the adult reproductive systems with a dissecting microscope.
9. N. Arantes-Oliveira, J. Apfeld, C. Kenyon, data not shown.
10. Supplemental material is available at *Science* Online at www.sciencemag.org/cgi/content/full/295/5554/502/DC1.
11. C. Kenyon, J. Chang, E. Gensch, A. Rudner, R. Tabtiang, *Nature* **366**, 461 (1993).
12. M. K. Barton, J. Kimble, *Genetics* **125**, 29 (1990).
13. T. Schedl, J. Kimble, *Genetics* **119**, 43 (1988).
14. R. E. Ellis, J. Kimble, *Genetics* **139**, 561 (1995).
15. T. Karashima, A. Sugimoto, M. Yamamoto, *Development* **127**, 1069 (2000).
16. J. R. Priess, H. Schnabel, R. Schnabel, *Cell* **51**, 601 (1987).
17. L. W. Berry, B. Westlund, T. Schedl, *Development* **124**, 925 (1997).
18. R. Francis, M. K. Barton, J. Kimble, T. Schedl, *Genetics* **139**, 579 (1995).
19. Animals were fed bacteria (BL21-DE3) harboring either a plasmid with T7 promoters opposing each other to express dsRNA of *daf-16* (pAD43), or an identical plasmid with no insert (control vector) (pAD12). dsRNA production was induced by administering 100 μl of 0.1 M IPTG to the bacterial lawn 2 to 3 hours before adding the worms. The animals were moved to fresh bacterial lawns every 4 to 7 days.
20. L. Timmons, D. L. Court, A. Fire, *Gene* **263**, 103 (2001).
21. G. J. Lithgow, T. M. White, S. Melov, T. E. Johnson, *Proc. Natl. Acad. Sci. U.S.A.* **92**, 7540 (1995).
22. P. L. Larsen, *Proc. Natl. Acad. Sci. U.S.A.* **90**, 8905 (1993).
23. J. R. Vanfleteren, *Biochem. J.* **292**, 605 (1993).
24. L. Guarente, C. Kenyon, *Nature* **408**, 255 (2000).
25. K. Lin, H. Hsin, N. Libina, C. Kenyon, *Nature Genet.* **28**, 139 (2001).
26. C. M. Sgro, L. Partridge, *Science* **286**, 2521 (1999).
27. J. Apfeld, C. Kenyon, *Cell* **95**, 199 (1998).
28. A *daz-1/+* heterozygote, *daz-1(tj3)/mIn1[dpy-10(e128)mIs14]*, lived slightly longer than the wild type. This effect was present in gonad-ablated animals (*9*), indicating that this life-span extension was independent of the gonad.
29. We also ablated the germ line of wild-type males and found that the ablation increased life-span significantly (36% mean increase, $P \leq 0.0001$). In this experiment, a large number of animals (23/73 intact, and 40/69 ablated) crawled off the plates. Many of the ablated animals crawled off the plates at old ages, suggesting that the animals that would have lived the longest were lost.
30. T. Schedl, in C. elegans *II*, D. L. Riddle, T. Blumenthal, B. J. Meyer, J. R. Priess, Eds. (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1997), pp. 241–269.
31. B. Gerisch *et al.*, *Dev. Cell* **1**, 841 (2001).
32. We thank the Kenyon lab members for very helpful discussions, A. Sugimoto for *daz-1*, and the *Caenorhabditis* Genetics Center for other strains. Supported by a predoctoral fellowship from the Portuguese Foundation for Science and Technology and by the Gulbenkian Foundation (N.A.-O.); by a Howard Hughes Medical Institute predoctoral fellowship (J.A.); and by a postdoctoral fellowship from the Damon Runyon–Walter Winchell Foundation (A.D.). This work was supported by a grant from the NIH to C.K.

27 August 2001; accepted 15 November 2001

# Abnormal Vascular Function and Hypertension in Mice Deficient in Estrogen Receptor β

**Yan Zhu,[1] Zhao Bian,[2,3] Ping Lu,[1] Richard H. Karas,[1] Lin Bao,[1] Daniel Cox,[1] Jeffrey Hodgin,[4] Philip W. Shaul,[5] Peter Thorén,[3] Oliver Smithies,[4] Jan-Åke Gustafsson,[2] Michael E. Mendelsohn,[1]***

**Blood vessels express estrogen receptors, but their role in cardiovascular physiology is not well understood. We show that vascular smooth muscle cells and blood vessels from estrogen receptor β (ERβ)–deficient mice exhibit multiple functional abnormalities. In wild-type mouse blood vessels, estrogen attenuates vasoconstriction by an ERβ-mediated increase in inducible nitric oxide synthase expression. In contrast, estrogen augments vasoconstriction in blood vessels from ERβ-deficient mice. Vascular smooth muscle cells isolated from ERβ-deficient mice show multiple abnormalities of ion channel function. Furthermore, ERβ-deficient mice develop sustained systolic and diastolic hypertension as they age. These data support an essential role for ERβ in the regulation of vascular function and blood pressure.**

Steroid hormones regulate a wide range of cellular events by activating a receptor family of transcription factors (*1*). Estrogens influence gene expression, growth, and cellular differentiation in target tissues by activating one or both of two estrogen receptors, ERα and ERβ (*2, 3*). Estrogen receptors have been studied intensely in female reproductive physiology, but functional estrogen receptors are also present and physiologically important in other tissues of both sexes, including the liver, brain, bone, and the cardiovascular system (*4*).

ERα and ERβ both are expressed in vascular endothelial and smooth muscle cells, and in myocardial cells (*5*). ERβ expression is induced in vascular cells following injury (*6*). Both estrogen receptors are necessary and sufficient for estrogen-mediated protection against measures of vascular injury in mice (*7, 8*). Estrogen receptors also regulate the expression of a number of vasodilator and vasoconstrictor proteins, including multiple components of the renin-angiotensin system (*5*). An association between an ERβ gene polymorphism and systemic blood pressure in postmenopausal Japanese women (*9*) is the only genetic data to implicate estrogen receptors in blood pressure regulation.

Vascular tone is regulated by a complex set of variables that determine the contractile state of vascular smooth muscle (*10, 11*). Inherited forms of hypertension have been shown to involve mutations in genes regulating renal salt reabsorption (*12*), but the pathogenesis in most individuals with hypertension remains unknown. Both acute and longer term changes in vascular tone due to estrogen are mediated principally by nitric oxide (*5, 13–15*). Endothelial-dependent vascular relaxation is mediated by production of nitric oxide (NO) by endothelial nitric oxide synthase (eNOS) (*16*). Estrogen enhances production of NO by endothelial cells by increasing eNOS activity or expression of the eNOS gene, or both (*17, 18*). In vivo, estrogen enhances vasodilatation in both primates and humans with normal and abnormal endothelial function (*5, 19, 20*). Endothelial cell-independent vascular contraction is mediated by the direct actions of contractile agonists on vessel wall smooth muscle cells (*11*). Estrogen reduces vasoconstriction in vessels from which the endothelium has been removed from both humans and wild-type (WT) animals (*21, 22*), an effect that is blocked by pharmacologic inhibition of inducible nitric oxide synthase (iNOS) (*22*). To examine the role of estrogen receptors in vascular physiology, the existence of an estrogen effect on vasoconstriction was explored in vessels from WT mice.

We studied endothelial cell-independent vascular contraction (*11, 22*) in endothelium-denuded vascular rings from WT mice. Treatment of endothelial-denuded aortic rings from WT mice with 17β-estradiol (E2) for 18 to 20 hours attenuated constriction to the alpha (1B)-adrenergic receptor agonist phenylephrine (PE) [decrease of 45% and 69%, respectively, $P < 0.05$ for both, (Fig. 1, A and B) (*5, 21, 22*)]. The attenuating effect of E2 on PE-induced constriction was partially reversed by treatment with the general inhibitor of nitric oxide synthases, N(G)-nitro-L-arginine (L-NNA) (Fig. 1A), and with the

[1]Molecular Cardiology Research Institute, New England Medical Center and Department of Medicine, Tufts University School of Medicine, Boston, MA 02111, USA. [2]Department of Medical Nutrition and Center for Biotechnology, Novum, Huddinge University Hospital, 141 86 Huddinge, Sweden. [3]Department of Physiology and Pharmacology, 171 77, Karolinska Institute, Stockholm, Sweden. [4]Department of Pathology, University of North Carolina, Chapel Hill, NC 27599, USA. [5]Department of Pediatrics, University of Texas Southwestern Medical Center, Dallas, TX 75390, USA.

*To whom correspondence should be addressed. E-mail: mmendelsohn@lifespan.org

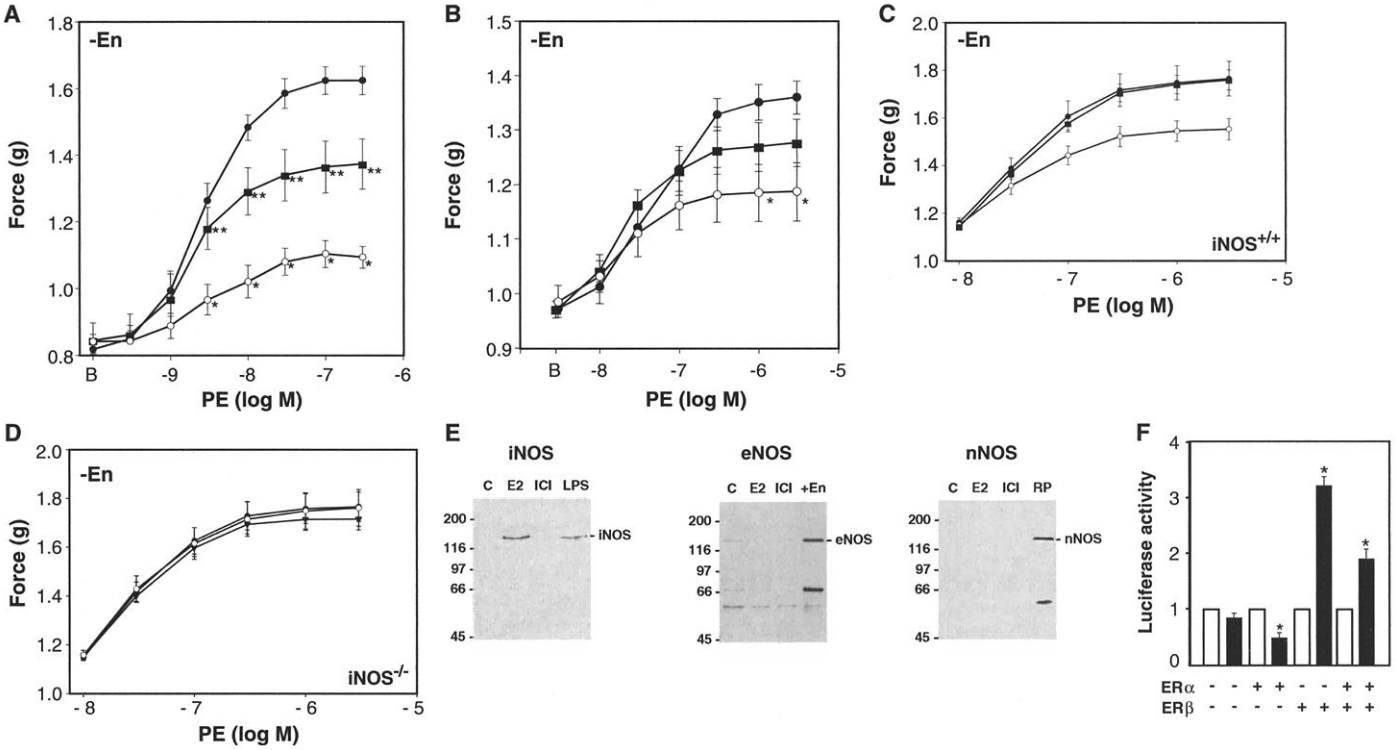


**Fig. 1.** Mechanism of estrogen inhibition of endothelial cell-independent vasoconstriction in vascular rings from WT mice. Effect of E2 on vascular constriction in endothelium-denuded (–En) WT aortic rings. Rings were treated with the indicated concentration of PE (closed circles) in the absence or presence of E2 ($10^{-8}$ M) alone (open circles) or E2 + NOS inhibitors (triangles) L-NNA ($10^{-4}$ M, $n = 6$) (**A**) or aminoguanidine ($10^{-4}$ M, $n = 9$) (**B**). *$P < 0.05$ +E2 versus vehicle alone. **$P < 0.05$ E2 + inhibitor versus +E2 alone. Vehicle alone versus E2 + AG, $P$ = NS. Effect of E2 on vascular constriction in endothelium-denuded (–En) aortic rings from (**C**) WT mice ($iNOS^{+/+}$) ($n = 13$) or (**D**) mice deficient in iNOS ($iNOS^{-/-}$) ($n = 8$), treated with vehicle alone (closed circles), E2 ($10^{-8}$ M, open circles), or E2 ($10^{-8}$ M) + ER antagonist ICI 182,780 ($10^{-7}$ M, triangles). *$P < 0.05$ + E2 versus vehicle alone, **$P < 0.05$ E2 + ICI versus +E2 only. Methods described in (*24*). (**E**) Expression of NOS isoform proteins in endothelium-denuded WT rings after incubation with E2 ($10^{-8}$ M) (E2) or E2 + ICI-182,780 ($10^{-7}$ M) (ICI) for 24 hours. Positive controls: lipopolysaccharide (10 μg/ml) (LPS), protein from endothelium-intact aortic ring (+En), rat pituitary lysate (RP), $n = 2$ to 5. Methods described in (*24*). (**F**) Effects of ERα and ERβ on iNOS reporter activity in vascular smooth muscle cells. Primary human VSMC lacking detectable mRNA or protein for either ERα or ERβ (*25*) were transfected with human iNOS-luciferase reporter plasmid (*27*) and ERα, ERβ, or both, in the absence (open bars) or presence (filled bars) of $10^{-8}$ M E2. *$P <$ 0.001 versus control.

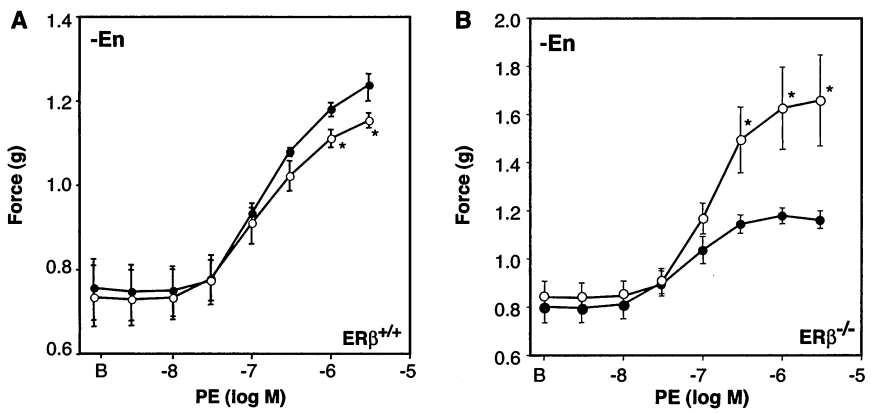


**Fig. 2.** Endothelial-independent constriction in untreated and E2-treated vascular rings from ERβKO mice and their WT littermates. (**A**) Concentration-constriction curves to PE for aortic rings from WT littermates of ERβKO mice incubated with vehicle (closed circles) or E2 ($10^{-8}$ M, open circles). (**B**) PE Concentration-constriction curves of aortic rings from ERβKO mice incubated with vehicle (closed circles) or E2 ($10^{-8}$ M, open circles) ($n = 4$). *$P < 0.05$ +E2 versus vehicle alone. Methods described in (*24*).

relatively selective iNOS inhibitor, aminoguanidine (Fig. 1B).

Vasoconstriction was examined in denuded aortic rings from mice lacking iNOS ($iNOS^{-/-}$) and WT controls (Fig. 1, C and D). Treatment of the rings with estrogen attenuated PE-induced constriction in vascular rings from WT animals (21%, $P < 0.05$). This effect was completely prevented by simultaneous treatment of the rings with the ER antagonist ICI 182,780 (Fig. 1C). In contrast, E2 failed to attenuate PE-induced constriction in aortic rings from $iNOS^{-/-}$ mice (Fig. 1D). These data support the hypothesis that E2, acting via estrogen receptors, attenuates vasoconstriction by an iNOS-dependent pathway in denuded WT blood vessels. The eNOS and neuronal NOS proteins were barely detected or undetected in untreated rings, and their expression was unaffected by E2 (Fig. 1E). The iNOS protein also was barely detected in untreated rings, but treatment of endothelium-denuded rings with E2 led to increased accumulation of the 130-kD iNOS protein, which was fully inhibited by simultaneous treatment of the rings with the ER antagonist ICI-182,780 (Fig. 1E). In primary human VSMC transfected with ERβ, E2 treatment caused a 3.3-fold activation of an iNOS reporter gene (Fig. 1F). In contrast, E2 treatment of cells transfected with ERα resulted in suppression of iNOS reporter activity. Transfection of both estrogen receptors resulted in an intermediate activation of the reporter (Fig. 1F). Thus, in normal vessels, the estrogen-stimulated increase in iNOS

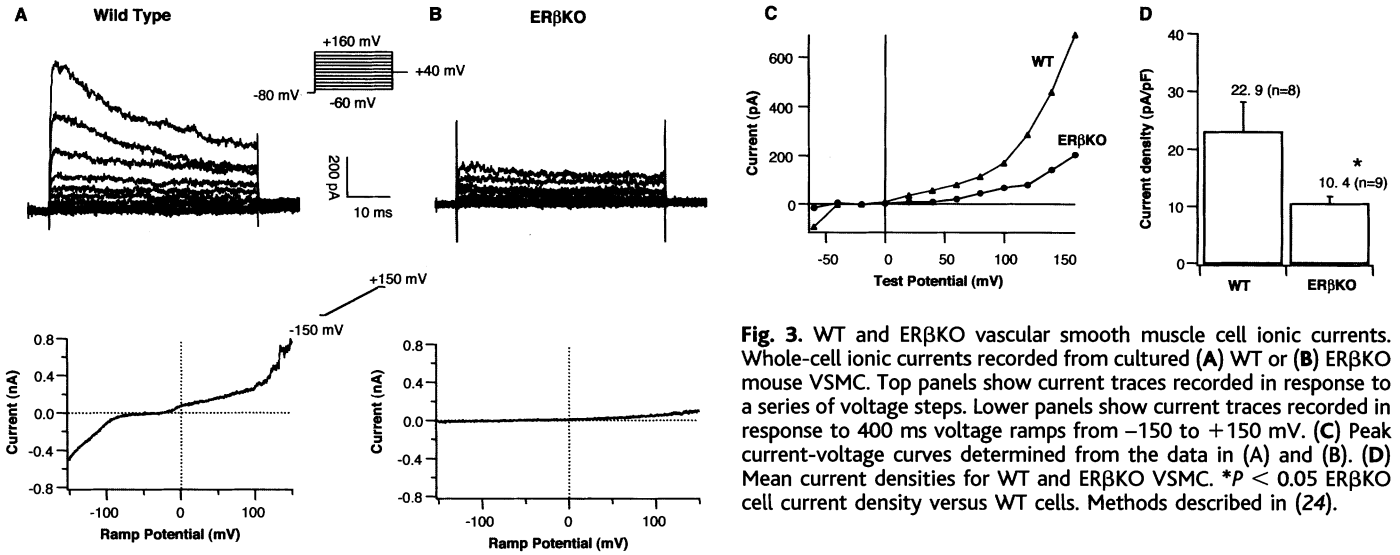


**Fig. 3.** WT and ERβKO vascular smooth muscle cell ionic currents. Whole-cell ionic currents recorded from cultured **(A)** WT or **(B)** ERβKO mouse VSMC. Top panels show current traces recorded in response to a series of voltage steps. Lower panels show current traces recorded in response to 400 ms voltage ramps from −150 to +150 mV. **(C)** Peak current-voltage curves determined from the data in (A) and (B). **(D)** Mean current densities for WT and ERβKO VSMC. $*P < 0.05$ ERβKO cell current density versus WT cells. Methods described in (*24*).

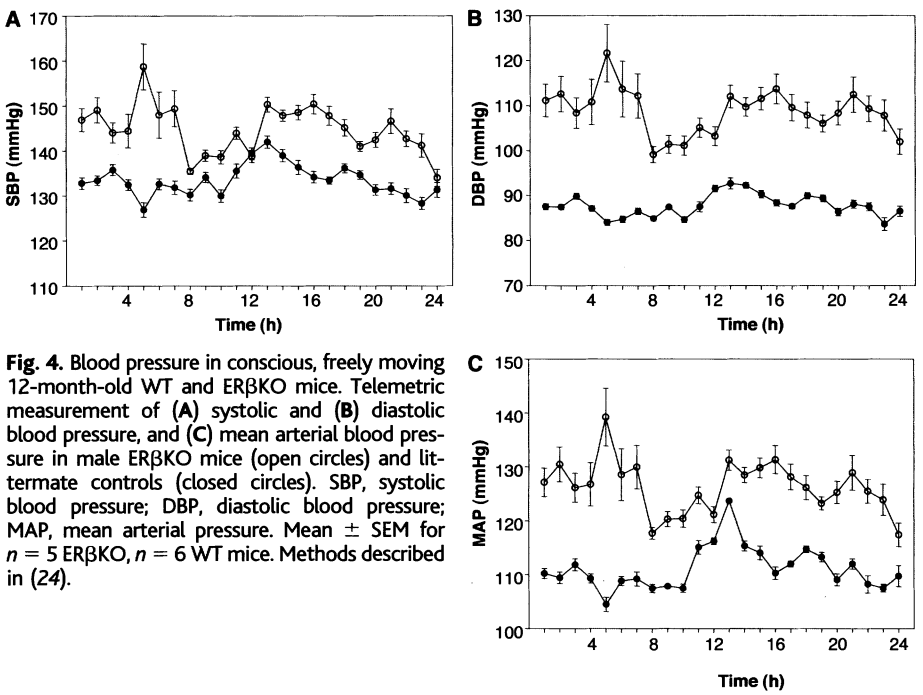


**Fig. 4.** Blood pressure in conscious, freely moving 12-month-old WT and ERβKO mice. Telemetric measurement of **(A)** systolic and **(B)** diastolic blood pressure, and **(C)** mean arterial blood pressure in male ERβKO mice (open circles) and littermate controls (closed circles). SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure. Mean ± SEM for $n = 5$ ERβKO, $n = 6$ WT mice. Methods described in (*24*).

gene transcription appears to be mediated by ERβ and antagonized by ERα, and ERβ-mediated induction of iNOS protein appears to attenuate vascular contraction.

We studied intact and endothelium-denuded vascular rings from ERβ-deficient mice [$ERβKO_{CH}$ (*8, 23*); ERβKO] and from their WT littermates. Contraction and relaxation in response to PE were identical in intact vascular rings from ERβKO mice or their WT littermates and were not altered by E2 treatment (*24*). However, endothelial cell-independent constriction was altered in vascular rings from ERβKO mice. Contraction of vascular rings from WT littermates of the ERβKO mice was diminished after treatment with E2 (15% reduction, $P < 0.05$) (Fig. 2A), though the effect in this cohort was less pronounced than in other WT mouse strains. In contrast, E2 treatment of endothelial cell-denuded rings from ERβKO mice augmented PE-induced vasoconstriction nearly twofold (183%, $P < 0.05$) compared to untreated WT controls (Fig. 2B). Endothelial cell-denuded rings from WT and ERβKO mice relaxed similarly in response to the endothelium-independent vasodilator sodium nitroprusside (*25*). Disruption of ERβ therefore leads to loss of the E2 effect to attenuate vasoconstriction, and net enhancement of the contractile response to PE in mouse vascular rings.

We used the whole-cell patch clamp technique to examine membrane currents from WT and ERβKO vascular smooth muscle cells (VSMC). WT VSMC were generally about twice as large as the ERβKO cells [WT 41.3 ± 2.8 picofarads (pf), $n = 8$; ERβKO 17.7 ± 1.3 pf, $n = 9$], and displayed larger voltage-dependent, outward currents in response to voltage steps (Fig. 3, A through C). Three of eight WT cells displayed evidence of inward rectifier potassium channels (Fig. 3A, lower panel), but such currents were not observed in ERβKO cells (Fig. 3B, lower panel) (*24*). Furthermore, after normalization for cell capacitance (and thus membrane area), WT cells had a significantly larger outward current density (Fig. 3D). The loss of outward current observed could be due to loss of either voltage-activated ($K_v$ type), or $Ca^{2+}$ and voltage-activated ($BK_{Ca}$ type) potassium channels, both of which have been implicated in the control of vasomotor tone (*24*). To test whether ERβKO animals exhibit generalized vasomotor abnormalities, we made serial tail-cuff measurements of blood pressure (BP) in intact ERβKO animals and their WT littermates. Animals had normal BP until 5 to 6 months of age, when BP increased in approximately half of a cohort of 20 ERβKO mice (*24*). Direct hemodynamic BP measurements of these mice at 6 to 7 months of age revealed elevated BP in the majority (*24*). Twelve-month-old WT and ERβKO male mice were studied with implantable, telemetric blood pressure monitoring devices. The average blood pressures recorded over an 8-day period in WT and ERβKO mice were 108 ± 2.9 and 120 ± 9.7 mm-Hg, respectively [$n = 5$ (*24*)]. Over 24-hour recording periods, both systolic and diastolic blood pressures were increased in ERβKO mice (Fig. 4, A and B), as was mean arterial pressure (Fig. 4C). No differences in heart rate were noted between the ERβKO mice and WT littermates (*24*). In studies of separate ERβKO animals, blood pressure remained elevated for as long as 22 months of age (*25*).

Our results identify a clear physiologic function for ERβ. Estrogen receptors are known to regulate a number of genes that affect vascular function (*5, 26*). Multiple ERβ-regulated gene products may contribute to the abnormal vascular contraction, ion channel dysfunction and hypertension observed in ERβKO animals. Our findings also support the concept that the transcription factor ERβ controls expression of genes critical to normal vascular physiology in both males and females. Gene targets of ERβ in relevant target tissues may provide insights into the pathophysiology and treatment of hypertension.


**References and Notes**

1. R. M. Evans, *Science* **240**, 889 (1988).
2. P. Walter *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **82**, 7889 (1985).
3. G. G. J. M. Kuiper, E. Enmark, M. Pelto-Huikko, S. Nilsson, J. Å. Gustafsson, *Proc. Natl. Acad. Sci. U.S.A.* **93**, 5925 (1996).
4. D. P. McDonnell, *Trends Endocrinol. Metab.* **10**, 301 (1999).
5. M. E. Mendelsohn, R. H. Karas, *N. Engl. J. Med.* **340**, 1801 (1999).
6. V. Lindner *et al.*, *Circ. Res.* **83**, 224 (1998).
7. S. Bakir *et al.*, *Circulation* **101**, 2342 (2000).
8. R. H. Karas *et al.*, *Circ. Res.* **89**, 534 (1999).
9. S. Ogawa *et al.*, *J. Hum. Genet.* **45**, 327 (2000).
10. A. P. Somlyo, A. V. Somlyo, *Nature* **372**, 231 (1994).
11. M. J. Davis, M. A. Hill, *Phys. Rev.* **79**, 387 (1999).
12. R. P. Lifton, A. G. Gharavi, D. S. Geller, *Cell* **104**, 545 (2000).
13. M. Y. Farhat, M. C. Lavigne, P. W. Ramwell, *FASEB J.* **10**, 615 (1996).
14. K. Kauser, G. M. Rubanyi, *J. Vasc. Res.* **34**, 229 (1997).
15. M. E. Mendelsohn, *Circ. Res.* **87**, 956 (2000).
16. T. Michel, O. Feron, *J. Clin. Invest.* **100**, 2146 (1997).
17. C. P. Weiner *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **91**, 5212 (1994).
18. R. L. Lantin-Hermoso *et al.*, *Am. J. Physiol. Lung Cell. Mol. Physiol.* **273**, L119 (1997).
19. J. K. Williams, M. R. Adams, D. M. Herrington, T. B. Clarkson, *J. Am. Coll. Cardiol.* **20**, 452 (1992).
20. V. Guetta *et al.*, *Circulation* **96**, 2795 (1997).
21. A. Mügge, M. Barton, H. G. Fieguth, M. Riedel, *Pharmacology* **54**, 162 (1997).
22. J. Binko, H. Majewski, *Am. J. Physiol. Heart Circ. Physiol.* **274**, H853 (1998).
23. J. H. Krege *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **95**, 15677 (1998).
24. Supplemental methods, results, figures, and references are available on *Science* Online at www.sciencemag.org/cgi/content/full/295/5554/505/DC1
25. Y. Zhu, M. E. Mendelsohn, unpublished results.
26. M. E. Mendelsohn, R. H. Karas, *Curr. Opin. Cardiol.* **9**, 619 (1994).
27. A. Y. Kolyada, N. Savikovsky, N. E. Madias, *Biochem. Biophys. Res. Commun.* **220**, 600 (1996).


28. We are grateful to N. Madias for the iNOS-luciferase reporter construct, N. Flavahan for guidance in establishing the vascular ring methods, and M. Aronovitz for superb technical assistance. Supported in part by NIH grants P50 HL63494, R01 HL55309, and R01 HL56069 (M.E.M.); NIH grant HL56235 and a Grant-in-Aid from the American Heart Association (Y.Z.); NIH grant R01 HL61298 (R.H.K.); NIH grants HD30276 and HL53546 (P.W.S.); NIH grant GM20069 (O.S.); and grants from KaroBio AB and the Swedish Cancer Fund (J-Å.G.), MFR04764 and Heart and Lung Foundation (P.T.), and from Karolinska Institute (1306/2000FFU) (Z.B.). The reported findings are solely the responsibility of the authors and do not necessarily represent the official views of the NIH.

9 August 2001; accepted 27 November 2001


REPORTS

# Alternative Splicing and Neuritic mRNA Translocation Under Long-Term Neuronal Hypersensitivity


**Eran Meshorer,[1] Christina Erb,[1] Roi Gazit,[2] Lev Pavlovsky,[3] Daniela Kaufer,[1]* Alon Friedman,[3] David Glick,[1] Nissim Ben-Arie,[2] Hermona Soreq[1]†**



**To explore neuronal mechanisms underlying long-term consequences of stress, we studied stress-induced changes in the neuritic translocation of acetylcholinesterase (AChE) splice variants. Under normal conditions, we found the synaptic AChE-S mRNA and protein in neurites. Corticosterone, anticholinesterases, and forced swim, each facilitated a rapid (minutes), yet long-lasting (weeks), shift from AChE-S to the normally rare AChE-R mRNA, promoted AChE-R mRNA translocation into neurites, and induced enzyme secretion. Weeks after stress, electrophysiological measurements in hippocampus slices displayed apparently normal evoked synaptic responses but extreme hypersensitivity to both anticholinesterases and atropine. Our findings suggest that neuronal hypersensitivity under stress involves neuritic replacement of AChE-S with AChE-R.**


Traumatic stress is often followed by long-term pathological changes (*1, 2*). In humans, extreme cases of such changes are clinically recognized as posttraumatic stress disorder (PTSD) (*3*). Although the immediate response to acute stressful insults has been extensively studied, the molecular mechanisms leading to the long-term neuronal hypersensitivity that is characteristic of PTSD are yet unknown. Stimulus-induced changes in alternative splicing have recently emerged as a major mechanism of neuronal adaptation to stress, contributing to the versatility and complexity of the expression patterns of the human genome (*4–6*). Another stimulus-induced post-transcriptional process is dendritic mRNA translocation, which has been described for several transcripts (*7–12*). Because psychological, physical, and chemical stressors all cause neuronal activation and hyperexcitation, dendritic translocation of specific target mRNAs may follow.

Acetylcholinesterase (AChE) modulations provide an appropriate case study for exploring long-term stress effects. Chemical, psychological, and physical stresses all shift splicing from the primary mRNA product that encodes the synaptic membrane AChE-S multimeric protein to the normally rare "readthrough" AChE-R transcript, which yields soluble monomers (*13*). We thus examined neuronal distributions of the two splice variants, which have characteristic 3′ regions (Fig. 1A). A comprehensive search of the NCBI GenBank and EST databases revealed several AChE-S mRNAs but only a single AChE-R mRNA of rodent brain origin (GenBank accession number X70141), attesting to the scarcity and/or instability of neuronal AChE-R mRNA under normal conditions. To study changes in gene expression at the subcellular level, we used double-label fluorescence in situ hybridization (FISH) of specific AChE mRNA splice variants (*14*) and confocal microscope image analysis.

FISH detection efficiencies likely depend on probe sequences, but subcellular distributions can be reliably compared for single transcripts in different cells and conditions. Cultured PC12 cells (*15*), primary cultures of mouse cerebellar neurons (*16*), and pyramidal neurons in paraffin-embedded sections of the prefrontal cortex (*17*) all displayed a larger fraction of AChE-S mRNA transcripts in neuronal processes than of AChE-R mRNA (Fig. 1, B through D). Also, both cell types displayed nuclear localization of AChE-R but not of AChE-S mRNA (Fig. 1, B and C) (*18*). To test whether labeling properties prejudiced this conclusion, we reversed the fluorophores on the two probes (Fig. 1, B and C). In paraffin-embedded brain sections from naïve mice, cortical pyramidal neurons presented dispersed AChE-S mRNA through-


Departments of [1]Biological Chemistry and [2]Cell and Animal Biology, The Institute of Life Sciences and The Eric Roland Center for Neurodegenerative Diseases, The Hebrew University of Jerusalem, Israel 91904. [3]Departments of Physiology and Neurosurgery, Zlotowsky Center of Neuroscience, Ben Gurion University and Soroka Medical Center, Beersheva, Israel 84105.

*Present address: Department of Biological Sciences, Stanford University, Stanford, CA 94305, USA.
†To whom correspondence should be addressed. E-mail: soreq@shum.huji.ac.il