Heat-shock factor-1, steroid hormones, and regulation of heat-shock protein expression in the heart

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Received 27 March 2000; accepted in final form 9 August 2000

Knowlton, A. A., and Limin Sun. Heat-shock factor-1, steroid hormones, and regulation of heat-shock protein expression in the heart. Am J Physiol Heart Circ Physiol 280: H455–H464, 2001.—Heat-shock proteins (HSPs) are an important family of endogenous, protective proteins. Overexpression of HSPs is protective against cardiac injury. Previously, we observed that dexamethasone activated heat-shock factor-1 (HSF-1) and induced a 60% increase in HSP72 in adult cardiac myocytes. The mechanism responsible for this effect of dexamethasone is unknown. Because HSP90 is known to bind the intracellular hormone receptors, we postulated that the interaction between HSP90, the receptors, and HSF was an important element in activation of HSF-1 by hormones. We hypothesized that there is an equilibrium between HSP90 and the various receptors/enzymes that it binds and that alteration in levels of certain hormones will alter the intracellular distribution of HSP90 and activate HSF-1. We report that, in adult cardiac myocytes, HSF-1 coimmunoprecipitates with HSP90. HSP90 redistributes in cardiac myocytes after treatment with 17β-estradiol or progesterone. Estrogen and progesterone activate HSF-1 in adult male isolated cardiac myocytes, and this is followed by an increase in HSP72 protein. Testosterone had no effect on HSP levels; however, no androgen receptor was found in cardiac myocytes; therefore, testosterone would not be expected to effect binding of HSP90 to HSF. Geldanamycin, which inactivates HSP90 and prevents it from binding to receptors, activates HSF-1 and stimulates HSP72 synthesis. Activation of HSF-1 by steroid hormones, resulting from a change in the interaction of HSP90 and HSF-1, represents a novel pathway for regulating expression of HSPs. These findings may explain some of the gender differences in cardiovascular disease.

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We report that HSF-1 coimmunoprecipitates with HSP90. Furthermore, HSP90 moves from the cytoplasmic fraction to the nuclear fraction of cardiac myocytes after treatment with 17β-estradiol or progesterone, but not with 5α-dihydrotestosterone. Estrogen and progesterone activate HSF-1 in adult, male isolated cardiac myocytes, and this is followed by an increase in HSP72 protein. 5α-Dihydrotestosterone does not have the same effect, but no AR was identified in the adult male cardiac myocytes; however, adult male cardiac myocytes were found to have ER and PR. Geldanamycin, which inactivates HSP90, preventing it from binding to receptors, was used as an alternative agent to test our hypothesis (2, 32). Treatment with geldanamycin decreased the coimmunoprecipitation of HSF-1 with HSP90. Geldanamycin treatment resulted in activation of HSF-1 and stimulation of HSP72 synthesis. Activation of HSF-1 by steroid hormones, resulting in a change in the interaction of HSP90 and HSF-1, represents a novel pathway for activating HSF and upregulating the expression of HSPs. We propose a model where HSP90 exists in homeostasis with intracellular hormone receptors and a number of other proteins including HSF-1. Altering this homeostasis, by changing the cellular distribution of HSP90 or by inactivating HSP90’s ability to bind to these proteins by treatment with geldanamycin, results in freeing of HSF-1 from binding to HSP90. Once unbound, HSF-1 is activated, stimulating transcription and ultimately upregulation of HSP synthesis.

METHODS

Isolation of adult rat cardiac myocytes. Adult rat cardiac myocytes were isolated from 3- to 4-mo-old male Sprague-Dawley rats weighing 250–300 g according to a method described by Ford and Rovetto (4) with modification as previously described (31). This procedure yielded on average 97% cardiac myocytes, which were >97% cardiac myocytes (31).

The animal protocol was approved by the Baylor College of Medicine Animal Research Committee in accordance with the Guide for the Care and Use of Laboratory Animals [DHHS Publ. No. (NIH) 85-23, Revised 1985, Office of Science and Health Reports, Bethesda, MD 20892].

Cardiac myocyte culture and hormone treatment. Freshly isolated cardiac myocytes were cultured in M199 (GIBCO-BRL, Grand Island, NY) supplemented with 100 U of penicillin, 100 μg of streptomycin, 20 μl of pyrogen-free human serum albumin, 5 μg of insulin, and 5 μg of transferrin per milliliter in petri dishes precoated with 0.2% laminin (GIBCO-BRL) at 37°C in a humidified incubator with 5% CO₂-95% air. After 2–4 h, when the cells became adherent to the dishes, the culture medium was changed to fresh M199 containing 0.1 μM (low dose) or 10 μM (high dose) 17β-estradiol, progesterone, or 5α-dihydrotestosterone (Sigma Chemical, St. Louis, MO) or an equal volume of diluent. In additional experiments, cells were treated with 1 μg/ml geldanamycin (1.78 μM; Sigma Chemical), a concentration known to bind to and inactivate HSP90 (2, 32, 33).

Gel shift. The mobility shift assay was used to detect activation of HSF by detecting binding of HSF to the HSE. This is the standard approach for detecting activation of this normally inactive transcription factor. For the mobility shift assay, we used 5′-CTAGAAGCTTCTAGAAGCTTCTAG-3′ end-labeled with [γ-32P]ATP as our consensus HSE, as previously described (31). Because HSF is normally present in the cell in an inactive form, we were able to use whole cell lysates for our studies. Supershift studies were carried out using a mouse monoclonal anti-HSF-1 (Affinity Bioreagents) and anti-HSF-2 (generous gift of R. Morimoto, Northwestern University). The cell lysate-HSE mixes were incubated with antibody at 1:5 and 1:10 dilutions for 30 min. For cold competition experiments, the samples were incubated with a 50-fold molar excess of unlabeled HSE for 15 min before the addition of labeled HSE. Images were collected using a PhosphorImager (Molecular Dynamics, Sunnyvale, CA).

Western blot analysis. Western blotting was performed as previously described (13). The cells were washed twice with PBS, solubilized by scraping into ice-cold RIPA buffer [50 mM Tris, 150 mM NaCl, 2.5 mM deoxycholic acid, 1 mM EGTA, and 10 μl/ml Nonidet P-40 (NP-40), pH 7.4] supplemented with protease inhibitors [2.5 μg/ml antipain, 2.5 μg/ml leupeptin, 1.75 μg/ml pepstatin A, 0.95 μg/ml aprotinin, and 2.5 mM phenylmethylsulfonyl fluoride (PMSF)], and sonicated. Protein concentrations were determined with a bicinchoninic acid assay (Pierce). Samples were stored at −80°C until analyzed. The antibodies to HSPs were purchased from StressGen (Victoria, BC, Canada). These included rabbit polyclonal antibody to HSP72 protein (1:5,000 dilution), mouse monoclonal antibody to HSP60 protein (clone LK-2, 1:70,000 dilution), and rabbit polyclonal antibody to HSP25 protein (1:5,000 dilution). The anti-HSP90 (H38220, 1:500 dilution) was a mouse monoclonal antibody (Transduction Laboratories). Binding of anti-HSP72 and anti-HSP27 was detected in Western blots with anti-rabbit IgG-horseradish peroxidase (HRP) at 1:2,000 dilution (Amersham, Arlington Heights, IL). Anti-HSP60 and anti-HSP90 were developed with anti-mouse IgG-HRP at 1:1,000 (Amersham). To detect hormone receptors, we used an anti-ER antibody (mouse monoclonal, clone C-542, StressGen), an anti-AR antibody (rabbit polyclonal, N-20, Santa Cruz), and an anti-PR antibody (rabbit polyclonal, H-190, Santa Cruz) at 1:1,000, 1:500, and 1:200 dilutions, respectively, according to the manufacturer’s recommendations. Secondary antibodies were as described above. For the anti-ER only, more stringent conditions were used in processing as follows: 1) washing was done with Tris-buffered saline as previously described, but 0.2% NP-40 was used as the detergent, rather
than Tween 20, and 2) the secondary antibody was used in a 1:5,000 dilution. Blots were washed and developed using a chemiluminescent system (ECL, Amersham). The films were scanned for densitometric analysis (SigmaGel, SPSS, Chicago, IL). A control sample of human AR was the generous gift of Dr. Marco Marcelli.

**Immunocytochemistry.** Cells were fixed and blocked as previously described (11). Anti-ER (Affinity Bioreagents) and anti-PR antibodies (as described above) were each used in a 1:50 dilution. Affinity-purified anti-rabbit (for PR) and anti-mouse (for ER) antibodies labeled with FITC were used as secondary antibodies at 1:200 dilution (Binding Site, San Diego, CA). Secondary antibody alone was used as a control. Slides were analyzed with an Olympus BX60 fluorescence microscope.

**Immunoprecipitation.** After they were washed with PBS, the cardiac myocytes were incubated for 5 min at room temperature in PBS containing 2 mM dithiobis(succinimidyl propionate), as described by Zou et al. (35). Glycine (10 mM) was added to quench the cross-linking reaction. The cells were then washed with PBS and collected in RIPA buffer with protease inhibitors as described above with 100 mM sodium orthovanadate and 10 mM NaF. After sonication, the lysate was precleared with protein G-agarose (Sigma Chemical). The cells were then immunoprecipitated with anti-HSP90 overnight at 4°C. Immunoprecipitation with anti-rat intercellular adhesion molecule (ICAM)-1 antibody (mouse monoclonal, Serotec, Raleigh, NC) was used as a control. Protein G-agarose was used to precipitate the antibody-antigen complex in an overnight incubation at 4°C. The beads were pelleted in a microfuge and washed with RIPA buffer.

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**Fig. 2.** Cell fractionation studies. To determine whether treatment with the various hormones altered distribution of HSP90 between cytoplasm and nucleus, nuclear and cytoplasmic fractions were generated from the isolated myocytes with and without treatment. A: results of 3–5 experiments for each treatment. Results are expressed as nuclear-to-cytoplasmic (N/C) ratio less control value for each experiment. B: representative Western blot showing cytoplasmic and nuclear fractions. C, control (vehicle only); T, 5α-dihydrotestosterone; E, 17β-estradiol; P, progesterone. *P < 0.05 vs. controls.

**Fig. 3.** Gel mobility shift assay showing activation of HSF. Lane 1, probe alone. C, control lysates from untreated cells (lanes 2 and 3); P, progesterone (lanes 4 and 5); CC, cold compete (lane 6); T, 5α-dihydrotestosterone (lanes 7 and 8); E, 17β-estradiol (lanes 9 and 10); SS1, supershift (lanes 11 and 12), 17β-estradiol-treated samples plus antibody to HSF-1, positive for supershift; SS2, supershift, same samples plus antibody to HSF-2, no supershift present; G, geldanamycin-treated sample (1 μg/ml, lane 15). Left arrow, positive shift band; right arrow, positive supershift bands. For each hormone pair, the first lane is low dose and the second lane is high dose. All cells were treated for 3 h with various hormones or geldanamycin.
three times. Samples were separated on a 10% SDS-PAGE and transferred to nitrocellulose. The Western blot was developed with anti-HSF-1 at 1:500 dilution following our standard protocol and then with anti-mouse IgG-HRP at 1:1,000 dilution. The blot was exposed to enhanced chemiluminescence as described above.

**Fig. 4.** Effect of progesterone treatment on HSP levels. Cells were treated for 10 h with 0.1 or 10 μM progesterone. Levels of HSPs were analyzed by Western blot, and density was normalized to controls. A: results from 4–5 experiments. HSP72 levels increased with low and high doses of progesterone. HSP27 levels decreased slightly with high-dose progesterone. HSP60 and HSP90 were unaffected. *P < 0.05 vs. control. B: representative Western blot for HSP72 showing effects of low-dose (0.1 μM) and high-dose (10 μM) progesterone vs. control (C), untreated cells. C: representative Western blot for HSP27. C, control; T0.1, 0.1 μM 5α-dihydrotestosterone; T10, 10 μM 5α-dihydrotestosterone; P0.1, 0.1 μM progesterone; P10, 10 μM progesterone. Only the higher dose of progesterone had any effect on HSP27 levels, with a reproducible decrease of ~25%. 5α-Dihydrotestosterone had no effect on HSP72.

**Fig. 5.** A: representative Western blot for HSP60. No change in HSP60 levels occurred for any of the treatments. B: representative Western blot for HSP90. No change in HSP90 levels occurred for any of the treatments.

**Cell fractionation studies.** Cell fractionation studies were done using the approach described by Huang et al. (8). Cells were washed with PBS twice and then scraped into 1 ml of PBS containing protease inhibitors (1 μg/ml each of aprotinin, leupeptin, and pepstatin and 1 mM PMSF). The cells were then centrifuged at 270 g at 4°C for 10 min. The supernatant, cytoplasmic protein, was saved as fraction A. The pellet was resuspended in 600 μl of nuclei isolation buffer (60 mM KCl, 15 mM NaCl, 15 mM HEPES, 2 mM EDTA, 0.5 mM EGTA, 0.15 mM spermine, 0.5 mM spermi-
dine, 14 mM β-mercaptoethanol, 10% sucrose, and 0.1% NP-40) and placed on ice for 5 min. The preparation was centrifuged at 220 g at 4°C for 5 min. The pellet was resuspended in 300 μl of glycerol storage buffer (50% glycerol, 20 mM Tris, pH 7.9, 75 mM NaCl, 0.5 mM EDTA, 0.85 mM dithiothreitol, and 0.1 mM PMSF) and then centrifuged at 13,000 g for 1 min at 4°C. The pellet was resuspended in 500 μl of PBS with protease inhibitors to which 1% NP-40 had been added. The preparation was then centrifuged for 2 min at 13,000 g at 4°C. The pellet was resuspended in 600 μl of RIPA buffer. After the integrity of the nuclei was verified by examination with light microscopy, the nuclei were lysed by sonication. The preparation was then centrifuged at 16,000 g at 4°C, and the supernatant saved as the nuclear fraction.

Statistics and data analysis. Values are means ± SE of three or more experiments with multiple data determinations in each experiment. Data were compared by one-way ANOVA, followed by a Student-Newman-Keuls test. Data comparing normalized values with control values were compared with an ANOVA on ranks (Kruskal-Wallis) followed by a Dunn’s test; if data samples passed a test of normality and were of equal variance, one-way ANOVA was performed. All statistical analysis was performed with SigmaStat (SPSS). P < 0.05 was considered significant.

RESULTS

We postulated that known interaction between 17β-estradiol, progesterone, ER, PR, and HSP90 resulted in displacement of HSF from HSP90 when these hormones were added. To test this theory, we immunoprecipitated HSP90 from adult cardiac myocytes to show that HSP90 bound HSF-1 in the normal cardiac myocyte. As shown in Fig. 1, HSF-1 coimmunoprecipitated with HSP90 (lane 1). Immunoprecipitation with an-
other, nonspecific antibody (anti-ICAM-1) did not precipitate HSF (lane 2). Thus HSP90 binds HSF-1 in the normal adult cardiac myocyte.

HSP90 binds to the receptors for hormones including the ER, the PR, and the AR (3, 10, 26, 30). It is thought that when glucocorticoids bind the receptor, the entire complex of HSP90, hormone, and receptor moves to the nucleus. Thus hormone binding to the receptors may change HSP90 distribution. This phenomenon has been studied in cell lines, but not in cardiac myocytes. To determine whether redistribution of HSP90 occurs in cardiac myocytes, cells were treated with hormone for 4 h and then separated into nuclear and cytoplasmic fractions. A representative Western blot of these fractions is shown in Fig. 2. With 17β-estradiol and progesterone treatment, there is accumulation of HSP90 in the nucleus (P < 0.05 vs. control cells). In contrast, 5α-dihydrotestosterone treatment has no effect on localization of HSP90. Thus treatment with progesterone or 17β-estradiol changed the intracellular distribution of HSP90, while 5α-dihydrotestosterone had no effect.

Isolated adult cardiac myocytes were treated with physiological levels of three different hormones, 17β-estradiol, progesterone, and 5α-dihydrotestosterone, to determine whether this hormone treatment activated HSF, the transcription factor for HSPs. Gel mobility shift assays were performed to determine whether progesterone, 17β-estradiol, and 5α-dihydrotestosterone activated HSF. As shown in Fig. 3, progesterone and 17β-estradiol activated HSF by 3 h. Low (0.1 μM) and high (10 μM) doses of hormone activated HSF. Competition with unlabeled probe (cold compete) showed the observed band to be specific. 5α-Dihydrotestosterone at low and high doses had no effect on HSF activation. Supershift studies were done using anti-HSF-1 and anti-HSF-2. As shown in Fig. 3, 17β-estradiol treatment activated HSF-1, but not HSF-2. Progesterone also activated HSF-1, as determined by supershift assays (data not shown).

Activation of HSF-1 is not necessarily followed by an increase in all HSP synthesis. Four different HSPs were assessed by Western blotting after each of the hormone treatments. To facilitate comparison of Western blot data from different experiments, results were converted to percentage of control untreated cell density. As shown in Fig. 4, which summarizes the results of four experiments, low-dose progesterone treatment increased HSP72 levels by 40% at 10 h, and high-dose treatment nearly doubled HSP72 levels (P < 0.05). In contrast, HSP27 levels were unaffected at low-dose progesterone and were reproducibly decreased by ~25% at the high dose. HSP60 and HSP90 were unchanged (Fig. 5).

Low-dose 17β-estradiol increased HSP72 levels by 70%, and the high dose resulted in a 99% increase (Fig. 6). HSP27 levels were unchanged. Likewise, HSP60 and HSP90 levels were unaffected, and a representative Western blot is shown in Figs. 5 and 6. Thus estrogen and progesterone substantially increased HSP72 levels but had minimal to no effect on the other HSPs. Not surprisingly, 5α-dihydrotestosterone, which did not activate HSF, had no effect on levels of any of the HSPs (Figs. 5 and 7).

For binding of progesterone or 17β-estradiol to change the equilibrium among HSP90 and the proteins it binds, there must be receptors for both of these hormones in the adult male cardiac myocytes we studied. Likewise, the absence of any effect for 5α-dihydrotestosterone was puzzling. As shown in Fig. 8, A and B, we found receptors for progesterone and estrogen in the adult male cardiac myocytes, but no receptor for androgen (Fig. 8C, lanes 2 and 3). To ensure that this was not an artifact, prostate tissue was collected from the rats when the hearts were taken for myocyte isolation. As shown in Fig. 8C, lanes 4 and 5, prostate...
tissue was positive for the presence of the AR, but adult male cardiac myocytes were negative. Although the ER and PR are nuclear proteins in many cell types, the ER has been found to be distributed throughout the cell in the cardiac myocyte (5). We confirmed this finding by immunocytochemistry for ER and found that PR had similar distribution (Fig. 9).

The inhibitor geldanamycin, which binds and inactivates HSP90 and inhibits tyrosine kinases, was tested as a control. After HSP90 is inactivated, it is unable to bind to the various receptors. In immunoprecipitation studies, treatment with geldanamycin greatly reduced coimmunoprecipitation of HSF-1 with HSP90, as shown in Fig. 10, similar to the experiments shown in Fig. 1. Immunoprecipitation with a nonspecific antibody did not precipitate HSF-1 (lane 2). Geldanamycin pretreatment reduced immunoprecipitation of HSF-1 with HSP90 by 40%, as shown in lane 3. Geldanamycin activated HSF by 3 h, as would be expected (Fig. 3, lane 15). In fact, geldanamycin activated HSF as early as 1 h (data not shown). Supershift assays showed that geldanamycin activated HSF-1, but not HSF-2 (data not shown). Furthermore, geldanamycin markedly increased levels of HSP72 (Fig. 11). Geldanamycin, similar to 17β-estradiol and progesterone, had no effect on levels of HSP27, HSP60, or HSP90 (data not shown).

**DISCUSSION**

Previously we observed (31) that dexamethasone activates HSF-1 and upregulates HSP72 but has no effect on HSP60 or HSP27. We postulated that HSP90 and HSF interact to form a complex in the cardiac myocyte and that HSP90 and HSF, ER, PR, and GR were in equilibrium. This would be consistent with the findings of Zou et al. (35) and Ali et al. (1) in studies of HeLa cells and *Xenopus oocytes* that show that HSP90 binds HSF-1. Under steady-state conditions, changing one protein's interaction with HSP90 would alter the interaction of the others, specifically HSF-1. Further support for our hypothesis comes from the observation of Xiao and DeFranco (34) that overexpression of GR results in activation of HSF. In the present study, we observed that 17β-estradiol and progesterone activate HSF-1 and upregulate HSP72. Addition of either hormone caused redistribution of HSP90 in the cell. In contrast, 5α-dihydrotestosterone had no effect. All these results are consistent with our hypothesis, except the absence of effect for 5α-dihydrotestosterone.

For the hormones to have the postulated effects, the appropriate receptor must be present in these adult cardiac myocytes. Western blotting was carried out to confirm the presence of ER and PR in the male cardiac myocytes. This is consistent with previous reports of the presence of ER in adult male cardiac myocytes (5, 6). We are unaware of previous reports

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**Fig. 10.** A: results of 3 different experiments. Treatment with geldanamycin reduced coimmunoprecipitation of HSF-1 with HSP90 by 40%. C, control; G, geldanamycin. *P < 0.05 vs. control. B: representative Western blot of immunoprecipitates developed with anti-HSF-1; composite is from a single Western blot. Lane 1, untreated cells immunoprecipitated with anti-HSP90; lane 2, immunoprecipitation with anti-ICAM-1 antibody; lane 3, immunoprecipitation with anti-HSP90 antibody after treatment with geldanamycin. After immunoprecipitation, samples were separated by SDS-PAGE and then transferred to nitrocellulose. The nitrocellulose membrane was developed with anti-HSF-1 antibody. Treatment with geldanamycin decreased the amount of HSF-1 immunoprecipitating with HSP90.

**Fig. 11.** Effect of geldanamycin treatment on HSP levels. Cells were treated for 10 h with 1 μg/ml (1.78 μM) geldanamycin. Levels of HSPs were analyzed by Western blot, and density was normalized to controls. A: HSP72 levels increased markedly with geldanamycin treatment. C, control, untreated cells. *P < 0.05 vs. control. B: representative Western blot for HSP72 showing effects of geldanamycin (G) vs. control (C), untreated cells.
of a PR in adult male cardiac myocytes. We were unable to demonstrate the presence of AR in the adult male cardiac myocytes, although prostate samples from the same rats were positive. Other investigators have reported that AR are present in whole rat heart (31). The fact that we did not observe AR may mean that it is present in very low amounts (although we ran very high amounts of protein on our gels) or that it is present in the heart, as others have observed, but not in the myocytes themselves. Rather, AR may be present in fibroblasts, endothelial cells, and/or smooth muscle cells in the heart. The absence of an AR is consistent with finding no effect on HSF when the cells were treated with 5α-dihydrotestosterone. In contrast, there are receptors for 17β-estradiol and progesterone in cardiac myocytes, and these receptors are distributed throughout the cell, rather than being confined to the nucleus as in many other cell types. Addition of progesterone or 17β-estradiol changed cellular distribution of HSP90 and activated HSF-1. However, adding 5α-dihydrotestosterone, for which we found no receptor in cardiac myocytes, did not alter cellular distribution of HSP90, nor did it activate HSF-1.

To investigate our hypothesis further, geldanamycin, an inhibitor of HSP90, which blocks HSP90 from binding to the various receptors, was used (1, 32). Having shown that three different hormones, 17β-estradiol and progesterone here and the glucocorticoid dexamethasone in a previous study (31), bind intracellular receptors, we wanted to test our hypothesis by an alternative approach. Geldanamycin decreased binding of HSF-1 by HSP90, as shown by the coimmunoprecipitation studies. Geldanamycin treatment resulted in activation of HSF-1 and a marked increase in HSP72 levels. Thus inactivation of HSP90 had effects on HSF-1 and HSP72 similar to treatment with hormones to change the homeostasis among the substances bound to HSP90.

HSP27, HSP60, and HSP90 levels were not increased by progesterone or 17β-estradiol, and in fact HSP27 was decreased by high-dose progesterone. These findings are consistent with the observation that herbimycin A, a benzoquinoid ansamycin antibiotic similar to geldanamycin that binds HSP90, also induces HSP72 protein, but not other HSPs (7, 19, 33). It is likely that herbimycin A increases HSP72 through a mechanism similar to that of geldanamycin, which
inactivates HSP90, allowing the activation of HSF-1. HSP72 may be more readily upregulated in the heart than other HSPs. Similarly, with dexamethasone treatment, we reported an increase in HSP72, although not as large as with progesterone or estrogen. There was no change in other HSPs (31). Other agents may be needed to increase expression of these other HSPs in cardiac myocytes. Further studies are needed to illuminate the differential regulation of HSPs in the heart.

Our hypothesis is summarized by the schema shown in Fig. 12. For simplicity, only GR, ER, and HSF are shown. HSP90 exists in an equilibrium with all these proteins as well as PR, Src, and a number of other proteins. For example, when 17β-estradiol is added, it binds to the ER, which is bound to HSP90. This complex of 17β-estradiol, the ER, and HSP90 moves to the nucleus. That this occurs is supported by the increase in nuclear HSP90 after treatment with 17β-estradiol. This binding of 17β-estradiol to the ER changes the equilibrium between HSP90 and the proteins it binds; the intracellular distribution of HSP90 changes, and more HSP90 is found in the nuclear fraction. Therefore, less HSP90 is present in the cytoplasm, there is less HSP90 to bind HSF-1, and there is an increase in unbound HSF-1. Likewise, the addition of geldanamycin inactivates HSP90 and prevents it from binding to the various receptors. Any of these treatments shifts the equilibrium between free HSF and HSF bound to HSP90; this results in more unbound HSF, which is then readily activated, and upregulation of HSP72 follows.

In summary, we show that multiple different steroid hormones activate HSF-1 and upregulate HSP72 in isolated adult male cardiac myocytes. All these hormones have receptors that are known to bind to HSP90. We hypothesized that HSP90 is in equilibrium with various receptors/enzymes, including GR, PR, ER, and AR (3, 10, 26, 30). HSF-1 has been shown to bind HSP90 in Cos cells and Xenopus oocytes, and we demonstrate that HSF-1 binds HSP90 in cardiac myocytes (1, 35). Overexpression of free steroid receptors in Cos cells activated HSF-1 in the absence of stress (25). Injection of antibodies to HSP90 into Xenopus oocytes also activated HSF-1 (1). A change in localization and/or binding of HSP90 could potentially change its equilibrium with other proteins including HSF-1. Treatment with steroid hormones must free HSF-1, and in its unbound state, in the cardiac myocyte, HSF-1 appears to be readily activated.

Treatment with 17β-estradiol or progesterone activated HSF-1 and increased HSP72. At the higher doses, HSP72 levels were doubled. These results raise a potential new method for induction of HSPs electively. These increases are sufficient to cause cardioprotection (23, 31). It is interesting to consider that these effects of estrogen and progesterone may account for some of the unexplained differences observed in female and male cardiovascular disease (17). To our knowledge, there are no published reports of male vs. female HSP levels in the heart. Further work is needed to determine whether these acute changes are paralleled in chronic settings.

The authors thank Marco Marcelli (Baylor College of Medicine) and John Stallone (Texas A & M University) for continuing critical review of this work and many helpful suggestions, Roger Rossen for critical review of the manuscript, and Andrew Schafer for continued support and guidance. This work was supported by National Heart, Lung, and Blood Institute Grant HL-58515 (A. A. Knowlton).

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