) at 37 C.

Northern analysis

Total RNA was extracted from frozen cells by the guanidinium-thiocyanate-phenol-chloroform method (20). RNA was quantified spectrophotometrically, and 15 μg RNA were denatured at 50 C for 1 h in 0.9 M glyoxal-50% dimethylsulfoxide. The RNA was run on a 1% agarose gel in a 10 mM sodium phosphate buffer. The fractionated RNA was then transferred to Zetabind (Cuno Life Sciences, Meriden, CT) in 20 × SSC (3 M NaCl and 0.3 M sodium citrate, pH 7.0) and immobilized by UV cross-linking (Stratalinker, Stratagene, La Jolla, CA). The membranes were prehybridized, hybridized, and washed, as previously described (21). Differences in RNA loading were accounted for by hybridizing membranes with a 26-base single-stranded oligonucleotide probe for 28S ribosomal RNA, as described by Barbu and Dautry (22). Labeled blots were visualized by autoradiography using Kodak XAR film (Eastman Kodak). Densitometric evaluations were performed on a Visage 2000 Gel Scanner (Bioimage, Millipore, Bedford, MA).

The probe used for detecting HSP-70 transcript was a 30-mer oligonucleotide (21, 23) similar to that shown to discriminate rodent inducible from constitutive HSP-70 (24). The probe was 5′-labeled using [^{32}P]ATP and T4 polynucleotide kinase (Boehringer Mannheim Corp., Indianapolis, IN).

Gel retardation assay

Total cellular protein was isolated from luteal cells by homogenization on ice in 20 mM HEPES (pH 7.5), 1.5 mM $MgCl_2$, 0.2 M EDTA, 0.2 M dithiothreitol, 0.4 M NaCl, 20% glycerol, 0.5 mM PMSF, and 0.5 mM leupeptin (25). Cellular debris was pelleted by centrifugation at 140,000 × *g* (4 C; 30 min), and the supernatant fraction was assayed for protein content, as described above. Ten micrograms of protein samples were incubated with 2 μg poly(dI-dC) and 1 ng (20,000 cpm) [^{32}P]ATP end-labeled double stranded HSE (26) oligonucleotide probe in 15 μl binding buffer [12 mM HEPES (pH 7.9), 12% glycerol, 2 mM $MgCl_2$, 60 mM KCl, 0.12 mM EDTA, 0.3 mM PMSF, and 0.3 mM dithiothreitol] (27) for 30 min at 25 C and fractionated on nondenaturing (4.5%) polyacrylamide gels in TGE buffer (40 mM Tris, 270 mM glycine, and 2 mM EDTA, pH 8.0). To determine nonspecific binding, a 200-fold molar excess of the unlabeled probe was incubated for 15 min with the sample before addition of the labeled probe (28).

Statistics

In each experiment, luteal cells were pooled from several animals, and aliquots of equal numbers of cells were exposed to various treatments. All experiments were repeated at least three times, and for functional studies, each treatment was replicated three times in each experiment. Statistical significance ($P < 0.05$) within an experiment was determined by one-way repeated measure analysis of variance, and that between multiple experiments was determined with the Neuman-Keuls *post-hoc* test.

Results

HSP-70 induction by $PGF_{2\alpha}$, PMA, ionomycin, and TNFα is concurrent with their inhibition of LH-sensitive progesterone synthesis

The analysis of the [^{35}S]methionine pulse-labeled protein pattern of cells revealed dramatically increased synthesis of a protein at 70 kilodaltons (kDa) after treatment with various

agents that block hormone-stimulated steroidogenesis. $PGF_{2\alpha}$ (1 μM), PMA (3 μM), ionomycin (1 μM), and TNFα (100 ng/ml; Fig. 1, lanes 3, 5, 6, and 7, respectively) increased the synthesis of a 70-kDa protein. However, 1 μM PGE_2 (lane 4) caused only a slight increase in the synthesis of this protein compared to that in control cells, shown in lane 1. These preliminary studies led to more important Western blotting studies, in which the amount of protein loaded is easier to control.

To confirm the identity of the 70-kDa protein as a HSP, we subjected cell extracts from all groups to Western analysis with antibodies specific for the inducible form of HSP-70. Figure 2A shows a representative blot in which the HSP antibody recognized a protein whose synthesis was increased several-fold within 1 h by heat shock (lane 2) and $PGF_{2\alpha}$ (lane 3). In Fig. 2B, we show that both of these treatments simultaneously impaired LH-dependent progesterone production. Heat shock completely inhibited the approximately 5-fold and maximal increase in progesterone synthesis induced by LH (1 μg/ml; $P < 0.05$). $PGF_{2\alpha}$ also caused a decrease, although less precipitous, in LH-stimulated progesterone synthesis concurrent with its induction of HSP-70. Basal production of progesterone (absence of LH stimulation) was unaffected by all treatments, and LH did not affect HSP-70 synthesis (results not shown). However, PGE_2, which failed to elevate levels of HSP-70 (lane 4), caused only a slight decrease in the ability of LH to stimulate steroidogenesis.

Figure 3 shows that other factors that impair luteal cell function also caused an increase in HSP-70 levels coincident with inhibition of LH stimulation. Figure 3A shows increased HSP-70 levels within 1 h in luteal cells treated with PMA, ionomycin, and TNFα. All of these agents significantly decreased LH-dependent progesterone production within 1 h (Fig. 3B).

Inhibitors of steroidogenesis rapidly increase the HSP-70 transcript level in luteal cells

To assess whether increased mRNA levels is associated with increased HSP synthesis, we measured HSP-70 mRNA with a probe specific for the inducible form of HSP-70 in luteal cells in which function was compromised. An increase in inducible HSP-70 mRNA occurred in cells treated with heat shock and $PGF_{2\alpha}$, but not in cells treated with PGE_2 (Fig. 4A). Small differences in the amount of RNA loaded in each lane were compensated for by hybridization of the same blot with an oligonucleotide probe for 28S ribosomal RNA. Densitometry results, shown in Fig. 4B, represent the mean values of three separate experiments, expressed as a percentage of the integrated optical density HSP-70 mRNA/integrated optical density 28S mRNA for the heat-shocked sample. Figure 5 shows that PMA, ionomycin, and TNFα similarly increased mRNA levels of the inducible form of HSP-70, although densitometry (Fig. 5B) shows that ionomycin and TNFα did not increase HSP transcript levels as effectively as heat shock and PMA.

The kinetics of HSP-70 induction by $PGF_{2\alpha}$ were studied to explore whether HSPs might be involved in the regulation

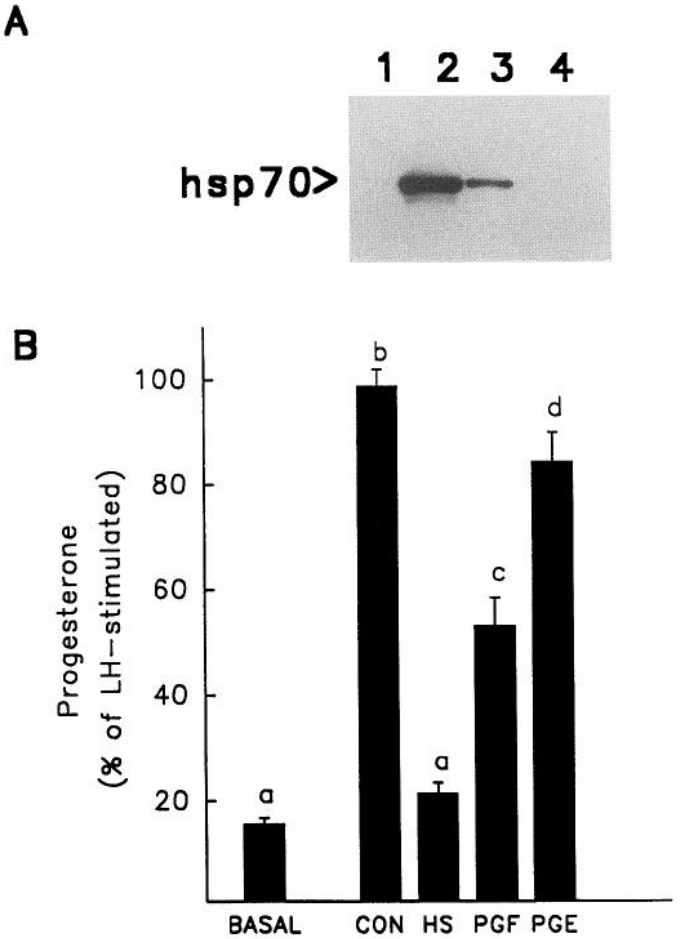

FIG. 2. A, A representative Western analysis, demonstrating the expression of HSP-70 in luteal cells after PG treatment. Proteins were extracted from control cells (lane 1), and heat shock-treated (lane 2), $PGF_{2\alpha}$-treated (lane 3), or 1 μM PGE_2-treated cells (lane 4), 1 h after treatment. B, Effects of the same treatments on LH-stimulated progesterone secretion, expressed as a percentage of the LH-stimulated progesterone levels in untreated cells. The basal level of progesterone was 9.8 ± 1.2 ng. Shown are the mean ± SEM for three independent experiments, with triplicate determinations within each experiment. Means with different superscripts are significantly different ($P < 0.05$).

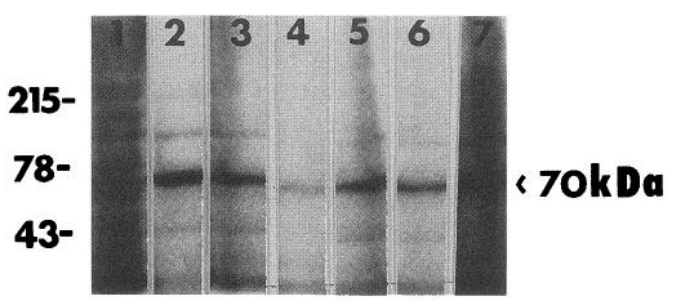

FIG. 1. The effects of treatments that inhibit LH-dependent steroidogenesis on protein synthesis in luteal cells. Cells were allowed to recover from isolation and then treated for 10 min with 45 C heat shock (lane 2), 1 μM $PGF_{2\alpha}$ (lane 3), 1 μM PGE_2 (lane 4), 3 μM PMA (lane 5), 1 μM ionomycin (lane 6), or 100 ng/ml TNFα (lane 7). After 3-h recovery, the cells were labeled with [^{35}S]methionine for 1 h, and the labeled proteins were analyzed by SDS-PAGE and autoradiography. Labeled proteins from untreated cells are shown in lane 1.

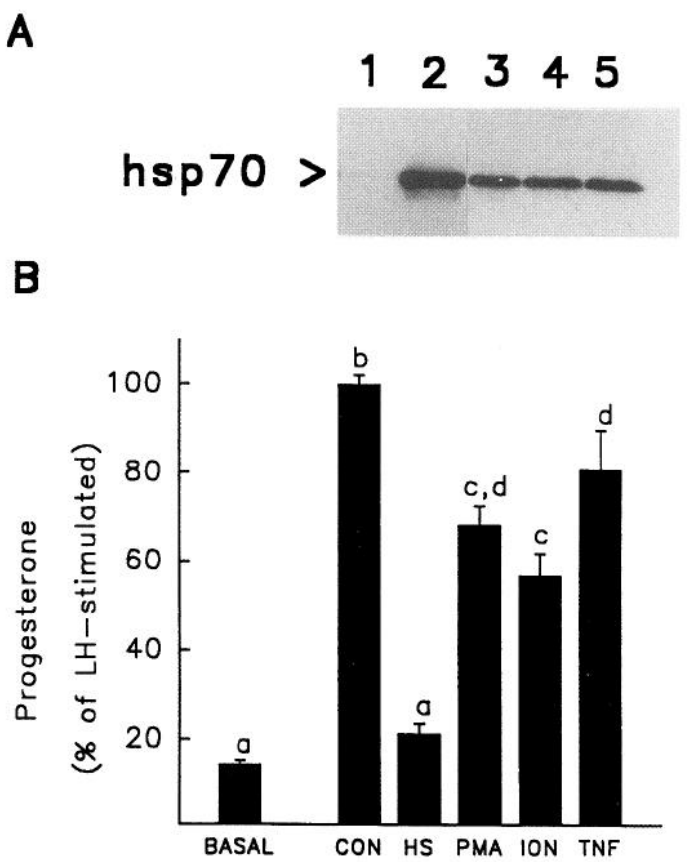

FIG. 3. The effects of PMA, ionomycin, and TNFα on inducible HSP-70 in rat luteal cells. A, Western analysis of proteins from luteal cells treated with PMA (lane 3), ionomycin (lane 4), and TNFα (lane 5) compared with those from untreated (lane 1) and heat-shocked (lane 2) cells. B, Effects of the same treatments on LH-stimulated steroidogenesis. Progesterone levels are expressed as a percentage of the LH stimulation in control cells. The basal progesterone level was 9.8 ± 1.2 ng. Each *point* represents the mean ± SEM of three experiments, performed in triplicate. Means with different superscripts are significantly different ($P < 0.05$).

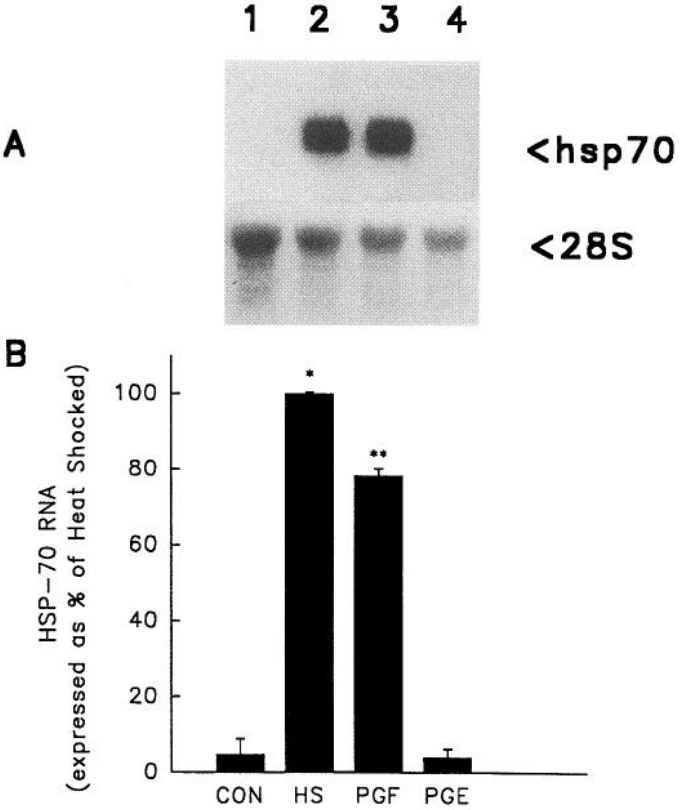

FIG. 4. A, Levels of HSP-70 mRNA after heat shock and PG treatment. mRNA was isolated from cells 1 h after treatment and electrophoresed, and the Northern blots were hybridized with labeled oligonucleotide probe for inducible HSP-70. Lane 1, Control; lane 2, heat shock; lane 3, $PGF_{2\alpha}$; lane 4, PGE_2. The same blot was hybridized with an oligonucleotide probe for the 28S ribosomal RNA. B, The autoradiographs in A were quantified by densitometry. The densitometry results for HSP-70 were normalized to the 28S ribosomal RNA densities, and the ratios expressed as a percentage of HSP-70 transcripts found in heat-shocked cells. Shown are the mean ± SEM for three experiments. The *error bar* for the HS sample is the coefficient of variation for the mean values of three experiments. *, $P < 0.05$ *vs.* control; **, $P < 0.05$ *vs.* control and heat shock.

of steroidogenesis. We measured HSP-70 mRNA in cells 15, 30, 60, and 120 min after PGF_α treatment. Inducible HSP-70 mRNA was found in luteal cells within 15 min of $PGF_{2\alpha}$ treatment (Fig. 6, lane 2). Peak HSP-70 mRNA was found 1 h (lane 4) after treatment, and although still present at 2 h (lane 5), HSP-70 mRNA levels were clearly reduced.

HSP-70 induction in luteal cells occurs via rapid activation of HSF

Luteal cells were treated with heat shock, $PGF_{2\alpha}$, PMA, or TNFα, and HSF binding to HSE was assessed 1 h after treatment. HSF activation, seen as a labeled high molecular weight complex, was observed in all treatment groups (labeled HS, PGF, PMA, and TNF in Fig. 7), but was negligible in untreated cells (Cont in Fig. 7). The DNA-binding activity induced by these agents was specific for the HSE, because competition by unlabeled HSE sequences eliminated labeling of the high mol wt complex (Fig. 7, lanes marked with a +).

Figure 8 shows that HSF activation followed a time course similar to that of HSP-70 mRNA during $PGF_{2\alpha}$ treatment. After treatment, HSF binding was evident within 15 min. HSF levels peaked at 1 h and fell within 2 h to levels found at 30 min. Competition with unlabeled HSE totally eliminated the high mol wt complex.

Discussion

In this study we show that two physiological inhibitors of LH-dependent steroidogenesis, $PGF_{2\alpha}$ and TNFα, induce HSP-70 synthesis in rat luteal cells by rapidly activating HSF binding to HSE and increasing HSP mRNA levels. As an increased intracellular calcium concentration and protein kinase-C activity are known to accompany loss of hormone sensitivity, we mimicked these conditions in luteal cells by treatment with ionomycin or PMA. All of these agents also increased the synthesis of HSP-70 in luteal cells concurrent with their inhibition of hormone-dependent steroidogenesis. PGE_2, however, neither impaired hormone stimulation nor induced stress proteins. For the first time, we show that inhibition of progesterone synthesis in luteal cells by these

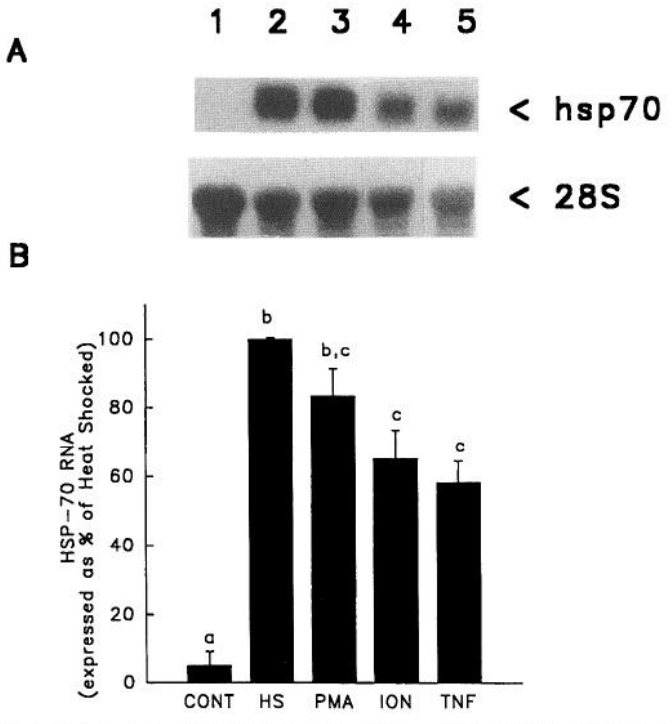

FIG. 5. Representative Northern blot of rat luteal cells treated with inhibitors of LH-dependent steroidogenesis. A, RNA from cells treated with PMA (lane 3), ionomycin (lane 4), and TNFα (lane 5) was hybridized with probes for HSP-70 and 28S ribosomal RNA, and compared with RNA expressed in control (lane 1) and heat-shocked (lane 2) cells. B, The autoradiographs in A were quantified by densitometry and normalized as described in Fig. 4. HSP mRNA levels were expressed as a percentage of HSP-70 transcripts found in heat-shocked cells. Shown are the mean ± SEM for three experiments. The *error bar* on the heat-shocked sample represents the coefficient of variation for the mean values of three independent experiments. Means with different superscripts are significantly different ($P < 0.05$).

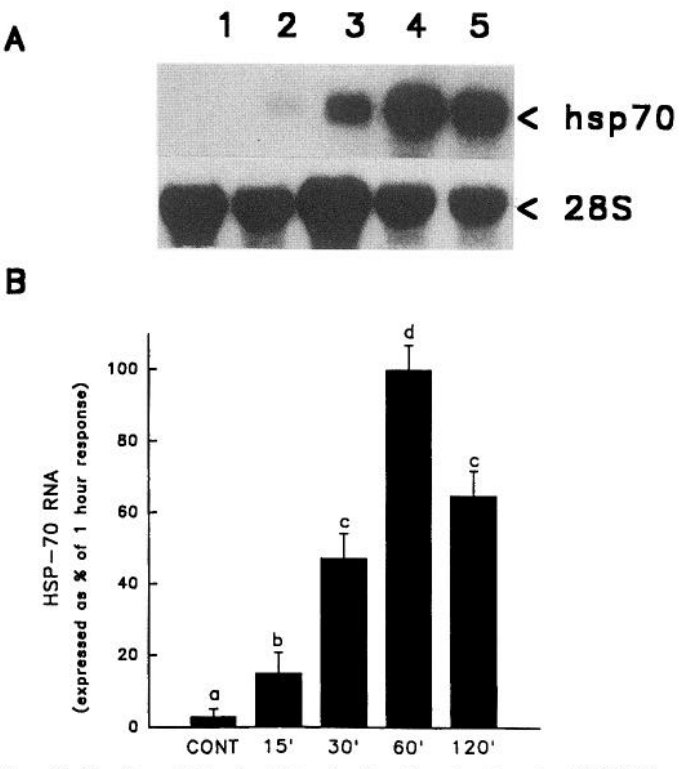

FIG. 6. Northern blot of rat luteal cells after treatment with $PGF_{2\alpha}$. RNA from $PGF_{2\alpha}$-treated cells was isolated 15, 30, 60, and 120 min (lanes 2, 3, 4, and 5, respectively) after treatment and hybridized with an oligonucleotide probe for inducible HSP-70, then compared with HSP mRNA expression in the control (lane 1). Hybridization of the same blot with an oligonucleotide probe for the 28S ribosomal RNA is also shown. B, The autoradiographs in A were quantified by densitometry. The densitometry results for HSP-70 were normalized to the 28S ribosomal RNA densities, and the ratios expressed as a percentage of HSP mRNA levels in the 1 h response. Shown are the mean ± SEM for three experiments. The *error bar* for the 1 h sample represents the coefficient of variation for the mean values of three independent experiments. Means with different superscripts are significantly different ($P < 0.05$).

agents involves a stress response and the production of HSPs.

The present studies show that transcriptional activation mediated by the specific HSF controls HSPs in rat luteal cells. HSF binding occurs within 15 min after treatment with $PGF_{2\alpha}$, as does accumulation of HSP-70 mRNA. This rapid induction is consistent with the observation that, due to lack of introns and the presence of flanking regions that confer translational efficiency, stressed cells selectively transcribe heat shock genes (29). However, our results do not eliminate the possibility of increased mRNA stability or posttranslational control, both of which have been reported for HSPs (30). The rapid detection of HSP-70 in luteal cells after treatment with luteolytic agents makes HSP-70 induction a potential candidate as a mediator of the arrest of hormone-sensitive steroidogenesis caused by antisteroidogenic agents.

Although all of the agents that inhibited LH-dependent progesterone production used in this study induced HSP-70 rapidly, the degree of induction did not correspond to the degree of inhibition of LH-sensitive progesterone synthesis. For example, although both TNFα and ionomycin activated HSF and induced HSP-70 synthesis, the latter inhibited steroidogenesis more effectively. This finding indicates that even if stress protein induction is involved in rapid inhibition of luteal steroidogenesis, a number of other factors are probably also involved. In an earlier study we showed that heat shock, although inhibiting hormone-dependent progesterone synthesis and inducing HSP-70, did not reduce hormone-stimulated cAMP accumulation in response to LH (1). Because agents such as $PGF_{2\alpha}$ inhibit cAMP accumulation in addition to progesterone production, the activation of stress proteins may represent only one of many effects these agents produce in steroidogenic cells.

Stress proteins may mediate cell surface autoimmune recognition during regression (2). For example, cells expressing surface HSPs may become targets of the immune system (31), and some agents, such as PMA, stimulate surface expression of HSP-70 (32). $PGF_{2\alpha}$ stimulates leukocyte invasion into the corpus luteum at the time of regression (33). It has been suggested recently that because $PGF_{2\alpha}$ induced stress proteins before leukocyte invasion, these proteins might mediate cell surface autoimmune mechanisms underlying luteal regression (2). However, the rapid induction of HSP-70 seen in our studies suggests other, more immediate functions for HSPs in rat luteal cells.

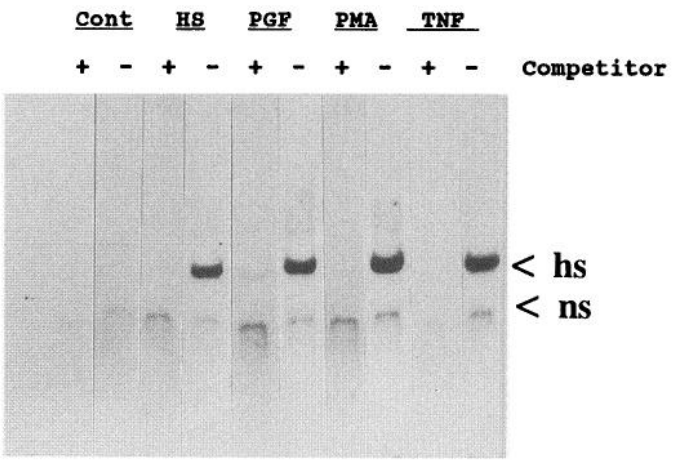

FIG. 7. Activation of HSF binding by agents that inhibit hormone-dependent steroidogenesis. Luteal cells were incubated with heat shock, $PGF_{2\alpha}$, PMA, or TNFα and after 1 h were processed to measure HSF binding, as described in *Materials and Methods*. To assess specific binding, the same lysates were preincubated with or without 200 ng unlabeled competitor HSE oligonucleotide. HS, Specific HSE-binding activity; NS, nonspecific binding activity.

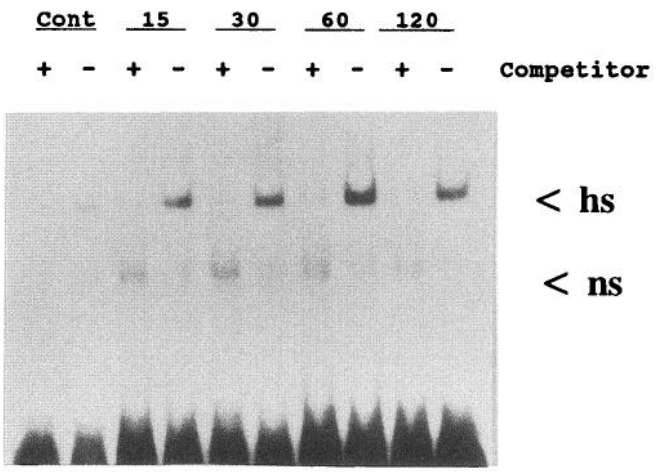

FIG. 8. Time course of $PGF_{2\alpha}$ activation of HSF. Lysates were prepared from cells, as described in *Materials and Methods*, 15, 30, 60, and 120 min after treatment with $PGF_{2\alpha}$. Specific binding was assessed by incubating the same lysates with or without 200 ng unlabeled competitor HSE oligonucleotide. HS, Specific HSE-binding activity; NS, nonspecific binding activity.

The precise site at which $PGF_{2\alpha}$ blocks steroidogenesis is unknown, although it is known to inhibit steroidogenesis by rapidly uncoupling adenylyl cyclase from the LH receptor (34, 35) and inhibiting a post-cAMP site (36). TNFα, released by activated macrophages, has been shown to inhibit LH stimulation of steroidogenesis in bovine luteal cells (12) and rat or porcine granulosa cells (37, 38). However, the identity of the intracellular mediators of both of these agents remains unknown. Our present studies suggest HSPs as possible candidates for mediators of functional luteolysis.

HSP-70 may mediate inhibition of LH-stimulated steroidogenesis during stress by impairing cholesterol transport to the inner membrane of the mitochondria. Steroidogenesis requires transport of cholesterol into the mitochondria. Cholesterol transport to the inner membrane of the mitochondria depends on protein synthesis and intact cytoskeletal elements (39, 40). It is provocative that, in addition to being induced by a number of luteolytic agents, HSP-70 is known to be involved in mitochondrial protein import (8) and cytosolic protein transport (41) by acting as an ATP-dependent unfoldase (42). It is possible that HSP-70, once induced, may sequester or unfold the rapidly turning over protein that appears to translocate cholesterol to cholesterol side-chain cleavage cytochrome P450. As HSP-70 also binds cytoskeletal elements (43, 44), it may impair cholesterol transport by such an action. Although these functions are commonly attributed to the constitutive form of HSP-70 (hsc70), during stress conditions such as luteal regression, the inducible form we detect in luteal cells may also accomplish these tasks (45).

In addition to unfolding incorrectly folded proteins, HSP-70, with other molecular chaperons, may be vital for correct folding of nascent polypeptides as they are released from the ribosome (7, 46). It is, therefore, possible that HSP-70 acts to stabilize the structure of proteins induced by treatments found to inhibit LH-dependent progesterone synthesis.

In summary, agents that inhibit LH-dependent progesterone production rapidly induce heat shock proteins in rat luteal cells. The physiological relevance of HSP induction in natural luteal regression is not known. However, the fact that HSP induction occurs in response to physiological and pharmacological agents that impair LH-sensitive steroidogenesis suggests that arrest of steroidogenesis during functional luteal regression shows elements of a stress response, and that HSP may be an early mediator of this response.

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