2. Mitochondrial Influences on the Neurovascular Unit

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Abstract

It is now appreciated that mitochondria are involved in diverse cellular activities beyond energy production and that mitochondrial status and responsiveness can be affected by physiological, pharmacological, and pathological processes. We and others have shown that the selective targeting of mitochondria protects the neurovascular unit against lethal stresses and can independently promote changes in cerebrovascular tone. Specifically, the activation of ATP-dependent potassium channels on the inner mitochondrial membrane (mito-K<sub>ATP</sub> channels) induces immediate and long term protection of neurons, astroglia, and cerebral vascular endothelium. Thus, mitochondrial depolarization, via opening of mito-K<sub>ATP</sub> channels, activates mechanisms that protect the neurovascular unit by suppressing the availability of reactive oxygen species and/or cytosolic calcium (Ca<sup>2+</sup>) during exposure to potentially lethal stress. Activation of mito-K<sub>ATP</sub> channels in isolated cerebral arteries causes vasodilation, with specific contributions from endothelium, vascular smooth muscle (VSM), and perivascular nerves. Furthermore, in an experimental stroke model pre-existing insulin resistance (IR) exacerbates brain injury and impairs the development of cellular preconditioning. Similar to preconditioning, IR impairs relaxation of mitochondrial centered responses in cerebral arteries. Future studies will be directed toward developing therapies to target mitochondrial specific responses in patients and to reverse the impairment of mitochondrial function by disease processes.

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Key words: preconditioning, anoxia, oxygen glucose deprivation, ischemia, brain, mitochondria, cerebral arteries, ATP-sensitive potassium channels, excitotoxicity, necrosis, apoptosis

Introduction

Mitochondria are double membrane organelles which generate chemical energy in the form of adenosine triphosphate (ATP) which is distributed within cells to promote various activities of the di-
tion, the mitochondria often form a network within cells (Fig. 1) and the mitochondrial outer membrane is often connected with the membranes of the endoplasmic reticulum (ER), a structure involved in calcium storage and release (Zick et al., 2009).

While traditionally relegated to an energy producing role, it is now appreciated that mitochondria are involved in diverse cellular activities and that mitochondrial status and responsiveness can be affected by pharmacological agents, disease processes, as well as aging (McBride et al., 2006). The purpose of this review is to critically examine the role of mitochondrial activation on initiation of responses that will impact the neurovascular unit. Specifically, we will examine how the selective targeting of mitochondria leads to protection of the neurovascular unit against otherwise lethal stress and/or will lead to changes in cerebral vascular tone. Furthermore, we will show that even relatively mild metabolic stress, such as that which occurs with insulin resistance (IR), can dramatically affect mitochondrial related events in the brain and cerebral vasculature. While these three apparently separate topics were originally independent research efforts in our laboratory, subsequent findings have resulted in a convergence of themes for an integrated examination of the ubiquitous roles of mitochondria in the functioning of the neurovascular unit during health and disease.

**Preconditioning**

Preconditioning represents the condition in which transient exposure of cells to an initiating event leads to protection against subsequent, potentially lethal stimuli. Two forms have been described. Immediate preconditioning occurs within minutes of the initiating stimulus and lasts for several hours before disappearing, while delayed preconditioning takes several hours to develop and persists for several days (Kirino, 2002; Gidday, 2006). Given the temporal aspects, it seems likely that immediate preconditioning primarily involves changes in the activity or function of enzymes, second messengers, and ion channels already present while delayed preconditioning principally is due to de novo protein synthesis (Busija et al., 2008). Although the mechanisms involved are not fully understood, the result of preconditioning is that the cells are able to limit the influx of calcium and the availability of reactive oxygen species (ROS) during stress (Kis et al., 2003; Gaspar et al., 2008 a.; Domoki et al., 2004). Postconditioning, in which the initiating event occurs following the onset of the potentially lethal event (Zhao, 2009) also has been reported but will not represent a major focus in this review because many of the mechanisms are similar to preconditioning. However, one important issue that has resulted from the research on postconditioning is that timing is critical since postconditioning must be applied within several minutes of reperfusion to be effective against transient middle cerebral artery occlusion (MCAO) in rats (Robin et al., 2011).

Recent studies, beginning with our original observation (Domoki et al., 1999), have established that mitochondrial-centered mechanisms are important initiators of the preconditioning response in neurons, astroglia, and cerebral endothelial cells (Domoki et al., 1999; Rajapakse et al., 2002, 2003; Liu et al., 2002; Busija et al., 2004; Nagy et al., 2004; Lenzser et al., 2005; Domoki et al., 2005; Farkas et al., 2006; Busija et al., 2008). These mechanisms can be directly or
secondarily activated by physiological and pharmacological stimuli. For example, anesthetic gases (Blanck et al., 2000; Horiguchi et al., 2005 b), drugs (Gaspar et al., 2007), neuronal depolarization (Horiguchi et al., 2005 a, b), anoxia (Dahl and Balfour, 1964; Perez-Pinzon, 2007), and lipopolysaccharide administration (Longhi et al., 2011) can promote preconditioning via multiple mechanisms that probably involve mitochondrial as well as non-mitochondrial the dependent effects. However, in order to elucidate mechanisms involved, we will focus exclusively on preconditioning responses originating in mitochondria and elicited by pharmacological agents specific for mitochondria. Several mitochondrial specific targets for inducing preconditioning include: 1) potassium channels located on the inner mitochondrial membrane, 2) respiratory chain enzymes, and 3) oxidative phosphorylation.

Mitochondrial potassium channels

Different types of potassium channels have been identified in the inner mitochondrial membrane (Busija et al., 2008). It is our view that the ATP-sensitive potassium (K_{ATP}) channel is the most important individual target for the induction of preconditioning especially in neurons (Busija et al., 2008). The physical structure of mitoK_{ATP} channels is not yet known with certainty but appears to differ substantially from the previously described plasmalemmal K_{ATP} channels (Scheraseyoun et al., 2000; Ardehali and O'Rourke, 2005). Nonetheless, the pharmacological identification of the mitoK_{ATP} channels is convincing to most investigators. Thus, isolated mitochondria, mitochondria in cultured cells, as well as tissue slices depolarize in a dose-dependent manner to well-characterized mitoK_{ATP} channel openers such as diazoxide and BMS-191095 (Busija et al., 2005; Mayanagi et al. 2007a; Gaspar et al., 2008 a) and are responsive to other factors such as peroxynitrite (Lacza et al., 2003; Busija et al., 2008) and protein kinase C epsilon (PKCe) (Perez-Pinzon et al., 2005). The classical blocker glibenclamide as well as the more problematic 5-HD, which needs to be metabolized before becoming active (Hanley et al., 2005), block the actions of diazoxide, BMS-191095 and/or PKCe (Busija et al., 2005; Perez-Pinzon et al., 2003; Gaspar et al., 2008 a).

Diazoxide, a drug previously used against acute hypertension or hypoglycemia in people, is the most commonly used mitoK_{ATP} channel opener (Farkas et al., 2006), but it has the additional effect of inhibiting succinate dehydrogenase (SDH; complex II of the electron transport chain), especially at high doses (Kis et al., 2003). Nonetheless, the primary actions of diazoxide on the cells of the neurovascular unit are still specific to mitochondria (Kis et al., 2003; Busija et al., 2008). In contrast, BMS-191095 is very selective for mitoK_{ATP} channels and has no known non-specific effects which would complicate the interpretation of the results (Grover et al., 2001, 2003; Busija et al., 2008). While applications of diazoxide or BMS 191095 depolarize mitochondria, diazoxide but not BMS also causes the liberation of ROS (Busija et al., 2008), which we believe is due to SDH inhibition. This view is supported by examination of the effects of the specific inhibitor of SDH, 3-nitropropionic acid (3-NPA), which increases ROS production by mitochondria (Busija et al., 2005).

Our finding that it is possible to depolarize mitochondria, using BMS-191095, without associated release of ROS, challenges the accepted tenet that both events are functionally linked and is supported by work from other laboratories (Tretret and Adam-Vizi, 2007). We have taken advantage of the very selective effects of BMS-191095 to study the isolated effects of mitochondrial depolarization from other events as the initiator of preconditioning in the neurovascular unit. Previous studies using diazoxide to protect the neurovascular unit have shown that neurons, astroglia and endothelial cells are suitable cellular targets for protecting the neurovascular unit because of their adaptability (Farkas et al., 2005; Busija et al., 2008).

We have demonstrated that BMS-191095 also is able to confer both immediate and delayed preconditioning in cultured rat neurons (Gaspar et al., 2008 a; Kis et al., 2004). Similar to diazoxide, BMS-191095 reduces the ROS availability upon exposure to potentially lethal stimuli (Gaspar et al., 2008 a; Kis et al., 2004). In contrast to diazoxide, BMS-191095 does not increase mitochondrial ROS levels upon its application to either isolated mitochondria (Busija et al., 2005), cultured neurons (Gaspar et al., 2008 a), or vascular smooth muscle or endothelium (Katakam et al., 2010 b; 2011 a). Thus, mitoK_{ATP} channel opening can
be dissociated from mitochondrial ROS production which demonstrates that mitoK<sub>ATP</sub> channel opening alone, as an initiation target, is sufficient for inducing preconditioning. Additionally, BMS-191095 was also able to protect the adult rat brain in vivo by reducing infarct volume in a transient stroke model in rats and these effects are blocked by co-treatment with 5-HD (Mayanagi et al., 2007b). Nonetheless, it seems likely that mitoK<sub>ATP</sub> channel opening and ROS production are synergistic in inducing preconditioning (Shimizu et al., 2002) and in the case of diazoxide, it may not be possible to separate these dual effects. It also seems reasonable to suggest that endogenous activators of mitochondria will have multiple effects on these organelles.

**Respiratory chain enzymes**

Although representing a very efficient system, superoxide anion is continuously released by the electron transport chain at different sites under normal conditions (Fig. 2; Zhang and Gutterman, 2007) and is thought to be an important signaling agent in promoting basal functions of cells. Superoxide anion acting either at sites within the mitochondria or following conversion to hydrogen peroxide by manganese superoxide dismutase, can influence events in the
cytosol. Superoxide anion normally is unable to leave the matrix (Jastrow et al. 2010) but hydrogen peroxide is able to traverse the inner mitochondrial membrane through aquaporin-like channels (Kaaksi et al. 2007). The primary sites of ROS production and release are Complex I, Complex II, and Complex III. Complex I (NADH-ubiquinone oxidoreductase) and Complex II (succinate dehydrogenase, SDH) are where electrons are accepted from NADH + H+ and FADH2 respectively, and transferred to Complex III (ubiquinol-cytochrome c oxidoreductase) and finally to Complex IV (cytochrome c oxidase), where the final electron acceptor is oxygen and the final product is water (Zhang and Guterman, 2007). Partial inhibition of SDH with 3-NPA is able to induce delayed preconditioning and thus reduce brain infarct volume by about 20% in rats when administered 3 days prior to temporary occlusion of the MCAO (Horiguchi et al., 2003). On the other hand, 3-NPA does not induce immediate preconditioning in this same model (unpublished observations). Since 3-NPA induced alterations of mitochondrial membrane potential in cultured neurons but not in isolated brain mitochondria (Horiguchi et al., 2003; Busija et al., 2005), it appears that cytosolic mechanisms are necessary for mitochondrial-derived ROS to activate mitoKATP channels in intact neurons. These results strongly suggest that secondary opening of mitoKATP channels plays a key role as the trigger in the development of 3-NPA-induced tolerance in the brain against ischemic stress.

We made the first observation that NS 1619 was neuroprotective (Veltkamp et al., 1998). Our more recent studies using NS 1619 indicate that this agent induced neuronal preconditioning by increasing ROS production and mitochondrial depolarization independent of activation of a putative mitoKATP channel (Gaspar et al., 2008 b). Similar to 3-NPA, these effects appear to be secondary to respiratory chain inhibition (Debska et al., 2003; Kicinska and Szewczyk, 2004). Although investigators have referred to NS 1619 as selective for BKCa channels in general or more recently as a selective mitoKATP channel opener, our evidence and that of other authors indicate otherwise (Holland et al., 1996; Debska et al., 2003; Kicinska and Szewczyk, 2004; Gaspar et al., 2008 b). Our results support the view that the primary mitochondrial effect of NS 1619 is inhibition of Complex I of the electron transport chain rather than activation of a putative mitoKATP channel. On the other hand, irrespective of the site of action, NS 1619 is an effective agent for causing immediate and delayed preconditioning and protects cultured neurons against oxygen-glucose deprivation, H2O2, and glutamate excitotoxicity (Gaspar et al., 2008 b, 2009).

Substrate limitation

Brief periods of ischemia or anoxia were the original stimuli found to lead to the development of preconditioning and thus led to the descriptive terms of “ischemic preconditioning” or “ischemic tolerance” (Dahl and Balfour, 1964; Trendelenburg and Dírnagl, 2005). The original description of anoxic preconditioning apparently was performed Dr. Nancy Dahl at the University of Kansas in the laboratory adjacent to the one in which one of us (DWB) received his Ph. D. The global nature of these stimuli leads to the activation of multiple signaling pathways and it is unclear to what extent factors such as anoxia and energy substrate depletion contribute to preconditioning. We determined whether transient withdrawal of glucose, the major energy substrate, would induce neuroprotection in primary neuronal cultures (Gaspar et al., 2006). The acute effect of energy deprivation by glucose withdrawal was mitochondrial membrane depolarization and reduced ATP production. While the transient incubation of cultured neurons in a glucose-free solution did not provide acute protection, it resulted in delayed tolerance against various insults such as oxygen-glucose deprivation, glutamate excitotoxicity and exogenous hydrogen peroxide toxicity (Gaspar et al., 2007). While severe hypoglycemia in people cause neurophysiologic and intellectual deficits, mild metabolic stress such as that caused by dietary restriction or fasting can protect the brain against ischemic stress (Perez-Pinzon, 2007). Thus, reduced caloric intake, moderate hypoglycemia, decreased amino acid availability and brief periods of ischemia or anoxia can reduce ischemic brain damage, decrease the risk of neurodegenerative diseases, and also increase the lifespan of laboratory animals (Gaspar et al., 2007; Mattson and Wan, 2005; Mattson, 2008).
Mechanisms of Preconditioning

The critical events leading to death of the cells comprising the neurovascular unit are described in several recent review articles (Choi, 1995; Hales-trap, 1999; Kristian and Siesjo, 1998; Nicholls et al, 1999). Briefly, Ca"" influx from the extracellular space into the cytosole and mitochondria and increased generation of ROS are considered to be the primary causative agents promoting cellular death. Our findings from various models of preconditioning indicate that one or both of these primary agents are attenuated following mitochondrial activation.

BMS-191095 induces both immediate and delayed preconditioning in cultured neurons and brain (Kis et al, 2004; Mayanagi et al, 2007b; Gaspar et al, 2008 a) as well as in heart and skeletal muscle (Grover et al, 2001, 2003). Several pro-survival signaling events occur immediately following activation of mitoK,ATP channels and mitochondrial depolarization with BMS-191095 application in neurons, such as a transient but modest increase in the level of free cytosolic calcium and the phosphorylation of Akt, Gsk 3β, PKC, but not ERK 1/2, JNK 1/2, or p38 (Fig. 1) (Gaspar et al, 2008 a; Kis et al, 2004). These changes probably also explain immediate preconditioning with BMS-191095 although we have not examined in depth the precise factors involved. Another pro-survival pathway linked to mitochondrial activation includes the sirtuin pathway (Morris et al, 2011). Nonetheless, our results using diazoxide suggest that attenuation of calcium influx and prevention of mitochondrial swelling in response to stress are important, final components of immediate preconditioning (Domoki et al, 2004). Furthermore, these initial signaling events also lead to delayed preconditioning in which the neurons are protected against lethal stresses such as glutamate excitotoxicity for several days following the application of BMS-191095 (Kis et al, 2004; Gaspar et al, 2008 a). The principal events associated with delayed protection against neuronal cell death include sustained mitochondrial depolarization, attenuation of the increase in the ROS surge, phosphorylation and inhibition of Gsk 3β and maintenance of ATP levels (Gaspar et al, 2008 a). Lack of an increase in ROS production by neurons or isolated brain mitochondria following BMS-191095 application and the failure of a ROS scavenger to block preconditioning when co-applied with BMS-191095 support the concept that only mitoK,ATP channel opening rather than an increase in ROS levels is the primary, initiating event associated with effects of this drug (Gaspar et al, 2008 a). While we do not know all the mechanisms involved, augmented cellular levels of catalase, an important ROS scavenger, appears to be a major factor in delayed neuroprotection. BMS-191095 application leads to enhanced levels of mRNA for catalase, increased protein levels of catalase, and augmented activity of catalase in neurons (Gaspar et al, 2008 a). The link between BMS-191095 application and catalase up regulation probably involves the phosphoinositide 3 kinase (PI3K) system. Inhibition of PI3K with wortmannin did not block BMS-191095 effects on mitochondrial depolarization, but prevented the increased catalase levels and blocked protection of neurons against glutamate excitotoxicity. Additionally, inhibition of catalase with 3-aminotriazole in BMS-191095 treated neurons dose-dependent increased glutamate-mediated neuronal cell death (Gaspar et al, 2008 a). Thus, sustained depolarization and ATP homeostasis together with the activation of the PI3K-Akt-Gsk 3 beta axis and upregulation of catalase represent multiple effects of a single initiating event, namely mitoK,ATP channel opening, which combine to protect neurons against otherwise lethal stress (Fig. 2) (Gaspar et al, 2008a). Nonetheless, we believe that other mechanisms such as sustained effects on mitochondrial membrane potential as well as yet undefined signaling events, such as modulation of astrocytic gap junctions by mitoK,ATP channels in vivo (Jiang et al, 2011), also contribute to development of neuronal protection.

Cerebral Vascular Dilation

Our studies of preconditioning have led us to the realization that many of the signaling events associated with the induction of mitochondrial-targeted cellular protection also may affect cerebrovascular tone (Fig. 2). While it was already known that extra-cellular ATP, from either glycolysis or oxidative phosphorylation, could affect cerebrovascular tone via plasmalemmal purinergic receptors (Dietrich et al, 2009), only a few studies have examined mitochondrial influences on the diameter of cerebral re-
assistance vessels (Cheranov and Jaggar, 2004; Xi et al, 2005; Duckles and Krause, 2007; Katakan et al, 2009). Thus, the study of mitochondrial effects on the cerebral vasculature is a relatively new field.

**Vascular smooth muscle (VSM)**

Jaggar and colleagues (Xi et al, 2005; Cheranov and Jaggar, 2004) showed that activation of mitochondria by diazoxide promoted relaxation of VSM cells in endothelium-denuded cerebral arteries or freshly dissociated VSM via a mechanism primarily involving ROS. Thus, diazoxide application enhanced the generation of ROS from mitochondria, which sequentially caused the activation of ryanodine-sensitive Ca\(^{2+}\) channels on the sarcoplasmic reticulum, the generation of Ca\(^{2+}\) transients called "Ca\(^{2+}\) sparks", and the opening of adjacent large-conductance Ca\(^{2+}\)-activated K\(^+\) (BK\(_{Ca}\)) channels on the plasma membrane. The resulting K\(^+\) efflux led to VSM hyperpolarization, decreased global intracellular Ca\(^{2+}\), and vasodilation. We have reported similar findings in endothelium denuded arteries with diazoxide (Katakan et al, 2009). The role of ROS in promoting VSM relaxation also is shown by application of 3-NPA (Katakan et al, 2009). However, we find that BMS-191095 has a similar effect in VSM without the involvement of ROS (Katakan et al, 2010 b, 2011a, b). Thus, BMS-191095 does not increase vascular cytosolic or mitochondrial levels of ROS and dilation to BMS-191095 is not affected by ROS scavengers, but calcium spark activity still increases. The reasons for these fundamental differences in findings are unclear but taken together the results underscore the importance and robustness of mitochondrial mechanisms in promoting relaxation of VSM.

**Endothelium**

We investigated the contribution of mitochondrial factors arising within the endothelium on the integrated response of intact cerebral arteries using several approaches. Removal of the endothelium altered the vasodilation to diazoxide, implying that traditional endothelium-derived factors such as nitric oxide (NO) and prostaglandins contribute to vasodilation (Katakan et al, 2009). However, the endothelial effect is complicated. In intact blood vessels, inhibition of NO synthase (NOS) with L-NAME administration reduced dilation to diazoxide, indicating a dilator role of NO. In contrast, inhibition of cyclooxygenase (COX) with indomethacin enhanced dilation, implying a role for constrictor prostanooids. Since indomethacin did not affect vasodilation in endothelium-denuded arteries, it appears that COX metabolites do not mediate vasodilation in endothelium-denuded arteries. Fluorescence measurements of NO in intact arteries or cultured cerebral microvascular endothelial cells confirmed the production of NO in response to diazoxide. Similar results were obtained with BMS-191095 with respect to NO (Katakan et al, 2010a). In addition, BMS-19105 fluorescence measurements showed that a global increase in free cytosolic calcium rather than calcium sparks was temporally associated with increased NO production. We have demonstrated previously that NO arises from cytosolic rather than mitochondrial sites during normal conditions (Lacza et al, 2004; 2006a, b). These results indicate that vasoactive factors from endothelium modify the direct mitochondrial effects on VSM in the determination of the final arterial response.

**Perivascular nerves**

The potential for mitochondrial influences from perivascular nerves or parenchymal neurons on cerebral vascular responses has never been specifically investigated. However, there are a number of factors that would suggest an important neuronal contribution. For example, mitochondrial density is relatively high in perivascular nerves (unpublished observations) and as seen in our pre-conditioning studies, mitochondria in neurons are responsive to both diazoxide and BMS-191095. These speculations are supported by our preliminary studies. Specific inhibition of neuronal NOS, located in perivascular nerves with 7-NI, or blockade of perivascular nerves with tetrodotoxin (TTX), inhibits dilation in both intact or denuded cerebral arteries to BMS-191095 (Katakan et al, 2010 b). Additionally, BMS-191095 depolarizes mitochondria in cultured cortical neurons and enhances NO production by these cells (unpublished observation). Thus, in addition to the endothelium, perivascular nerves, parenchymal neurons, and possibly astroglia (Jiang et al, 2011) as well,
Fig. 3. Schematic illustration showing interaction among mitochondrial influences originating from vascular smooth muscle, endothelium, perivascular nerves, and parenchymal astroglia and neurons. Factors produced following activation of mitochondria by physiological, pharmacological, and pathological stimuli in any of these cell types can affect vascular smooth muscle, and the interaction of these factors will determine the final, integrated arterial tone. Abbreviations: NO, nitric oxide; PGs, prostaglandins; EDHF, endothelial derived relaxing factor; EETs, epoxyeicosatrienoic acids, $K^+$, potassium ion.

can provide mitochondrially-initiated vasoactive signals to VSM for the final determination of integrated changes in cerebral vascular diameter (Fig. 3).

**Insulin Resistance**

Insulin resistance often precedes the development of type II diabetes by years or decades, and is considered a relatively silent phase of the metabolic syndrome despite vascular dysfunction and arterial hypertension. We and others have characterized many of the general cardiovascular and brain effects of this disease (Erdös et al., 2004a, b) which are due to tissue and vascular inflammation and increased basal levels of ROS (Erdös et al., 2004b, 2006).

**Disruption of preconditioning mechanisms**

We were the first investigators to demonstrate that several pathological stressors are able to disrupt normal functioning of plasmalemmal $K_{ATP}$ channels in cerebral arteries (Bari et al., 1996) and therefore it is not surprising that mito$K_{ATP}$ channels would be affected in a similar manner. For example, nutritional and genetic models of IR reduce function of several potassium channels in cerebral VSM, including $K_{ATP}$ channels, through mechanisms involving enhanced baseline vascular levels of ROS (Erdös et al., 2004a, b). Thus, ROS scavengers (Erdös et al., 2004b), or anti-inflammation therapies such as statin administration (Erdös et al., 2006), are able to restore normal dilation to potassium channel activators despite continued IR. Similarly, a more severe but acute event, namely ischemia/reperfusion is able to reduce $K_{ATP}$ channel-dependent dilation in cerebral arteries (Bari et al., 1996). Furthermore, our recent work has indicated that immediate preconditioning dependent upon activation of mito$K_{ATP}$ channels is abolished in hearts from IR rats (Katakami et al., 2007). Thus, ischemic- as well as diazoxide-induced preconditioning fail to protect hearts from ischemia/
Fig. 4. Schematic illustration showing that insulin resistance can affect a number of targets, including mitochondria, so that mitochondrial determined effects of cerebral vascular tone or the degree of preconditioning are reduced. In IR, mitochondrial structure often appears to be disrupted and mitoK<sub>ATP</sub> channels are not as responsive to diazoxide and BMS-191095 as in the normal situation. Paradoxically, following inhibition of succinate dehydrogenase with 3-NPA, cerebral vascular dilation is augmented in IR, but the reason for this unique response is not known.

reperfusion in IR rats while both of these approaches limit infarct size in hearts from non-IR rats. In these IR animals, prior to ischemia/reperfusion, a substantial number of the mitochondria in the heart were swollen or had disruption of the normal pattern for cristae. Furthermore, the isolated mitochondria from the hearts of insulin resistant animals had reduced responses to diazoxide. An additional finding from this study was that infarct size was enhanced in the hearts from Zucker obese compared to lean rats, which is similar to what we (Mayanagi et al, 2008) and others (Terao et al, 2008) have found in brains of IR, obese mice following MCAO. These results may indicate that normal protective mechanisms initiated at the level of the mitochondria are impaired in many common disease states and thus the brain and other organs are more at risk during ischemic episodes. The potential for the uncoupling of the tight relationship between metabolic need and blood flow in the brain due to cerebral vascular dysfunction associated with IR, as well as the elimination of normal, protective mechanisms involving mitochondria such as preconditioning, may account for the increased risk and severity of neurological diseases and strokes in patients suffering from the metabolic syndrome.

Disruption of arterial responses

Similar to preconditioning, mitochondrial-dependent responses in cerebral arteries are impaired in IR rats (Katakam et al, 2009). This attenuation of dilation appears to be due to reduced mitochondrial depolarization of VSM as well as reduced ROS generation in response to diazoxide. In addition, decreased NO production, despite increased endothelial NOS expression in IR arteries, appears to contribute to diminished relaxation of VSM. We have found similar impairment of cerebral vascular dilation to BMS-191095 in IR animals (Katakam et al, 2011b). An unexpected result was that in contrast to mitochondrial activation with diazoxide or BMS-19095, dilator responses to 3-NPA were modestly enhanced in arteries from IR rats (Katakam et al, 2009). Additionally, co-application of 3-NPA with diazoxide leads to more than just an additive dilator effect which was not attenuated in arteries from IR animals. The reason for this effect is unclear but it seems to indicate that IR interferes with some but not all vasoactive influences from mitochondria (Fig. 4).

Perspectives and Significance

There are three major results from our studies. First, mitochondrial influences are important initiators of preconditioning in the most vulnerable cell types within the neurovascular unit. Thus, targeting mitochondria might be a useful therapeutic approach
for protecting the cerebral vasculature and brain in people. Second, mitochondrial influences from endothelium, perivascular nerves, and perhaps parenchymal neurons and astroglia, are able to add to the direct mitochondrial-initiated dilator effects of VSM into a final, integrated change in cerebral vascular tone. Thus, activation of mitochondria in all or some of the cells in the neurovascular unit can induce changes in cerebral vascular tone and might represent the elusive signaling link between metabolic rate and blood flow. And third, important mitochondrial-derived mechanisms such as those that induce preconditioning or those that cause changes in cerebral vascular tone are easily disrupted by even relatively mild disease processes as well as by more severe, acute diseases such as strokes. We believe that these findings establish a new mechanism which contributes to the dysfunction of the neurovascular unit and places people at enhanced neurological risk during stroke and elevates the risk for the development of diseases such as dementia. Thus, reversing impairment of mitochondria in the absence of apparent disease, as in the early stages of IR, and also during the more established, later period of the metabolic syndrome, may represent an important, but not yet appreciated target for therapy in patients. Future treatment approaches targeting mitochondria may be more in line with prevention rather than remediation following the development of severe disease signs and symptoms in patients, or may involve the manipulation of mitochondria with the aim of taking advantage of unique features which are present in disease states.

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