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Microarray Analyses of Genes Regulated by Isoflurane Anesthesia *In Vivo*: A Novel Approach to Identifying Potential Preconditioning Mechanisms

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Abstract

Background—While general anesthetics are recognized for their potential to render patients unconscious during surgery, exposure can also lead to long-term outcomes of both cellular damage and protection. As regards the latter, delayed anesthetic preconditioning is an evolutionarily conserved physiological response that has the potential for protecting against ischemic injury in a number of tissues. While it is known that delayed preconditioning requires *de novo* protein synthesis, knowledge of anesthetic-regulated genes is incomplete. In this study we used the conserved nature of preconditioning to analyze differentially regulated genes in three different rat tissues. We hypothesized that by selecting those genes regulated in multiple tissues, we could develop a focused list of gene candidates potentially involved in delayed anesthetic preconditioning.

Methods—Young adult male Sprague Dawley rats were anesthetized with a 2% isoflurane/98% air mixture for 90 min. Immediately after anesthetic exposure, animals were killed and liver, kidney and heart were removed and total RNA was isolated. Differential gene expression was determined using rat oligonucleotide gene arrays. Array data were analyzed to select for genes that were significantly regulated in multiple tissues.

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Results—All three tissues showed differentially regulated genes in response to a clinically relevant exposure to isoflurane. Analysis of coordinately regulated genes yielded a focused list of 34 potential gene candidates with a range of ontologies including regulation of inflammation, modulation of apoptosis, regulation of ion gradients and maintenance of energy pathways.

Conclusions—We conclude that, through using an analysis approach focusing on coordinately regulated genes, we were able to generate a focused list of interesting gene candidates with potential to enable future preconditioning studies.

Introduction

General anesthetics, such as the inhaled drug isoflurane, are recognized and valued for their critical hypnotic actions in rendering patients unconscious during surgery. However, along with their acute effects studies have revealed that general anesthetics can also result in long-term changes (hours-weeks postexposure) in cellular and tissue function[1-3]resulting in either detrimental or potentially protective outcomes. Indeed, it is now well documented that even brief exposures to anesthetic drugs can have profound and lasting effects on gene expression in multiple tissues.[4-6] For instance, Sergeev et al. demonstrated in the heart that exposures to isoflurane triggered changes in the regulation of multiple genes involved in both cellular protection and damage, and discussed the former in relation to their potential roles in anesthetic preconditioning (APC) protection.[7]

Preconditioning is a biological phenomenon whereby nonlethal stressors, including brief bouts of ischemia[8] or drugs such as volatile anesthetics[9,10] are able to regulate endogenous pathways which can protect against subsequent, potentially lethal ischemia. Protection derived through preconditioning is typically biphasic. Early preconditioning (lasting 2-3 hrs) relies on rapid, post-translational modifications to existing proteins and begins almost immediately after application of the preconditioning stimulus.[11] By contrast, delayed preconditioning relies on *de novo* protein synthesis and has an extended protective window that begins 12-24 hrs post-stimulus, and lasts up to 96 hrs *in vivo.*[12]

Delayed preconditioning has been shown to provide protection against acute ischemic injury in a range of tissues including brain and heart in a number of *in vivo* and *in vitro* models[9,10,13-16] Furthermore, delayed APC can also provide improved long-term neurological and histological outcomes after cerebral ischemic injury.[17] Given the potential for both widespread protection from ischemic injury along with the relative safety of administering APC,[18] there is substantial interest in understanding the molecular mechanisms through which APC arises.[19,20]

Multiple pathways have been identified in APC that involve cyclooxygenase-2,[21] nuclear factor kappa B,[22] PI3 kinase,[23] adenosine triphosphate-sensitive potassium channels,[24] manganese superoxide dismutase,[25] 12-lipoxygenase[26] and nitric oxide.[20] Nevertheless, a comprehensive picture of how anesthetics induce protection has yet to be described. In the current study we aimed to generate a focused list of genes that might potentially play a role in the induction of delayed APC. To accomplish this we used DNA microarray to identify anesthetic-regulated genes[27] at an early window after anesthetic exposure. In order to analyze the regulated genes and to identify those that might be involved in APC, we used a novel approach in which we capitalized on the observation that delayed APC is conserved across a wide range of organisms (mouse,[1] rat,[9] rabbit,[10] and human[16])and tissues (heart,[10] brain,[9] skeletal muscle,[28] kidney[27]and spinal chord[22]). Indeed, APC has even been shown to extend beyond vertebrates to Caenerhabitis elegans.[29,30] By filtering our data to select for genes regulated in multiple tissues, we generated a focused list of genes coordinately regulated in response to isoflurane exposure.

Methods

Animal Care

Animals were housed and all experiments performed with protocols approved by Smith College Institutional Animal Care and Use Committee (Northampton, MA, USA) according to the Guide for the Care and Use of Laboratory Animals (National Institutes of Health, 1996). Male Spraque-Dawley rats were acquired from Jackson Laboratories (250 and 300 grams; Bar Harbor, ME) and allowed to acclimatize for two weeks before experiments. Animals were maintained under a standard 12/12 light/dark cycle at room temperature and given access to food and water *ad libitum*.

Arrays

Rat-specific DNA microarrays were obtained from the Rutgers Neuronal Gene expression Lab (NGEL; Piscataway, NJ). Briefly, each array was constructed from a collection of 4967 probes representing approximately 4900 genes printed onto a poly-L-lysine slide. Probes were 65-70 nucleotides in length and were designed to optimize melting temperature and to minimize homology between probes. Further information about these arrays can be found at the web site www.cag.icph.org/microarray_facility.htm.

Isoflurane Exposure and Tissue Collection

Animals receiving isoflurane treatment were placed in an induction chamber and induced with 3% isoflurane/97% medical grade air (vol/vol) until loss of righting reflex (2-3 mins). Anesthesia was then maintained at 2% isoflurane/98% air ~1.5 minimum alveolar concentration for the remainder of the 90-min exposure period. Body temperature was maintained at 37°C throughout the treatment using heated gel packs and oxygen levels were monitored using a model V3304 pulse oximeter (Surgivet; Waukesha, WI). Animals were observed in order to maintain > 95% oxygen saturation at all times. Upon completion of the anesthetic exposure, test animals were immediately decapitated. Samples from liver, kidney cortex and heart ventricle were collected and homogenized with a Polytron homogenizer (Brinkmann; Westbury, NY) in ice cold Trizol (Invitrogen; Carlsbad, CA). Since stress has been demonstrated to influence gene expression,[31] control animals were maintained in their cages until the time of death. Each control animal was handled gently and then rapidly decapitated. Tissue was processed in the same manner as for test animals. Tissue/Trizol homogenates were stored at −20°C until further processing. All animals were killed during the same daily 5-hr window in order to diminish confounding factors from diurnal variations in gene expression.[32]

RNA Preparation

Total RNA was isolated from Trizol/tissue homogenate by adding chloroform and performing a phase extraction. The aqueous phase was mixed with ethanol 1:3 v/v and added to an Rneasy column (Qiagen: Valencia, CA). Manufacturer's instructions were followed for column washing and elution of RNA. Purified RNA was quantified using a Nanodrop ND-1000 spectrophotometer (Nanodrop Technologies; Rockland, DE). All samples yielded A_{260}/A_{280} ratios between 1.9 and 2.1. Isolated RNA was stored at −80°C until further use. The same RNA preparations were used for both microarray experiments and subsequent quantitative reverse-transcriptase polymerase chain reaction (QRT-PCR) verification of selected genes.

Experimental Design

For each tissue, cDNA generated from an individual isoflurane-treated animal was competitively hybridized against control cDNA generated from a pool comprised of equal

amounts of RNA from 8 control individuals.[33] For each tissue from an individual, the array processing was performed twice using the same labeling schema, and a third time reversing the labeling schema as a dye-flip control to ensure no dye-specific artifacts. Three slides were repeated for each of 3 experimental animals, for a total of 9 arrays per tissue.

Target Labeling and Hybridization

Microarray targets were prepared using the Genisphere 350 dendrimer labeling system according to the manufacturer's instructions (Genisphere, Inc.; Hatfield, PA).[34] Briefly, 2.5μg of isoflurane-treated and pooled control RNA were reverse-transcribed to cDNA in separate tubes using Superscript II (Invitrogen), and oligo $d(T)_{18}$ primers containing unique, label-specific capture sequences. Samples were then alkaline hydrolyzed to destroy residual RNA. Control and test-tagged cDNAs were mixed, applied to arrays and sealed in watertight chambers (GeneMachines; Ann Arbor, MI), and allowed to hybridize for 48-72 hrs in a water bath at 55°C. Arrays were washed one time for 10 min (2× SSC, 55°C), followed by two washes for 10 min (0.2× SSC, room temperature). Arrays were then spin-dried before labeling. Hybridized arrays were labeled with fluorescent-labeled dendrimer and incubated 2 hrs at 61°C. Labeled arrays were washed and dried as described above and scanned using an Axon GenePix 4100 scanner and GenePix 4.0 software (Axon Instruments; Union City, CA).

Data Analysis

Scanned array images were processed using Genepix 4.0 (Axon Instruments). Spots were eliminated if the signal to noise ratio of the spot decreased below 2.0. Statistical analysis of each tissue group was performed using G-Processor (version 3.1, Zhong Guan, http://bioinformatics.med.yale.edu). This analysis package performs a Lowess normalization[33] and yields a differential expression ratio along with a Student's *t-test* p-value for each probe. For each tissue, the gene list was screened for statistical significance in Excel (Microsoft, Redmond, WA) using the following three criteria for each gene selected: p-value ≤ 0.05, spot data valid for ≥ 7 of the 9 arrays, and levels of differential expression ≥ 1.2 compared to control. The final list of “interesting genes” was compiled by choosing genes that were differentially expressed in more than one tissue and whose expression pattern (both up or both down) was consistent among the different tissues.

QRT-PCR

In order to verify results from our microarray, three of the highly regulated genes from the liver arrays were selected for verification by QRT-PCR (Table 1 for primer and probe sequences). All primer/probe sets were acquired from Integrated DNA technologies (IDT Inc., Coralville, IA). Each QRT-PCR reaction was performed in a single tube on an Applied Biosystems 7700 “Taqman” instrument (Applied Biosystems; Foster City, CA). The QRT-PCR reaction mix was comprised of 1X PCR buffer (Invitrogen, Carlsbad, CA), 6mM $MgCl_2$, 0.67 mM each dinucleotide, 6.75 pmols each of forward and reverse primers, 100 nM FAM/TAMRA probe, 1.5 units Superase-in (Ambion; Austin, TX), 15 units Superscript II (Invitrogen), 0.75 units Platininum Taq Polymerase (Invitrogen) to a final volume of 15μl. Each reaction was run one cycle of 30′ at 42°, 1 cycle of 3′ at 95° and 40 cycles of 1′ at 60° and 20″; at 95°. Universal mouse RNA (Stratagene, La Jolla CA) was used to create a standard curve for each gene of interest and for the normalization gene. Gene reactions were run in triplicate, averaged and normalized to rat protein tyrosine phosphatase, receptor type C, a gene that was chosen for the normalization since it showed no differential regulation on any of the tissue arrays.

Results

Based on our selection criteria, all of the tissues tested showed differential gene expression in response to a 90-min treatment with 2.0% isoflurane. Liver showed the most number of differentially regulated genes in response to isoflurane exposure with 725 of 4900 (~15%) genes showing significant up and down regulation, which was more than 1.2 fold as compared to the pooled control. Kidney had fewer anesthetic-regulated genes with 214 (~4%) and heart had the least with 137 (~3%).

Of the genes described above, 34 of the 4937 genes on the arrays were differentially regulated in the same direction in at least 2 tissues (Table 2 for gene descriptions). Of these, 19 were up-regulated and 15 were down-regulated. Figure 1 shows a Venn diagram of the distribution of the 34 co-regulated genes. From the genes described in Table 2, a number of ontologies were represented including: apoptosis, necrosis and survival signaling, regulation of energetic pathways, modulation of cellular redox, maintenance of cellular ion gradients, regulation of inflammatory response, and regulation of vesicular transport.

We selected 3 of the genes (metallothionein II, peroxiredoxin 5 and cyclin D1) that were significantly regulated on the liver arrays and assessed their expression levels by QRT-PCR using the same RNA as for the array experiments. As shown in Figure 2, there was agreement between the microarray and QRT-PCR differential expression levels, confirming the validity of the arrays for determining differential expression levels under the selected conditions.

Discussion

In the current study we exposed male Wistar rats to clinical levels of the anesthetic isoflurane and assessed changes to gene expression in rat liver, kidney and heart at an early time point after anesthetic exposure. From our array analyses we identified ~1000 rat genes whose expression was significantly impacted by a 90 min isoflurane exposure. Given the role of the liver in detoxification and metabolism, it is not surprising that the majority of these candidates were identified in liver compared to kidney and heart. Nevertheless, pronounced anesthetic-induced changes in gene expression suggest that isoflurane has an enduring impact on cellular function and metabolism post-exposure. On assessing the ontologies of the co-regulated transcripts from multiple tissues, it was particularly striking that genes related to apoptosis, cell survival, energy pathways, cellular redox and inflammation were highly represented. These findings support the notion that genes co-regulated by isoflurane in multiple tissues may be important to evolutionarily conserved physiological phenomena like APC.

While research on delayed APC has illuminated a number of the mechanisms involved in providing protection, a complete picture of the genetic pathways involved in the induction of APC has yet to emerge. Through using a novel approach to analyze microarray data from multiple exposed tissues we generated a list of 34 differentially regulated genes (<1% of the total)that are potentially involved in conserved pathways like preconditioning. While some of the genes from our list have been reported to be involved in APC, including interferon regulatory factor 1 (Irf1[35]) and voltage dependent anion channel 2 (VDAC2[36]), a number of the genes on our focused list have not previously been described.

In support of our view that some of these genes may be involved in conferring APC, we reported a functional study of two of the genes from our list, metallothioneins I + II (MT-I/II).[1] Using an *in vitro* model of ischemic brain injury, we found MTs play an important role in isoflurane-mediated APC [1]Due to heterogeneity of the tissue, we did not explore anesthetic-induced global changes in gene expression in brain in the present study.

Nevertheless, we have previously shown that MT I and II were up-regulated in response to isoflurane exposure in mixed neural cultures. In addition to the metallothioneins, a number of the other genes in the list have described functions that make them interesting candidates for participating in delayed APC-mediated protection.

Five of the genes we identified (Vdac2, BAD, Pld1, Slc25a3, Atp1a1) are broadly involved in apoptosis, necrosis and survival signaling. VDAC2 is a mitochondrial outer membrane protein. It is a component of the mitochondrial permeability transition pore that participates in transportation of metabolites and helps regulate cellular progression towards apoptosis.[37] VDAC2 has also been suggested to participate in APC.[11] VDAC2 activity is regulated, at least in part, by another in the gene list, bcl-2 associated death agonist (BAD).[37] BAD has been found to be modulated during early ischemic preconditioning and APC,[38,39] although to our knowledge differential regulation of BAD has not been associated with anesthetic exposure.

Phospholipase D1 is a pleotropic protein whose cleavage by caspases has been reported to promote apoptosis via a p53-dependent pathway.[40] Its activity during ischemia-reperfusion has also been tied to signaling for necrotic death.[41] Solute carrier family 25, member 3 (Slc25a3) is another pleotropic protein that has been reported to be a regulator of mitochondrial cytochrome-c release (a protein important for caspase-dependent apoptosis).[42] The ATPase, Na^+/K^+ transporting alpha 1 polypeptide (Atpa1), is the catalytic subunit of a membrane bound Na^+/K^+ transporter ATPase. Na^+/K^+ ATPases have been shown to have roles in survival signaling.[43]

Disruption of Na^+, K^+, and Ca^{2+} ion gradients is an important step in the progression of ischemia-reperfusion injury.[44] Two of the genes above (Pld1, and Atpa1) have described roles in the regulation of cellular Ca^{2+} and Na^+ and K^+ ion gradients, respectively.[41,45] Pld1 is a membrane protein that is involved in sarcolemmal Ca^{2+} control in heart and skeletal muscle.[41] Atpa1 is an integral membrane protein that is essential for establishing and maintaining Na^+ and K^+ gradients across the plasma membrane. Disregulation of Na^+/K^+ ATPases and the resultant loss of membrane potential is a central feature of the ischemic cascade and accompanying excitotoxicity.[46]

Inflammatory responses can play a significant role in later stages of ischemic injury.[46] Two of the genes we identified, Irf1 and basigin have been reported to participate in inflammatory response.[47,48] Irf1 regulates the transcription of interferons alpha and beta and acts as a transcriptional regulator of genes controlled by these interferons.[13] Knockout of Irf1 was shown to improve outcomes in a mouse model of ischemic stroke.[47,49] Basigin, also known as extracellular matrix metalloprotein inducer (EMMPRIN), has been reported to have a role in modulating an inflammatory response.[48] Additionally, basigin has been implicated in both diminished and improved outcomes after ischemic damage independent of its role in inflammation. Its role in tissue remodeling after ischemic damage in rat heart[50] and brain[51] has been associated with negative outcomes. However, basigin has also been associated with improved outcomes during hypoxia and ischemia through protection against lactic acid damage in neuronal and cardiac tissue.[52,53] Additionally, complexed with cyclophilin A, basigin has been reported to protect neurons from ischemic and oxidative stress.[54]

Disruption of energy pathways is key to the pathology of ischemic injury.[44] Four of the genes from our focused list (Vdac2, Prkab1, Slc25a3, and G6pc) have been shown to participate in the monitoring and regulation of energetic pathways. VDAC2, in addition to its role in apoptosis, helps to regulate the ingress and egress of small metabolites, including ATP and adenosine diphosphate from the mitochondrial space.[11] Protein kinase, AMP-

activated protein (Prkab1) has been shown to be involved in nutrient sensing.[55] Slc25a3 is a mitochondrial transport protein that is involved in the transport of inorganic phosphates into the mitochondrial space [56] and is therefore directly involved in regulating mitochondrial ATP production. Glucose-6-phosphatase is an endoplasmic reticulum protein that is involved in conversion of D-glucose-6-phosphate to D-glucose and inorganic phosphate in liver and kidney cortex.[57]

In conclusion, we have performed a novel comparative analysis of DNA microarray data in order to further our understanding of how clinically relevant anesthetic exposures can lead to protection against ischemic injury. By comparing expression patterns from multiple rat-derived tissues we have been able to generate a focused list of genes some of which may be important for delayed APC. Further functional studies will need to be performed to determine how these genes might be involved in the molecular pathways underlying anesthetic-mediated protection.

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References

1. Edmands SD, Hall AC. Role for Metallothioneins-I/II in Isoflurane Preconditioning of Primary Murine Neuronal Cultures. Anesthesiology. 2009; 110:538–47. [PubMed: 19225397]
2. Kalenka A, Hinkelbein J, Feldmann RE, Kuschinsky W, Waschke KF, Maurer MH. The effects of sevoflurane anesthesia on rat brain proteins: a proteomic time-course analysis. Anesth Analg. 2007; 104:1129–35. [PubMed: 17456663]
3. Jevtovic-Todorovic V, Hartman RE, Izumi Y, Benshoff ND, Dikranian K, Zorumski CF, Olney JW, Wozniak DF. Early exposure to common anesthetic agents causes widespread neurodegeneration in the developing rat brain and persistent learning deficits. J Neurosci. 2003; 23:876–82. [PubMed: 12574416]
4. Aravindan N, Cata JP, Hoffman L, Dougherty PM, Riedel BJ, Price KJ, Shaw AD. Effects of isoflurane, pentobarbital, and urethane on apoptosis and apoptotic signal transduction in rat kidney. Acta Anaesthesiol Scand. 2006; 50:1229–37. [PubMed: 16978161]
5. Carmel JB, Kakinohana O, Mestril R, Young W, Marsala M, Hart RP. Mediators of ischemic preconditioning identified by microarray analysis of rat spinal cord. Exp Neurol. 2004; 185:81–96. [PubMed: 14697320]
6. Kaneko T. Late preconditioning with isoflurane in cultured rat cortical neurones. Br J Anaesth. 2005; 95:662–8. [PubMed: 16143577]
7. Sergeev P, Da Silva R, Lucchinetti E, Zaugg K, Pasch T, Schaub MC, Zaugg M. Trigger-dependent gene expression profiles in cardiac preconditioning: evidence for distinct genetic programs in ischemic and anesthetic preconditioning. Anesthesiology. 2004; 100:474–88. [PubMed: 15108959]
8. Murry CE, Jennings RB, Reimer KA. Preconditioning with ischemia: a delay of lethal cell injury in ischemic myocardium. Circulation. 1986; 74:1124–36. [PubMed: 3769170]
9. Kapinya KJ, Löwl D, Fütterer C, Maurer M, Waschke KF, Isaev NK, Dirnagl U. Tolerance against ischemic neuronal injury can be induced by volatile anesthetics and is inducible NO synthase dependent. Stroke. 2002; 33:1889–98. [PubMed: 12105371]
10. Tanaka K, Ludwig LM, Krolikowski JG, Alcindor D, Pratt P, Kersten JR, Pagel PS, Warltier DC. Isoflurane produces delayed preconditioning against myocardial ischemia and reperfusion injury: role of cyclooxygenase-2. Anesthesiology. 2004; 100:525–31. [PubMed: 15108964]
11. Murphy E. Primary and secondary signaling pathways in early preconditioning that converge on the mitochondria to produce cardioprotection. Circ Res. 2004; 94:7–16. [PubMed: 14715531]
12. Bolli R. The early and late phases of preconditioning against myocardial stunning and the essential role of oxyradicals in the late phase: an overview. Basic Res Cardiol. 1996; 91:57–63. [PubMed: 8660262]

13. Bickler PE, Zhan X, Fahlman CS. Isoflurane preconditions hippocampal neurons against oxygen-glucose deprivation: role of intracellular Ca2+ and mitogen-activated protein kinase signaling. Anesthesiology. 2005; 103:532–9. [PubMed: 16129978]
14. Sakai H, Sheng H, Yates RB, Ishida K, Pearlstein RD, Warner DS. Isoflurane provides long-term protection against focal cerebral ischemia in the rat. Anesthesiology. 2007; 106:92. discussion 8-10. [PubMed: 17197850]
15. Zheng S, Zuo Z. Isoflurane preconditioning induces neuroprotection against ischemia via activation of P38 mitogen-activated protein kinases. Mol Pharmacol. 2004; 65:1172–80. [PubMed: 15102945]
16. De Hert SG. Volatile anesthetics and cardiac function. J Semin Cardiothorac Vasc Anesth. 2006; 10:33–42.
17. Zhao P, Peng L, Li L, Xu X, Zuo Z. Isoflurane preconditioning improves long-term neurologic outcome after hypoxic-ischemic brain injury in neonatal rats. Anesthesiology. 2007; 107:963–70. [PubMed: 18043065]
18. Zaugg M. Is protection by inhalation agents volatile? Controversies in cardioprotection. Br J Anaesth. 2007; 99:603–6. [PubMed: 17933798]
19. Lange M, Roewer N, Kehl F. Anesthetic preconditioning as the alternative to ischemic preconditioning. J Thorac Cardiovasc Surg. 2006; 131:252–3. [PubMed: 16399333]
20. Wakeno-Takahashi M, Otani H, Nakao S, Imamura H, Shingu K. Isoflurane induces second window of preconditioning through upregulation of inducible nitric oxide synthase in rat heart. Am J Physiol Heart Circ Physiol. 2005; 289:H2585–91. [PubMed: 16006547]
21. Ludwig LM, Tanaka K, Eells JT, Weihrauch D, Pagel PS, Kersten JR, Warltier DC. Preconditioning by isoflurane is mediated by reactive oxygen species generated from mitochondrial electron transport chain complex III. Anesth Analg. 2004; 99:1308–5. table of contents. [PubMed: 15502022]
22. Kim H, Yi JW, Sung YH, Kim CJ, Kim CS, Kang JM. Delayed preconditioning effect of isoflurane on spinal cord ischemia in rats. Neurosci Lett. 2008; 440:211–6. [PubMed: 18583046]
23. Kis A, Yellon DM, Baxter GF. Second window of protection following myocardial preconditioning: an essential role for PI3 kinase and p70S6 kinase. J Mol Cell Cardiol. 2003; 35:1063–71. [PubMed: 12967629]
24. Tonkovic-Capin M, Gross GJ, Bosnjak ZJ, Tweddell JS, Fitzpatrick CM, Baker JE. Delayed cardioprotection by isoflurane: role of K(ATP) channels. Am J Physiol Heart Circ Physiol. 2002; 283:H61–8. [PubMed: 12063275]
25. Chen CH, Liu K, Chan JY. Anesthetic preconditioning confers acute cardioprotection via up-regulation of manganese superoxide dismutase and preservation of mitochondrial respiratory enzyme activity. Shock. 2008; 29:300–8. [PubMed: 17693941]
26. Tsutsumi Y. Role of 12-lipoxygenase in volatile anesthetic-induced delayed preconditioning in mice. Am J Physiol Heart Circ Physiol. 2006; 291:H979–H983. [PubMed: 16648187]
27. Hashiguchi H, Morooka H, Miyoshi H, Matsumoto M, Koji T, Sumikawa K. Isoflurane Protects Renal Function Against Ischemia and Reperfusion Through Inhibition of Protein Kinases, JNK and ERK. Anesth Analg. 2005:1584–9. [PubMed: 16301223]
28. Carles M, Dellamonica J, Roux J, Lena D, Levraut J, Pittet JF, Boileau P, Raucoules-Aime M. Sevoflurane but not propofol increases interstitial glycolysis metabolites availability during tourniquet-induced ischaemia-reperfusion. Br J Anaesth. 2008; 100:29–35. [PubMed: 18029344]
29. Zaugg M. Anaesthetics and cardiac preconditioning. Part II. Clinical implications. Br J Anaesth. 2003; 91:566–76. [PubMed: 14504160]
30. Jia B, Crowder CM. Volatile anesthetic preconditioning present in the invertebrate Caenorhabditis elegans. Anesthesiology. 2008; 108:426–33. [PubMed: 18292680]
31. Morita K, Saito T, Ohta M, Ohmori T, Kawai K, Teshima-Kondo S, Rokutan K. Expression analysis of psychological stress-associated genes in peripheral blood leukocytes. Neurosci Lett. 2005; 381:57–62. [PubMed: 15882790]
32. Rönnbäck A, Dahlqvist P, Bergström SA, Olsson T. Diurnal effects of enriched environment on immediate early gene expression in the rat brain. Brain Res. 2005; 1046:137–44. [PubMed: 15927552]

33. Yang YH, Speed T. Design issues for cDNA microarray experiments. Nat Rev Genet. 2002; 3:579–88. [PubMed: 12154381]
34. Stears RL, Getts RC, Gullans SR. A novel, sensitive detection system for high-density microarrays using dendrimer technology. Physiol Genomics. 2000; 3:93–9. [PubMed: 11015604]
35. Stevens SL, Leung PY, Vartanian KB, Gopalan B, Yang T, Simon RP, Stenzel-Poore MP. Multiple Preconditioning Paradigms Converge on Interferon Regulatory Factor-Dependent Signaling to Promote Tolerance to Ischemic Brain Injury. J Neurosci. 2011; 31:8456–63. [PubMed: 21653850]
36. Clarke SJ, Khaliulin I, Das M, Parker JE, Heesom KJ, Halestrap AP. Inhibition of mitochondrial permeability transition pore opening by ischemic preconditioning is probably mediated by reduction of oxidative stress rather than mitochondrial protein phosphorylation. Circ Res. 2008; 102:1082–90. [PubMed: 18356542]
37. Tsujimoto Y, Shimizu S. Role of the mitochondrial membrane permeability transition in cell death. Apoptosis. 2007; 12:835–40. [PubMed: 17136322]
38. Miyawaki T, Mashiko T, Ofengeim D, Flannery RJ, Noh KM, Fujisawa S, Bonanni L, Bennett MV, Zukin RS, Jonas EA. Ischemic preconditioning blocks BAD translocation, Bcl-xL cleavage, and large channel activity in mitochondria of postischemic hippocampal neurons. Proc Natl Acad Sci USA. 2008; 105:4892–7. [PubMed: 18347331]
39. Raphael J, Abedat S, Rivo J, Meir K, Beeri R, Pugatsch T, Zuo Z, Gozal Y. Volatile anesthetic preconditioning attenuates myocardial apoptosis in rabbits after regional ischemia and reperfusion via Akt signaling and modulation of Bcl-2 family proteins. J Pharmacol Exp Ther. 2006; 318:186–94. [PubMed: 16551837]
40. Jang YH, Namkoong S, Kim YM, Lee SJ, Park BJ, Min DS. Cleavage of phospholipase D1 by caspase promotes apoptosis via modulation of the p53-dependent cell death pathway. Cell Death Differ. 2008; 15:1782–93. [PubMed: 18636075]
41. Tappia PS, Dent MR, Dhalla NS. Oxidative stress and redox regulation of phospholipase D in myocardial disease. Free Radic Biol Med. 2006; 41:349–61. [PubMed: 16843818]
42. Alcalá S, Klee M, Fernández J, Fleischer A, Pimentel-Muiños F. A high-throughput screening for mammalian cell death effectors identifies the mitochondrial phosphate carrier as a regulator of cytochrome c release. Oncogene. 2008; 27:44–54. [PubMed: 17621274]
43. Zhang S. Distinct Role of the N-terminal Tail of the Na,K-ATPase Catalytic Subunit as a Signal Transducer. J Biol Chem. 2006; 281:21954–62. [PubMed: 16723354]
44. Pacher P, Beckman JS, Liaudet L. Nitric oxide and peroxynitrite in health and disease. Physiol Rev. 2007; 87:315–424. [PubMed: 17237348]
45. Hammes A, Oberdorf-Maass S, Rother T, Nething K, Gollnick F, Linz KW, Meyer R, Hu K, Han H, Gaudron P, Ertl G, Hoffmann S, Ganten U, Vetter R, Schuh K, Benkwitz C, Zimmer HG, Neyses L. Overexpression of the sarcolemmal calcium pump in the myocardium of transgenic rats. Circ Res. 1998; 83:877–88. [PubMed: 9797336]
46. Singhal, AB.; Lo, EH.; Dalkara, T.; Moskowitz, MA.; González, RG.; Hirsch, JA.; Lev, MH.; Schaefer, PW.; Schwamm, LH., editors. Acute Ischemic Stroke. Springer Berlin Heidelberg; Berlin, Heidelberg: 2010.
47. Alexander M, Forster C, Sugimoto K, Clark HB, Vogel S, Ross ME, Iadecola C. Interferon regulatory factor-1 immunoreactivity in neurons and inflammatory cells following ischemic stroke in rodents and humans. Acta Neuropathol. 2003; 105:420–4. [PubMed: 12677441]
48. Schmidt R, Bültmann A, Fischel S, Gillitzer A, Cullen P, Walch A, Jost P, Ungerer M, Tolley ND, Lindemann S, Gawaz M, Schömig A, May AE. Extracellular matrix metalloproteinase inducer (CD147) is a novel receptor on platelets, activates platelets, and augments nuclear factor kappaB-dependent inflammation in monocytes. Circ Res. 2008; 102:302–9. [PubMed: 18048771]
49. Naschberger E, Werner T, Vicente A, Guenzi E, Tpolt K, Leubert R, Lubeseder-Martellato C, Nelson P, Stürzl M. Nuclear factor-κB motif and interferon-α-stimulated response element co-operate in the activation of guanylate-binding protein-1 expression by inflammatory cytokines in endothelial cells. Biochem J. 2004; 379:409. [PubMed: 14741045]
50. Schmidt R, Bültmann A, Ungerer M, Joghetaei N, Bülbül O, Thieme S, Chavakis T, Toole BP, Gawaz M, Schömig A, May AE. Extracellular matrix metalloproteinase inducer regulates matrix

metalloproteinase activity in cardiovascular cells: implications in acute myocardial infarction. Circulation. 2006; 113:834–41. [PubMed: 16461815]

51. Burggraf D, Liebetrau M, Martens HK, Wunderlich N, Jäger G, Dichgans M, Hamann GF. Matrix metalloproteinase induction by EMMPRIN in experimental focal cerebral ischemia. Eur J Neurosci. 2005; 22:273–7. [PubMed: 16029217]
52. Han M, Trotta P, Coleman C, Linask KK. MCT-4, A511/Basigin and EF5 expression patterns during early chick cardiomyogenesis indicate cardiac cell differentiation occurs in a hypoxic environment. Dev Dyn. 2006; 235:124–31. [PubMed: 16110503]
53. Zhang F, Vannucci SJ, Philp NJ, Simpson IA. Monocarboxylate transporter expression in the spontaneous hypertensive rat: effect of stroke. J Neurosci Res. 2005; 79:139–45. [PubMed: 15578721]
54. Boulos S, Meloni BP, Arthur PG, Majda B, Bojarski C, Knuckey NW. Evidence that intracellular cyclophilin A and cyclophilin A/CD147 receptor-mediated ERK1/2 signalling can protect neurons against in vitro oxidative and ischemic injury. Neurobiol Dis. 2007; 25:54–64. [PubMed: 17011206]
55. Feng Z, Hu W, De Stanchina E, Teresky A, Jin S, Lowe S, Levine A. The Regulation of AMPK 1, TSC2, and PTEN Expression by p53: Stress, Cell and Tissue Specificity, and the Role of These Gene Products in Modulating the IGF-1-AKT-mTOR Pathways. Cancer Res. 2007; 67:3043–53. [PubMed: 17409411]
56. Mayr JA, Merkel O, Kohlwein SD, Gebhardt BR, Böhles H, Fötschl U, Koch J, Jaksch M, Lochmüller H, Horváth R, Freisinger P, Sperl W. Mitochondrial phosphate-carrier deficiency: a novel disorder of oxidative phosphorylation. Am J Hum Genet. 2007; 80:478–84. [PubMed: 17273968]
57. van Schaftingen E, Gerin I. The glucose-6-phosphatase system. Biochem J. 2002; 362:513–32. [PubMed: 11879177]

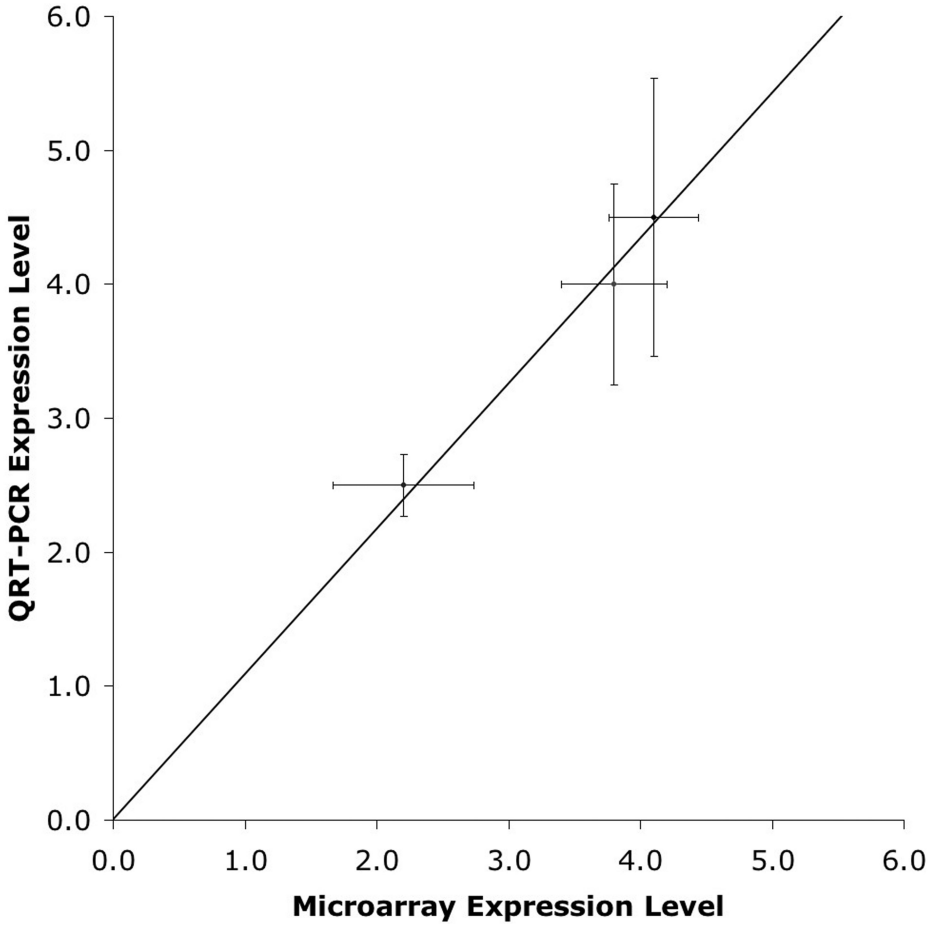

Figure 1.

Comparison of differential regulation of three genes by DNA microarray and by QRT-PCR. The trendline represents strong agreement between DNA microarray and QRT-PCR results. The same sample of RNA was used for both experiments. Microarray values shown are the average "median of ratios" value from 9 independent arrays ± SEM.

QRT-PCR values are the average ± SEM of 3 separate trials for each gene after being normalized to our control gene, rat protein tyrosine phosphatase, receptor type C.

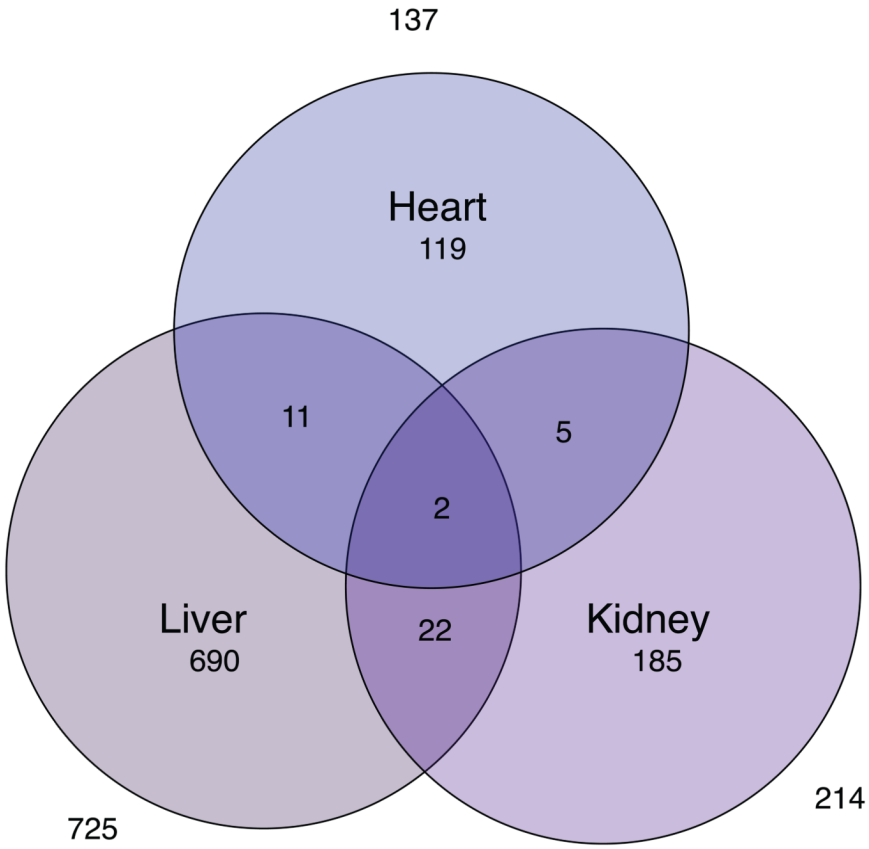

Figure 2.
Venn diagram of genes differentially expressed in rat liver kidney and heart.
Represented genes were differentially expressed on at least 7 of9 arrays for each tissue, were significant ($p < 0.05$), and were differentially regulated in more than one tissue.

Table 1

Primer/Probe sequences for quantitative reverse-transcriptase polymerase chain reaction confirmation of microarray data.

Gene	Description	GenBank ID	Forward Primer	Reverse Primer	FAM/TAMRA Probe
Ccnd1	CyclinD1	D14014	GGGTCTGCGAGCCATGCT	CCGCATGGATGGCACAAT	AGACCTGCGCGCCCTCCGTTT
Prdx5	Peroxiredoxin 5	AF110732	GCTCCGTGCATCGGCTACTT	ACCGCGGCACTGCTGAA	AGCAGGCCGGAAAGGAGCAGGTTG
Mt2	Metallothionein 2	NM_008630	GCAAATGCACCTCCTGCAA	ACACAGCCCTGGGCACATT	AAAGCTGCTGCTCCTGCTGCCCC
Ptprc	Protein tyrosine phosphatase, receptor type, C	M10072	ACCTACATTGGAATTGATGCCATGCT	CCTCCACTTGCACCATCAGACA	GAAGCAGAGGGCAAAGTGGATGTCTATGG

All sequences shown are 5′-3′. Probes sequenceswere modificed with a 5′ 6-carboxy flurescein andat the 3′ end with a 6-carboxy-tetramethyl rhodamine.

Primers and probes were designed in Primer express 1.0 (Applied Biosystems Inc,)

Table 2

Differentially regulated genes. Selected genes had to be present on at least 7 of 9 arrays, found to have statistically significant ($P \leq 0.05$) differential regulation compared to a pooled control in at least two separate tissues, and be regulated at least 1.2 either both up or both down in each of those tissues.

Ontology	Full Name	Code	GenBank accession	Liver Level	Kidney Level	Heart Level
Apoptosis and necrosis, energy metabolism	voltage-dependent anion channel 2	Vdac2	AB039663	1.53	1.23	NSR
Unknown neuronal growth and guidance	multiple EGF-like-domains 8 ninjurin 2	Megf8 Ninj2	AB011534 AB040815	-1.25 NSR	NSR -1.22	-1.36 -1.33
DNA repair, cell cycling cellular remodeling energy metabolism	cyclin-dependent kinase 7 basigin (EMMPRIN/Ox47/CE-9) solute carrier family 25 (mitochondrial carrier, phosphate carrier), member 3	Cdk7 Bsg Slc25a3	X83579 NM_012783 M23984	-1.61 2.22 1.46	-1.38 1.66 1.26	NSR NSR NSR
Neuronal growth and guidance energy metabolism	sterol-C4-methyl oxidase-like lipoprotein lipase 17	Sc4mol Lpl	D50559 NM_012598	-1.58 -1.50	NSR NSR	-1.25 -1.23
Energy metabolism	glucose-6-phosphatase, catalytic subunit	G6pc	NM_013098	2.82	1.43	NSR
Secretory	exocyst complex component 6	Exoc6	NM_019277	1.20	NSR	1.27
Ca2+ modulation	arginine vasopressin receptor 1A	Avpr1a	Z11690	NSR	-1.29	-1.24
Vesicular trafficking	coatomer protein complex, subunit beta 2 (beta prime)	Copb2	AF002705	-1.24	-1.20	NSR
Inflammatory response	cytochrome P450, family 4, subfamily f, polypeptide 1	Cyp4f1	NM_019623	1.61	1.29	NSR
Neuronal growth and guidance	glucosamine (UDP-N-acetyl)-2-epimerase/N-acetylmannosamine kinase	Gne	Y07744	1.38	1.21	NSR
Cell signaling	fatty acid binding protein 5, epidermal	Fabp5	U13253	-1.74	NSR	-1.27
Cell signaling	Rab acceptor 1 (prenylated)	Rabac1	AF025506	1.74	1.31	NSR
Nucleotide metabolism	ureidopropionase, beta	Upb1	M97662	1.32	1.39	NSR
Unknown	tripartite motif-containing 17	Trim17	AF156272	2.21	1.53	NSR
Cell death, exocytosis, cell signaling	phospholipase D1	Pld1	U69550	NSR	-1.20	-1.53
Protein transcription	ribosomal protein L8	Rpl8	X62145	1.55	1.25	NSR
Na, K transport and water regulation	ATPase, Na+/K+ transporting, alpha 1 polypeptide	Atp1a1	M28647	1.66	1.25	NSR
Energy metabolism, Apoptotic pathways	protein kinase, AMP-activated, beta 1 non-catalytic subunit	Prkab1	U42411	-1.45	NSR	-1.21
DNA damage response	Cas-Br-M (murine) ecotropic retroviral transforming sequence b	Cblb	AF199504	1.24	1.27	NSR
Transcription regulation	transcription elongation factor B (SIII), polypeptide 2	Tceb2	L42855	1.23	1.35	NSR
DNA damage response	interferon regulatory factor 1	Irf1	NM_012591	-1.72	NSR	-1.27

Ontology	Full Name	Code	GenBank accession	Liver Level	Kidney Level	Heart Level
Vesicle transport	SV2 related protein	Svop	AF060173	-1.41	-1.10	-1.24
Unknown	heart endothelial unknown protein		U44844	1.63	NSR	1.25
Unknown	Rat insulin-like growth factor I		M81184	-1.22	-1.25	NSR
Unknown	proline-rich protein 15	Prp15	NM_012632	1.74	NSR	1.20
Vesicle transport	synaptotagmin I	Syt1	X52772	1.62	1.31	NSR
Bilirubin catabolism	UDP glucuronosyltransferase 1 family, polypeptide A1	Ugt1a1	S70360	1.26	-1.24	-1.11
Apoptosis	Bcl2-associated death agonist	Bad	AF279910	-1.22	-1.22	NA
Zinc Metabolism	metallothionein 2	Mt2	NM_008630	3.77	1.41	NA
Zinc Metabolism	metallothionein 1a	Mt1a	J00750	2.31	1.35	NA

na=not applicable. nsr= no significant regulation.