Uncoupling Proteins 2 and 3 Function in Concert to Augment Tolerance to Cardiac Ischemia*

Christopher J. McLeod1†, Abdulhameed Aziz3†, Robert F. Hoyt, Jr.9, J. Philip McCoy, Jr.9, and Michael N. Sack1‡2

From the 1Cardiovascular Branch, 2Laboratory of Animal Medicine and Surgery, 3Flow Cytometry Core, NHLBI, National Institutes of Health, Bethesda, Maryland 20892-1454

Transient cardiac ischemia activates cell survival signaling, conferring subsequent ischemia tolerance to the heart. This biological phenomenon, termed ischemic preconditioning, results in improved clinical outcome and attenuated infarct size following myocardial infarction. To explore genomic modifications underpinning this ischemia tolerance, we delineated the regulation and function of the cardiac enriched mitochondrial uncoupling proteins 2 and 3 during delayed ischemic preconditioning in the rat. Cardiac transcripts of genes encoding uncoupling proteins 2 and 3 are up-regulated in parallel with infarct size reduction in preconditioned hearts. Mitochondria isolated from preconditioned hearts exhibit an augmented inducible proton leak. In parallel, following anoxia-reoxygenation these mitochondria generate less hydrogen peroxide compared with non-preconditioned mitochondria. Preconditioning in rat cardiac derived myoblasts is abolished following uncoupling protein-2 depletion by RNA-interference. RNAi of uncoupling protein-3 partially attenuates the capacity to precondition these cells. Functional characterization of anoxia and reoxygenation tolerance following uncoupling protein 2 or 3 and combined 2 and 3 RNAi shows the largest reduction in viability follows depletion of both homologues. Uncoupling protein-2 depletion alone significantly attenuates anoxia-reoxygenation tolerance but uncoupling protein-3 depletion does not reduce anoxia tolerance. In parallel combined uncoupling protein depletion and isolated uncoupling protein-2 depletion augments ROS production in viable cardiomyocytes following anoxia-reoxygenation. Concurrent antioxidant administration ameliorates the uncoupling protein-depleted anoxia-susceptible phenotype. In conclusion, mitochondrial uncoupling proteins are necessary components of ischemia tolerance and function as components of the cellular antioxidant defense program. In the cytoprotective hierarchy, uncoupling protein-2 appears to play a greater role than uncoupling protein-3 in modulating ischemia/anoxia tolerance in heart-derived cells.

The cell survival program, termed ischemic preconditioning, evoked by transient nonlethal tissue ischemia, is evolutionarily conserved and is evident across multiple organs/tissues. The signal transduction networks engendering this phenomenon are well described (1); however, functional cellular modifications conferring ischemia tolerance are less tangible. Interestingly, the invariable metabolic signature of preconditioning is abolished following ischemia reperfusion. Sham operations involved encircling the left coronary artery of Wistar rats as described previously (3). Here, the heart was subjected to five episodes of 3 min of ischemia coupled to 5 min of reperfusion. Sham operations involved encircling the left coronary artery without tightening the suture. Since inhibition of antioxidant signaling abolishes the preconditioning program, 2-mercaptopyrrolidone glycine (2-MPG) was administered to an additional group of rats (0.42 g/kg body weight, i.v., 1 h before the ischemic protocol).


doi:10.1074/jbc.M505258200

1 Funded by the Sarnoff Foundation.

2 To whom correspondence should be addressed: Cardiovascular Branch, NHLBI/National Institutes of Health, Bldg. 10-CRC, Rm. 5-3150, 10 Center Dr., Bethesda, MD 20892-1454. Tel.: 301-402-9259; Fax: 301-402-0888; E-mail: sackm@nhlbi.nih.gov.

3 The abbreviations used are: ROS, reactive oxygen species; 2-MPG, 2-mercaptopyrrolidone glycine; DCF, dichlorofluorescein; RNAi, RNA interference; 7-AAD, 7-amino-actinomycin D.

EXPERIMENTAL PROCEDURES

Animal Experiments—The ischemic preconditioning trigger was achieved by conferring transient regional ischemia to the left coronary artery of Wister rats as described previously (3). Here, the heart was subjected to five episodes of 3 min of ischemia coupled to 5 min of reperfusion. Sham operations involved encircling the left coronary artery without tightening the suture. Since inhibition of antioxidant signaling abolishes the preconditioning program, 2-mercaptopyrrolidone glycine (2-MPG) was administered to an additional group of rats (0.42 g/kg body weight, i.v., 1 h before the ischemic protocol).

Received for publication, May 12, 2005, and in revised form, July 28, 2005. Published, JBC Papers in Press, August 3, 2005.
Delayed preconditioned mitochondria exhibit a GDP-sensitive proton leak

| Succinate + oligomycin | Succinate + oligomycin + GDP | GDP-induced Δ in respiration |
|------------------------|-----------------------------|-----------------------------|
| nmmol O₂/mg protein/min | nmmol O₂/mg protein/min | %                          |
| Sham                   | 49 ± 5                      | 46 ± 9                      | 5 ± 4                     |
| PC                     | 64 ± 4*                     | 46 ± 2                      | 29 ± 4*                   |

*p < 0.05 versus nonpreconditioned mitochondria.
To establish whether the up-regulation of these inner mitochondrial carrier proteins modulate respiratory function, mitochondria were isolated from rat hearts 24 h following ischemic preconditioning and sham procedures. Basal glutamate/malate-driven mitochondrial oxygen consumption (State 3 respiration) and oligomycin-insensitive respiration (basal leak) are similar between preconditioned and nonpreconditioned mitochondria, and no differences in basal ROS levels were measured (data not shown). Interestingly, UCP2 and -3 have recently been shown to be quiescent during basal respiration but demonstrate a capacity to be up-regulated by delayed preconditioning. As the preconditioning trigger induces modifications in cellular tolerance to stress, mitochondria were isolated from rat hearts 24 h following ischemic preconditioning and sham procedures. Basal glutamate/malate-driven mitochondrial oxygen consumption (State 3 respiration) and oligomycin-insensitive respiration (basal leak) are similar between preconditioned and nonpreconditioned mitochondria, and no differences in basal ROS levels were measured (data not shown). Interestingly, UCP2 and -3 have recently been shown to be quiescent during basal respiration but demonstrate a capacity to be up-regulated by delayed preconditioning. 

FIGURE 1. Uncoupling protein gene expression and steady-state protein levels are up-regulated by delayed preconditioning. A, histogram shows the average ± S.E. mRNA levels of UCP2 and UCP3 as measured by quantitative PCR. The mRNA levels are compared 4 h following the ischemic preconditioning trigger versus the sham-operated control rats and in response to preconditioning in the presence of the preconditioning agonist and antioxidant 2-MPG. Values are expressed as arbitrary units normalized to sham = 1. *, p < 0.001 versus the sham control gene transcripts. B, inset shows a representative Western blot of UCP2 steady-state protein levels 24 h following an ischemic preconditioning trigger versus sham procedures. The histogram represents the mean ± S.E. UCP2 steady-state levels of all samples normalized to actin (n = 6 per group). AU, arbitrary units. *, p < 0.001 versus sham controls. PC, preconditioning.

greater inducible proton leak compared with sham control mitochondria (TABLE ONE). Notably, this enhanced inducible leak is inhibited by the UCP antagonist GDP (12, 13) (TABLE ONE). Since up-regulation of an inducible proton leak following delayed preconditioning would be compatible with an attenuation of mitochondrial ROS generation in response to anoxia-reoxygenation, we evaluated the generation of H2O2 at postanoxic reoxygenation. The delayed preconditioned mitochondria exhibited a 51.4 ± 7.6% reduction in H2O2 production at reoxygenation following 25 min of in vitro anoxia compared with sham control mitochondria as measured fluoroscopically (Fig. 2).

In order to establish the functional requirement of UCP2 and UCP3 in modulating tolerance to preconditioning and anoxia-reoxygenation, we employed RNA interference to deplete UCP2 and -3 in rat H9c2 cells. In parallel with the rat heart, UCP2 is the predominant uncoupling protein in H9c2 cells and is expressed in excess of 20-fold greater abundance than the UCP3 transcript (data not shown). RNAi resulted in a 75% reduction in UCP2 transcript and an 85% reduction in UCP3 and a similar combined depletion in response to RNAi of both constructs as measured by real time quantitative PCR (Fig. 3A). UCP2 steady-state protein levels were reduced to 39 ± 11% of base-line expression (Fig. 3B). Gene silencing was maintained from 24 to 72 h following transfection. RNAi and scrambled control RNAi constructs were maintained in equal molar amounts in all experimental groups, and cell viability was assessed by flow cytometry using the nuclear stain 7-AAD. Small interfering RNA treatment did not modulate cellular viability under non-stressed conditions over a 72-h period (data not shown).

The H9c2 cells exhibit anoxia-induced preconditioning with 84 ± 4% viability versus 22 ± 9% viability in nonpreconditioned controls in response to the index anoxia-reoxygenation insult (p < 0.001; Fig. 4). Prior depletion of UCP2 completely abolished preconditioning-mediated protection, whereas UCP3 depletion partially abolished the protective phenotype. Viability, however, was significantly greater than nonpreconditioned cells but yet significantly less than that seen in control RNAi preconditioned cells (Fig. 4).

As the preconditioning trigger induces modifications in cellular tolerance to subsequent ischemia-reperfusion injury, we then evaluated whether genomic depletion of the cardiac enriched UCPs directly modulates anoxia-reoxygenation injury following 16 h of anoxia and 2 h of
re-oxygenation. Viability was incrementally diminished from modest but nonsignificant effects on viability with UCP3 depletion to increasingly significant loss of viability following UCP2 and combined UCP2 and UCP3 depletion compared with scrambled RNAi transfected controls (Fig. 5, A and B). The cell viability diminished from 60 ± 4% in the scrambled RNAi controls to 47 ± 2% viability with UCP3 depletion (p = not significant) to 31 ± 7% following UCP2 RNAi (p < 0.05) to 19 ± 5% (p < 0.001) following the UCP2 and UCP3 depletion (Fig. 5, A and B). A putative mechanism whereby depletion of UCP2 and the combined UCP2 and UCP3 depletion would augment cell death in response to anoxia-reoxygenation is via increased ROS production. To assay ROS generation, the amount of dichlorofluorescein (DCF) fluorescence was measured post-anoxia-reoxygenation, using cells gated for viability (7-AAD-impermeable). The amount of DCF fluorescence inversely correlated with cell viability. The least ROS was present in cells transfected with scrambled RNAi, and the most ROS was found in cells depleted in both UCP2 and UCP3 (Fig. 5, C and D). Interestingly, in the knockdown experiments, viability was rescued when the cells were incubated with 1 mM 2-MPG throughout the anoxia-reoxygenation protocol (Fig. 6A). In concert, DCF fluorescence and hence ROS production was also attenuated with 2-MPG (Fig. 6B).

DISCUSSION

Our results show that the delayed preconditioning program up-regulates UCP2 and UCP3 in the rat heart. This genomic regulation is associated with an inducible mitochondrial GDP-dependent proton leak and an augmented capacity to ameliorate postanoxic H2O2 production. In parallel, the preconditioning phenotype is abolished following UCP2 RNAi depletion and partially blunted by UCP3 depletion in H9c2 cells. Functional characterization of these inner mitochondrial membrane proteins in the rat heart-derived myoblasts demonstrates that depletion of UCP2 or both UCP2 and UCP3 is associated with a diminished cellular tolerance to anoxia-reoxygenation injury and is associated with increased production of postanoxic ROS. Conversely, this susceptibility to anoxia-reoxygenation injury is annulled by the concurrent administration of the antioxidant 2-MPG. Together, these data strongly support a pivotal role of the cardiac enriched uncoupling proteins in innate cardiomyoblast ischemia tolerance and demonstrate that these
inner mitochondrial membrane carrier proteins are modulated as putative adaptive components of the delayed ischemic preconditioning cell survival program.

Twenty-four hours following the ischemic preconditioning trigger, the intact beating heart exhibits hemodynamic parity with nonpreconditioned hearts (3). This observation probably reflects similar energetic demand of preconditioned and nonpreconditioned hearts in the absence of additional biomechanical stress. Here, the observation that basal state 3 respiration in delayed preconditioned mitochondria is unaltered, despite the up-regulation of UCP2 and UCP3, is compatible with this hemodynamic profile. Furthermore, these data suggest that the preconditioning-mediated up-regulation of UCP2 and UCP3 are functionally quiescent during basal mitochondrial function. This quiescent nature of increased levels of uncoupling proteins aligns with the observations in transgenic murine hearts overexpressing UCP1 (21). Here Bouillaud and co-workers demonstrate that under normoxic conditions, the UCP1 transgenic mice have similar oxygen consumption, contractility, and energetic yield compared with littermate control mice (21).

Interestingly, recent studies demonstrate that UCP2 is activated/induced via ROS signaling (17, 18). Thus, we would postulate that a subsequent index ischemia-reperfusion-mediated oxidative stress could function as a trigger to mediate uncoupling protein activation. This concurs with observations that UCP-mediated proton leak is unmasked by ROS generation via reverse electron transfer through complex I of the electron transfer chain (19, 20). This modus operandi of UCPs as ROS detoxifying proteins supports their role as end effectors in the preconditioning-induced cell survival program. Moreover, it is feasible that this protein may function in an autoregulatory feed-forward manner to protect the mitochondria and cell from future excessive ROS generation (22).

The role of excessive ROS-mediated damage in cellular injury following ischemia-reperfusion is subject of an ongoing debate. Nevertheless, a significant body of evidence implicates a substantial role for ROS suppression in the attenuation of ischemia-reperfusion injury (23). Furthermore, previous studies have demonstrated that ROS scavenging proteins, such as SOD2, are up-regulated following delayed preconditioning (8). SOD2 overexpression in turn confers protection against cardiac ischemia-reperfusion injury (24). Our data provide additional evidence to support the control of ROS as a mechanism to sustain cellular viability in response to anoxia-reoxygenation. The reduction in postanoxic ROS in the isolated preconditioned mitochondria probably encompasses both the up-regulation of the uncoupling proteins and other known antioxidant defenses, such as SOD2, which are activated by the preconditioning program. However, our gene depletion studies do support the involvement of the UCP homologues in postanoxic ROS

![FIGURE 5. UCP isoform RNA silencing demonstrates differing viability and ROS production following anoxia-reoxygenation (A/R) in H9c2 cells. A, mean ± S.E. of nonincorporated 7-AAD fluorescence, indicating viable cells in response to the different RNAi transfection conditions. B, representative flow cytometric overlaid images, displaying impermeable (viable) and nucleus-incorporated (nonviable) 7-AAD peaks. Black histogram, anoxia-reoxygenation with control RNAi; orange histogram, anoxia-reoxygenation with UCP2 RNAi; purple histogram, anoxia-reoxygenation with UCP3 RNAi; red histogram, anoxia-reoxygenation with UCP2 and UCP3 RNAi. C, mean ± S.E. DCF fluorescence, derived primarily from intramitochondrial ROS. D, representative cytometric images of DCF fluorescence. Color coding parallels histograms in Fig. 5B. Data are shown as mean ± S.E. All of these experiments were performed at least four times in triplicate. *, p < 0.001; **, p < 0.0001 versus control RNAi cells.](image-url)
Mitochondrial Uncoupling Proteins and Ischemia Tolerance

UCP2 RNA silencing demonstrates reduced viability and augmented ROS production following anoxia-reoxygenation in H9c2 cells. Treatment with the antioxidant 2-MPG rescues the UCP2 knockdown phenotype. A, mean ± S.E. of nonincorporated 7-AAD fluorescence, indicating viable cells. B, mean ± S.E. DCF fluorescence, derived primarily from intramitochondrial ROS in H9c2 cells gated for viability via exclusion of 7-AAD. Data are shown as mean ± S.E. All of these experiments were performed at least four times in triplicate. *p < 0.001 versus control RNAi cells.

In conclusion, we previously demonstrated that isolated mitochondria following activation of the delayed preconditioning cell survival program are more tolerant of ex vivo anoxia-reoxygenation (3). Here we demonstrate that this resilient mitochondrial phenotype is associated with an up-regulation of the cardiac enriched UCP2 and UCP3 genes. In addition, preconditioned mitochondria exhibit a ROS-inducible modest uncoupling of oxidative phosphorylation as quantified by oligomycin-insensitive respiration in response to GDP inhibition. These preconditioned mitochondria also shown enhanced capacity to attenuate ROS generation following anoxia-reoxygenation. The genetic depletion of the UCP2 and UCP3 in mitochondria in situ further demonstrates that these inner mitochondrial membrane proteins are a necessary component of cellular antioxidant defense mechanisms, whose effects can be rescued by exogenous ROS scavenging. These findings strongly support UCP2 and UCP3 as endogenous cytoprotective proteins that are both regulated and induced in response to ischemic stress. Their cytoprotective effects parallel the relative expression of these two mitochondrial uncoupling protein homologues in that UCP2 depletion has greater susceptibility to anoxia than does UCP3 depletion. Finally, UCP2 and UCP3 are shown to be necessary components of innate cellular antioxidant defense. These data support a functional role of the uncoupling proteins in nonthermogenic tissue and implicate the mitochondrial uncoupling proteins as potential therapeutic targets for the management of ischemic diseases.
Mitochondrial Uncoupling Proteins and Ischemia Tolerance

Acknowledgments—We thank Professor Martin D. Brand (University of Cambridge), Professor Keith Garland (Portland State University), and Dr. Robert S. Balaban (NHLBI, National Institutes of Health) for helpful discussions and Dr. Paul Hwang and Dr. Toren Finkel (NHLBI) for critical review of the manuscript.

REFERENCES

1. Yellon, D. M., and Downey, J. M. (2003) Physiol. Rev. 83, 1113–1151
2. Opie, L. H., and Sack, M. N. (2002) J. Mol. Cell. Cardiol. 34, 1077–1089
3. McLeod, C. J., Jeyabalan, A. P., Minners, J. O., Clevenger, R., Hoyer, R. F., Jr., and Sack, M. N. (2004) Circulation 110, 534–539
4. McCarthy, J., McLeod, C. J., Minners, J., Essop, M. F., Ping, P., and Sack, M. N. (2005) J. Mol. Cell. Cardiol. 38, 697–700
5. Ozcan, C., Bienengraeber, M., Dzeja, P. P., and Terzic, A. (2002) Am. J. Physiol. 282, H531–H539
6. Oshima, Y., Fujio, Y., Nakashima, T., Itoh, N., Yamamoto, Y., Negoro, S., Tanaka, K., Kishimoto, T., Kawase, I., and Azuma, J. (2005) Cardiovasc. Res. 65, 428–435
7. Glantz, L., Avramovich, A., Trembovler, V., Gurvitz, V., Kohen, R., Eidelman, L. A., and Shohami, E. (2005) Exp. Neurol. 192, 117–124
8. Yamashita, N., Hoshida, S., Taniguchi, N., Kuzuya, T., and Hori, M. (1998) J. Mol. Cell Cardiol. 30, 1181–1189
9. Adell, H., Chen, Z., Ko, Y., Mejia-Alvarez, R., and Marban, E. (2004) Proc. Natl. Acad. Sci. U.S.A. 101, 11880–11885
10. St-Pierre, J., Lin, J., Krauss, S., Tarr, P. T., Yang, R., Newgard, C. B., and Spiegelman, B. M. (2003) J. Biol. Chem. 278, 26597–26603
11. Sordahl, L. A., Besch, H. R., Jr., Allen, J. C., Crow, C., Lindenmayer, G. E., and Shohami, E. (2005) J. Clin. Invest. 112, 1831–1842
12. Jaburek, M., Varecha, M., Gimeno, R. E., Dembski, M., Jezek, P., Zhang, M., Burn, P., Tartaglia, L. A., and Garlid, K. D. (1999) J. Biol. Chem. 274, 26003–26007
13. Zackova, M., Skobisova, E., Urbankova, E., and Jezek, P. (2005) J. Biol. Chem. 278, 20761–20769
14. Talbot, D. A., Lambert, A. J., and Brand, M. D. (2004) FEBS Lett. 556, 111–115
15. Bienengraeber, M., Ozcan, C., and Terzic, A. (2003) J. Mol. Cell Cardiol. 35, 861–865
16. Tang, X. L., Takano, H., Rizvi, A., Turrens, J. F., Qiu, Y., Wu, W. I., Zhang, Q., and Bolli, R. (2002) Am. J. Physiol. 282, H281–H291
17. Echtay, K. S., Murray, M. P., Smith, R. A., Talbot, D. A., and Brand, M. D. (2002) J. Biol. Chem. 277, 47129–47135
18. Krauss, S., Zhang, C. Y., Scorrano, L., Dalgaard, L. T., St-Pierre, J., Grey, S. T., and Lowell, B. B. (2003) J. Clin. Invest. 112, 1831–1842
19. Echtay, K. S., Rousset, D., St-Pierre, J., Jakobsen, M. B., Cadenas, S., Stuart, J. A., Harper, J. A., Roebuck, S. J., Morrison, A., Pickering, S., Clapham, J. C., and Brand, M. D. (2002) Nature 415, 96–99
20. Considine, M. J., Goodman, M., Echtay, K. S., Lalo, M., Whelan, J., Brand, M. D., and Sweetlove, L. J. (2003) J. Biol. Chem. 278, 22398–22302
21. Hoeter, J., Gonzalez-Barroso, M. D., Couplan, E., Mateos, P., Gelly, C., Cassard-Doulcier, A. M., Diolez, P., and Bouillaud, F. (2004) Circulation 110, 528–533
22. Krauss, S., Zhang, C.-Y., and Lowell, B. B. (2005) Nat. Rev. Mol. Cell Biol. 6, 248
23. Marzin, N., El-Habashi, N., Hoare, G. S., Bundy, R. E., and Yacoub, M. (2003) Arch. Biochem. Biophys. 420, 222–236
24. Chen, Z., Siu, B., Ho, Y. S., Vincent, R., Chua, C. C., Hamdy, R. C., and Chua, B. H. (1998) J. Mol. Cell Cardiol. 30, 2281–2289
25. Arsenijevic, D., Oruma, H., Pecqueur, C., Raimbaulh, S., Manning, B. S., Miroux, B., Couplan, E., Alves-Guerra, M. C., Goubert, M., Swiet, R., Bouillaud, F., Richard, D., Collins, S., and Riquier, D. (2000) Nat. Genet. 26, 435–439
26. Mattiasson, G., Shamloo, M., Gido, G., Mathi, K., Tomasevic, G., Yi, S., Warden, C. H., Castilho, R. F., Melcher, T., Gonzalez-Zulueta, M., Nikolich, K., and Wieloch, T. (2003) Nat. Med. 9, 1062–1068
27. Talbot, D. A., Hanusie, N., Rey, B., Rousset, J. L., Duchamp, C., and Brand, M. D. (2003) Biochem. Biophys. Res. Commun. 312, 983–988
28. Fink, B. D., Hong, Y. S., Mathals, M. M., Scholz, T. D., Dillon, J. S., and Sivitz, W. I. (2002) J. Biol. Chem. 277, 3918–3925
29. Teshima, Y., Akao, M., Jones, S. P., and Marban, E. (2003) Circ. Res. 93, 192–200
30. Lee, K. U., Lee, I. K., Han, J. H., Song, D. K., Kim, Y. M., Song, H. S., Kim, H. S., Lee, W. J., Koh, E. H., Song, K. H., Han, S. M., Kim, M. S., Park, I. S., and Park, J. Y. (2005) Circ. Res. 96, 1200–1207
31. Couplan, E., del Mar Gonzalez-Barroso, M., Alves-Guerra, M. C., Riquier, D., Goubert, M., and Bouillaud, F. (2002) J. Biol. Chem. 277, 26268–26275
32. de Bilbao, F., Arsenijevic, D., Vallet, P., Hjelle, O. P., Ottersen, O. P., Bouras, C., Raffin, Y., Abou, K., Langhans, W., Collins, S., Plamondon, J., Alves-Guerra, M. C., Hauenauer, A., Garcia, I., Richard, D., Riquier, D., and Giannakopoulos, P. (2004) J. Neurochem. 89, 1283–1292
33. Skulachev, V. P. (1998) Biochem. Biophys. Acta 1363, 100–124
34. Minners, J., van den Bos, E. J., Yellon, D. M., Schwahh, H., Opie, L. H., and Sack, M. N. (2000) Cardiovasc. Res. 47, 68–73
35. Minners, J., Lacerda, L., McCarthy, M., Meiring, J. I., Yellon, D. M., and Sack, M. N. (2001) Circ. Res. 89, 787–792
36. Maragos, W. F., and Korde, A. S. (2004) J. Neurochem. 91, 257–262
37. Schrauwen, P., Hardie, D. G., Roorda, B., Clapham, J. C., Abou, K., Langhans, W., Collins, S., Plamondon, J., Alves-Guerra, M. C., Hauenauer, A., Garcia, I., Richard, D., Riquier, D., and Giannakopoulos, P. (2004) J. Neurochem. 89, 1283–1292
38. Talbot, M., Miyamoto, S., De Mascio, P., Garlid, K. D., and Jezek, P. (2004) J. Biol. Chem. 279, 53097–53102