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MECHANISMS OF NEUROPROTECTION DURING ISCHEMIC PRECONDITIONING: LESSONS FROM ANOXIC TOLERANCE

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Abstract

Different physiological adaptations for anoxia resistance have been described in the animal kingdom. These adaptations are particularly important in organs that are highly susceptible to energy deprivation such as the heart and brain. Among vertebrates, turtles are one of the species that are highly tolerant to anoxia. In mammals however, insults such as anoxia, ischemia and hypoglycemia, all cause major histopathological events to the brain. However, in mammals even ischemic or anoxic tolerance is found when a sublethal ischemic/anoxic insult is induced sometime before a lethal ischemic/anoxic insult is induced. This phenomenon is defined as ischemic preconditioning. Better understanding of the mechanisms inducing both anoxic tolerance in turtles or ischemic preconditioning in mammals may provide novel therapeutic interventions that may aide mammalian brain to resist the ravages of cerebral ischemia. In this review, we will summarize some of the mechanisms implemented in both models of tolerance, emphasizing physiological and biochemical similarities.

INTRODUCTION

Different physiological adaptations for anoxia resistance have been described in the animal kingdom. These adaptations are particularly important in organs that are highly susceptible to energy deprivation such as the heart and brain. This vulnerability is especially evident in brain that, among body tissues, has the highest energy requirements and may be the most vulnerable to energy failure. It is well accepted that at least in mammals, brain is approx 3% of the body mass, yet it receives approx 15% of the total cardiac output and uses 20% of the body oxygen (Sokoloff 1989). In mammals, insults such as anoxia, ischemia and hypoglycemia, all cause the brain to become isoelectric within few minutes (Heiss et al. 1976; Astrup et al. 1977). If these insults are continued, the loss of electrical activity is followed by depletion of high energy intermediates, while lactate increases (Lowry et al. 1964), and by the loss of ion gradients (depolarization) (Hansen 1985). As ion gradients are lost, for example in ischemia, neurons release the excitatory neurotransmitter glutamate which may amplify or cause irreversible pathologies during energy failure (Greenmayre 1986; Choi 1987) (Figure 1).

Following these initial stages, the mechanisms leading to neuronal cell death after cerebral ischemia become more complex. A well established fact in this field is that cells continue to

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die over days and months after a stroke, a phenomenon that has been defined as delayed cell death. Although not clearly defined, neuronal cell death may result from either apoptosis, necrosis or a cell death mechanisms that is a mixture of these processes (Martin et al. 1998; Liou et al. 2003; Lo et al. 2003).

In addition numerous studies support the hypothesis that the reperfusion phase following cerebral ischemia contributes substantially to ischemic injury (Watson et al. 1989; Siesjo et al. 1991; Choi 1993; Chan 1994) and that mitochondrial dysfunction plays a central role (Abe et al. 1995; Ankarcrona et al. 1995; Schinder et al. 1996; White et al. 1996; White et al. 1997; Fiskum et al. 1999; Friberg et al. 2002).

In contrast to this susceptibility to energy deprivation in mammalian brain, the turtle brain is remarkably resistant to anoxia. Turtle brain survives anoxia by maintaining ATP levels necessary to avoid the loss of ion homeostasis and the uncontrolled release of excitotoxic neurotransmitters (Lutz et al. 2004; Nilsson et al. 2004). This delicate balance is achieved by increased glycolysis (Pasteur effect) and by decreasing energy demand.

Although mammalian brains are highly sensitive to loss of energy supply, as occurs during cerebral ischemia, in the last 20 years there has been a large number of studies demonstrating a unique mammalian brain adaptation that provide a metabolic state of resistance against cerebral ischemia, which is referred to as ischemic preconditioning (IPC). This state is reached when a brief ("sublethal") ischemic episode, followed by a period of reperfusion, increase an organ's resistance to injury (ischemic tolerance) following a subsequent ischemic event. This induction of tolerance against ischemia resulting from sublethal ischemic or anoxic insults has gained attention as a robust neuroprotective mechanism against conditions of stress such as anoxia/ischemia in heart and brain (Murry et al. 1986; Schurr et al. 1986; Kitagawa et al. 1990; Kato et al. 1992; Lin et al. 1992; Alkhulaifi et al. 1993; Lin et al. 1993; Walker et al. 1993; Perez-Pinzon et al. 1997c) and has the unique advantage over the non-vertebrate anoxia tolerance animal models in that it protects against ischemia, not just anoxia (see below). This is of particular importance in the field of stroke, because stroke remains the leading cause of disability and the third leading cause of death in USA and the Western world.

The present review examines similarities and differences among the mechanisms that promote both types of adaptations to anoxia in the turtle brain and to ischemia in the mammalian brain.

A key difference between anoxic and ischemic tolerance is that in the former, glucose is required. Thus, during cerebral anoxia in turtles, an enhanced glycolytic rate (Pasteur effect) is essential for the initial phases of tolerance. This is evident from studies demonstrating that inhibition of glycolysis with iodoacetic acid (IAA) (Sick et al. 1982b) or cerebral ischemia (Sick et al. 1985) or oxygen glucose deprivation (OGD) in vitro (as it would occur in ischemia) (Perez-Pinzon et al. 1992b) promote anoxic depolarization in turtle brains which although at a lower rate, then resemble the mammalian brain. In IPC, the Pasteur effect does not play a major role in ischemic resistance, since glucose is not available during cerebral ischemia.

A key factor required for both anoxic and ischemic tolerance is time. Turtle brains buy time by their intrinsic low metabolic rate, rapid inhibition of electrical activity and immediate increase in glycolytic rate upon anoxia. In mammalian ischemic tolerance, time is required after the induction of the sublethal insult before the right metabolic machinery is in place to promote tolerance against the lethal ischemic insult. This state of ischemic tolerance can now be achieved pharmacologically (Perez-Pinzon et al. 1996; Lange-Asschenfeldt et al. 2003; Raval et al. 2003; Raval et al. 2005).

Once ischemic tolerance is induced several mechanisms occur which are similar to anoxia tolerance in turtles. Thus, in this review we will focus on a) the common triggering mechanisms; and b) the two main sites where protection occurs (synaptic and mitochondrial sites).

a) Triggering mechanisms

a.1. Adenosine—Liu et al. (Liu et al. 1992) suggested that endogenous adenosine mediates IPC in cardiac muscle, since adenosine A1 receptor blockers eliminated the protective effect of IPC. Furthermore, acadesine (5-amino-4-imidazolecarboxamide riboside, AICAR), which increases local levels of adenosine delayed the natural decay of preconditioning (i.e., IPC protection was prolonged beyond 1 h) (Tsuchida et al. 1993; Tsuchida et al. 1994; Burckhardt et al. 1995). IPC was also enhanced by dipyridamole, which blocks adenosine uptake (Miura et al. 1992).

Recent evidence in brain also suggests that adenosine is involved in IPC (Figure 2). Supporting a role of adenosine also are findings that the nearly complete protection produced by IPC in the CA1 region of rat hippocampus 3 days after 6 min of global cerebral ischemia was abolished by DPCPX (1 mg/kg) (an adenosine A1 receptor antagonist). Infusion of cyclopentyl adenosine (CPA) (1 mg/kg) (an adenosine A1 receptor agonist) 15 min prior to ischemia, produced 70% protection of CA1 cells after 3 days of reperfusion (Heurteaux et al. 1995). We also demonstrated that both anoxic and ischemic preconditioning were neuroprotective via the adenosine A1 receptor in rat hippocampal brain slices (Perez-Pinzon et al. 1996; Lange-Asschenfeldt et al. 2003).

In turtles, adenosine plays a critical role in anoxia tolerance in the early stages of the insult. Upon the induction of anoxia there is significant rise in extracellular adenosine levels in turtle brain (Nilsson et al. 1992), and blockade of the adenosine A1 receptors pharmacologically provokes anoxic depolarization in the turtle brain in vitro (Perez-Pinzon et al. 1993). This rise in extracellular adenosine levels provoke an increase in cerebral blood flow early in anoxia (Hyland et al. 1994), a reduction in membrane K^+ leakage (Pek et al. 1997a), a reduction in glutamate efflux (Milton et al. 2002), and a down regulation in NMDA receptor activity and whole cell conductance (Buck et al. 1998).

a.2—Activation of the K^+_{ATP} channel, likely plays a role in at least some of the mechanisms of IPC. This is because blockade of the K^+_{ATP} channel abolished preconditioning and the protection afforded by adenosine and R-PIA (Schulz et al. 1994; Van Winkle et al. 1994). In contrast, a K^+_{ATP} channel opener (RP-52891, aprikalim) increased ischemic tolerance (Auchampach et al. 1992; Gross et al. 1992). Activation of K^+_{ATP} channel with bimakalin (agonist) during a preconditioning ischemic episode reduced the time necessary to produce preconditioning in dogs (Yao et al. 1994).

Recent evidence in brain also showed that transient infusion of K^+_{ATP} channel antagonist prior to ischemia can block IPC protection after forebrain ischemia (Heurteaux et al. 1995) or an agonist can emulate IPC in hippocampal slices (Perez-Pinzon et al. 1999a). However, the precise K^+_{ATP} channel involved remains undefined.

Recently, two ATP-sensitive potassium channels have been described. One of these channels resides in the plasma membrane; the other resides in the mitochondrial inner membrane. The mtK^+_{ATP} has been suggested to be the key channel involved in ischemic preconditioning, since the mtK^+_{ATP} blocker 5HD prevented IPC protection (Auchampach et al. 1992; Schultz et al. 1997). It has been hypothesized that opening of the mtK^+_{ATP} channel may depolarize mitochondrial membrane potential promoting an increase in the electron transport chain rate, and thus increasing ATP production (Inoue et al. 1991).

Because of the high bioenergetic component of ion pumping in brain, a reduction in ion permeability, often termed as “channel arrest”, has been proposed to be key for turtle brain survival during anoxia (Sick et al. 1982b; Lutz et al. 1985; Hochachka 1986). Much evidence supports this hypothesis and will be further described later in this review. However, K^+ membrane conductances in turtle brain are believed to play a role in channel arrest. In an ingenious experiment, Chih et al. (Chih et al. 1989) tested whether there was a change in the rate of K^+ leakage in normoxic vs anoxic turtle brains when the $Na^+-K^+-ATPase$ pump was inhibited with ouabain. The rates of K^+ leakage were significantly lower in brains subjected to 2 h of anoxia than in normoxic brains, supporting the suggestion that reduced ion leakage is an important mechanism for energy conservation during anoxia. These studies were followed by Pek and Lutz (Pek et al. 1997b), who determined that in the turtle cerebral cortex, intracellular to extracellular K^+ flux is reduced by about 50% over the first hour of anoxia and plateaus at about 30% of the normoxic flux over the next few hours. Further, they determined that opened K^+_{ATP} channels and activated adenosine A1 receptors mediated this down-regulation of K^+ flux.

b) Sites of protection

Numerous studies support the hypothesis that reperfusion following cerebral ischemia contributes substantially to ischemic injury (Watson et al. 1989; Siesjo et al. 1991; Choi 1993; Chan 1994) and that synaptic (Benveniste et al. 1989; Crepel et al. 1993) and mitochondrial dysfunction play a central role (Abe et al. 1995; Ankarcrona et al. 1995; Schinder et al. 1996; White et al. 1996; White et al. 1997; Fiskum et al. 1999; Friberg et al. 2002).

b.1: Synaptic protection—The link between synaptic dysfunction and brain ischemia is evident by the fact that about 50% of long-term survivors of cardiac arrest or transient ischemic attack exhibit impaired mental abilities, manifested as learning impairment, memory disturbance, concrete thinking, impaired attention and concentration, and reduced capacity to plan, initiate and carry out mental activities (Roine et al. 1993; Hankey 2003).

Cortical infarcts in focal cerebral ischemia models lead to an enhanced mode of synaptic plasticity, long term potentiation (LTP) in the area surrounding the ischemic core (Hagemann et al. 1998). In addition, previous studies demonstrated that $GABA_A$ receptor-mediated decreases in electrophysiological activities precede the morphologic deterioration in post-ischemic CA1 hippocampal neurons (Shinno et al. 1997). Seizures and altered synaptic plasticity (LTP) linked to cognitive deficits strongly suggest that alterations of synapses *per se* may be involved in these post-ischemic complications.

Finally, anoxic insults can elicit another form of LTP (Hsu et al. 1997). Anoxic LTP involves post-ischemic over-activation of NMDA receptor-mediated synaptic responses (Kawai et al. 1998). Since it has been postulated that over-activation of NMDA receptors may promote cell death, it was proposed that this type of synaptic derangement was key in delayed neuronal death (Kawai et al. 1998).

Excitotoxicity: Many groups have demonstrated that IPC promotes tolerance by ameliorating different aspects of neuronal excitotoxicity. For example, glutamate release during ischemia was ameliorated by cortical spreading depression (CSD) (Douen et al. 2000) and by hypoxia/hypoglycaemia (Johns et al. 2000) preconditioning. Decreases in glutamate release was accompanied by increases in GABA release, which is suggested to be neuroprotective (Johns et al. 2000), and linked to down-regulation of the glial glutamate transporter isoforms EAAT2 and EAAT1 (Douen et al. 2000). IPC also promoted an increase in GABA release and upregulation of $GABA_A$ receptors in the hippocampus (Sommer et al. 2002).

In a recent study, we found that IPC promoted a robust release of GABA in rats after lethal ischemia compared with controls (Dave et al. 2005). We also observed that the activity of glutamate decarboxylase (the predominant pathway of GABA synthesis in the brain) was higher in the IPC group compared with control and ischemic groups. Because GABA_A receptor up-regulation has been shown to occur following IPC, and GABA_A receptor activation has been implicated in neuroprotection against ischemic insults, we further tested the hypothesis that GABA_A or GABA_B receptor activation was neuroprotective during ischemia or early reperfusion by using an in vitro model (organotypic hippocampal slice culture). Only administration of the GABA_B agonist baclofen during test ischemia and for 1 h of reperfusion provided significant neuroprotection, but not the GABA_A agonist. We concluded that increased GABA release in preconditioned rats after ischemia might be one of the factors responsible for IPC neuroprotection and that GABA_B receptor may be the GABA receptor activated after IPC.

IPC-induced neuroprotection also appears to involve changes at the postsynaptic level. For example, IPC induced a small, transient down-regulation of GluR2 mRNA expression and greatly attenuated subsequent ischemia-induced GluR2 mRNA and protein down-regulation and neuronal death (which would promote AMPA receptors to be calcium permeable) (Tanaka et al. 2002). In addition, IPC was able to inhibit the induction of anoxic LTP in hippocampal slices (Kawai et al. 1998). These results suggested that protection by IPC may involve inhibition of postischemic overactivation of NMDA receptors.

In contrast to mammals, the turtle brain limits the exacerbated increases in excitatory neurotransmitters during anoxia (Nilsson et al. 1991; Milton et al. 1998). This decrease in excitatory neurotransmitter release may be due to the effect of channel arrest. As described above, changes in K⁺ conductances (Pek et al. 1997b) may play a role in the electrical activity depression observed in turtle brain during anoxia (Feng et al. 1988a; Feng et al. 1988b; Perez-Pinzon et al. 1991). Another explanation for this depression of electrical activity is that there is a 40% decrease in the density of voltage gated Na⁺ channels in the anoxic turtle cerebellum in vitro (Perez-Pinzon et al. 1992c) which correlated to an elevation in the action potential threshold (Perez-Pinzon et al. 1992a).

Another mechanism by which the turtle brain may reduce energy expenditure during anoxia is by releasing more of the inhibitory neurotransmitters. For example, extracellular GABA is elevated at approx 2 hours of anoxic exposure (Nilsson et al. 1991). In addition, there is an increase in GABA_A receptor density, that continues to increase for at least 24 hours (Lutz et al. 1995). These changes in extracellular neurotransmitter levels are accompanied by a reduction in the NMDA glutamate receptor activity in the anoxic turtle brain (Bickler et al. 2000).

b.2: Mitochondrial dysfunction—In recent years a specific cellular site, namely mitochondria, has received special attention as a key player in cell death pathways (Fiskum et al. 1999; Murphy et al. 1999; Liou et al. 2003). This is evidenced by numerous studies that support the hypothesis that reperfusion following cerebral ischemia contributes substantially to ischemic injury (Watson et al. 1989; Siesjo et al. 1991; Choi 1993; Chan 1994) and that mitochondrial dysfunction plays a central role (Abe et al. 1995; Ankarcrona et al. 1995; Schinder et al. 1996; White et al. 1996; White et al. 1997; Fiskum et al. 1999; Friberg et al. 2002).

For example, following cerebral ischemia, a prominent change in redox activity of mitochondrial respiratory chain components has been observed (Welsh et al. 1982; Welsh et al. 1991; Rosenthal et al. 1995; Perez-Pinzon et al. 1997a; Perez-Pinzon et al. 1997b; Rosenthal et al. 1997). Previous studies suggested that this hyper-oxidation may result from loss of

electron carriers from mitochondria following cerebral ischemia, such as cytochrome *c* (Perez-Pinzon et al. 1999b). The loss of cytochrome *c* from mitochondria might affect respiratory chain activity and/or trigger the apoptotic cascade (Nitatori et al. 1995; Charriaut-Marlangue et al. 1996). The release of cytochrome *c* has been linked to apoptosis (Ankarcrona et al. 1995; Schinder et al. 1996; Kluck et al. 1997; Yang et al. 1997).

Mitochondria isolated from ischemic brain exhibited decreases in “state 3” respiratory rates of approximately 70% with NAD-linked respiratory substrates (Sciamanna et al. 1992). Cafe *et al.* (Cafe et al. 1994) showed that non-synaptosomal mitochondria were insensitive to ischemia itself; however, mitochondria became dysfunctional in the late reperfusion phase.

Mitochondria from synaptic terminals were greatly affected by ischemia, but partially recovered during reperfusion. Sims and Pulsinelli (Sims et al. 1987) also reported that the rate of oxygen consumption decreased in the CA1, CA3 and CA4 regions in the late reperfusion phase. Mitochondrial dysfunctions are reported in core and penumbra regions after MCAO (Kuroda et al. 1996; Anderson et al. 1999). Isolated mitochondria exhibited decrease in ADP stimulated respiration during early reperfusion period (Kuroda et al. 1996). However, activities of mitochondrial electron transport chain (ETC) complex I, II–III, and IV were preserved in penumbra as well as in the core region during early reperfusion following MCAO (Canevari et al. 1997). During late reperfusion phase following MCAO, the activity of mitochondrial ETC complex I is decreased (Davis et al. 1997). In summary, there are two phases of mitochondrial dysfunctions. In the first phase (early reperfusion), mitochondrial dysfunctions are mild (decreased activity of complex IV). However, in the second phase (late reperfusion), mitochondrial dysfunction is more pronounced.

IPC significantly protected against delayed cell death, and this protection was correlated with mitochondrial protection against the deficits in respiration affecting complexes I – IV (Dave et al. 2001; Perez-Pinzon et al. 2002). As described above, many studies have demonstrated that reactive oxygen species (ROS) and the resulting oxidative stress play a pivotal role in neuronal cell death (Flamm et al. 1978; Fridovich 1979; Siesjo et al. 1985; Kontos 1989; Vlessis et al. 1990; Hall et al. 1993). There are two major regions in the electron transport chain where ROS are produced. One is complex I and the other is complex III (Chance et al. 1956). Since oxidative stress is implicated in the pathophysiology that ensues after cerebral and cardiac ischemia (Kannan et al. 2000), one can surmise that a key mechanism by which IPC protects hippocampus against delayed neuronal cell death is by protecting mitochondrial oxidative phosphorylation, diminishing ROS production and by limiting pro-apoptotic molecules to be released from mitochondria. In fact, all three mechanisms have been observed to occur in different models of IPC. The first one was demonstrated by our group (described above) (Dave et al. 2001; Perez-Pinzon et al. 2002). In addition, hypoxic preconditioning 24 h prior to OGD significantly reduced cell death from 83% to 22% in the necrotic model and 68% to 11% in the IPC model (Arthur et al. 2004). In this IPC model, the activity of the antioxidant enzymes glutathione peroxidase, glutathione reductase, and Mn superoxide dismutase were significantly increased. Furthermore, superoxide and hydrogen peroxide concentrations following OGD were significantly lower in the IPC group. Finally, cytochrome *c* release from mitochondria to the cytosol was suppressed in the ischemia-tolerance-induced hippocampal CA1 region (Nakatsuka et al. 2000).

Despite the important role of mitochondria in cell death pathways, not much has been done in the turtle brain during anoxic conditions. In turtle brain, cytochrome aa3 appeared more reduced than rat cytochrome aa3 in normoxia, which may be due either to lower affinity for O₂ or to enhanced substrate supply (Sick et al. 1982a). These results were suggestive that the resistance to anoxia observed in turtles is likely due to differences in redox activities of cytochrome aa3 and/or in substrate use play a role in the relative insensitivity of turtle brain to O₂ deprivation (Sick et al. 1982a). In turtle heart, Cai and Storey (Cai et al. 1996) reported the

upregulation of mitochondrial genes for NADH-ubiquinone oxidoreductase subunit 5 and cytochrome c oxidase subunit 1 after prolonged anoxia in turtles, which, if translated into protein levels, would suggest increase efficiency of the electron transport chain of mitochondria and may render brain mitochondria resistant to 'lethal' ischemia.

Conclusions

There are some similarities in some of the neuroprotective strategies involved in both anoxic tolerance in turtle brain and ischemic tolerance in mammalian brain. For example, adenosine release and activation of adenosine receptors have been proven to play a role in both models of tolerance.

Although electrical activity depression and "channel arrest" are well established as a strategy for tolerance in turtle brains during anoxia, it is not clear in ischemic preconditioning in mammals. However, both types of tolerance attempt to shift excitotoxicity by limiting glutamate release and enhancing GABA release. This strategy appears well established in turtle brain, but in ischemic tolerance in mammals remain controversial.

Finally, much evidence suggests that IPC targets brain mitochondria as a site for neuroprotection given the fact that this organelle is highly sensitive to cerebral ischemia and that it can elicit the apoptotic pathway during the reperfusion phase. Turtle brain mitochondria in turn, may be spared from anoxic stress by enhancing the glycolytic capacity and better maintenance of ion homeostasis.

Although the turtle brain is a remarkable model of anoxic tolerance, the fact that it does not resist ischemic insults suggests that it is a good model for the development of novel therapeutic interventions in hypoxic/anoxic pathologies, but not in stroke. Ischemic preconditioning is better suited for the development of therapies against stroke. Finally, a new ischemic tolerance model that has recently emerged is the arctic ground squirrel. We recently demonstrated that these species' brain is able to survive a clinically relevant model of ischemia, cardiac arrest (Dave et al. 2006). This finding is even more remarkable because brain ischemic resistance was found in euthermic conditions.

Peter L. Lutz—Peter was my mentor in graduate school and continued to mentor me as I continued my professional academic career. To me Peter was the old fashion scientist, more interested in understanding globally how the world works. I think he really disliked the super specialization of science, but to survive, he managed to keep up. Sadly, today's scientific environment is not conducive for this approach to science.

Peter's interests were not limited to science as every time I would meet him outside the laboratory enclaves (most likely at a bar/pub!) the conversations began with science, but ended up in politics, philosophy or religion. These were lively conversations/arguments and highly stimulating and motivated me to go home with the need to read few books before I met him again to continue our arguments. And this is how he faced science, with the same fervor and enthusiasm, with the same skepticism and always trying to connect the dots, even when they seemed impossible to connect! Speculation was his nature. He did not mind to be wrong or that you cannot publish speculative ideas, but he loved to speculate. It was part of his vivid imagination and curiosity. I surely miss Peter and Peter's personality.

I surely miss his friendship! But he will remain a part of me.

Abbreviations

IPC

	ischemic preconditioning
OGD	oxygen-glucose deprivation
LTP	long-term potentiation
CSD	cortical spreading depression
MCAo	middle cerebral artery occlusion
ETC	electron transport chain
ROS	reactive oxygen species
IAA	iodoacetic acid
CPA	cyclopentyl adenosine
DPCPX	8-cyclopentyl-1,3-dipropylxanthine

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Figure 1.

Schematic representation of a simplified cerebral ischemia induced cascade on synaptic dysfunction. Energy deprivation upon an ischemic insult promotes an exacerbated release of excitatory neurotransmitters that in turn overactivate glutamate receptors with a consequent cytosolic calcium overload that initiates aberrant signaling pathways that promote mitochondrial dysfunction and further synaptic dysfunction. DAG, diacylglycerol; STP, signal transduction pathway; Cyt c, cytochrome c.

Figure 2. Simplified scheme depicting the basic signaling pathways defined in ischemic preconditioning in mammals. Triggering pathways include activation of the NMDA and adenosine A1 receptors which in turn are involved in activating the epsilon isoform of protein kinase C. This pathway is known to protect mammalian neurons from excitotoxicity and against mitochondrial dysfunction.

Figure 3.
My mentor and friend, Dr. Peter L. Lutz in Edinburgh, Scotland during my first scientific conference in Europe.