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Research Report

Real-time monitoring of spatial and temporal metabolic changes during focal cerebral ischemia in rats

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ABSTRACT

Focal cerebral ischemia creates a gradual injury, ranging from severe injury in the core towards moderate damage in the penumbra. Disruption of blood supply leads to shortage in oxygen supply, resulting in mitochondrial disruption in the ischemic area. The present work study the mitochondrial function and microcirculatory blood supply in the core and penumbra of the ischemic tissue following different ischemic durations. Focal ischemia was obtained by middle cerebral artery occlusion (MCAO). Monitoring of the brain was conducted using a unique multi-site–multi-parametric (MSMP) monitoring system, which enables real-time, in vivo, simultaneous and continuous monitoring of mitochondrial NADH and CBF. Short sessions of anoxia before ischemia and following reperfusion were used to test the ability of the tissue to respond to such metabolic challenges. Following focal ischemia, CBF levels decreased and NADH levels increased and recovered at reperfusion. These changes were more severe in the core compared to the penumbra. Longer ischemic duration led to an increase in oxygen demand following reperfusion and to vast disruption of blood supply, as seen during short anoxic exposures. In conclusion, the ability of mitochondrial activity and blood supply to recuperate following ischemia, as well as the ability of the tissue to cope with metabolic challenges, varies in the core and the penumbra and depends on ischemic duration. The MSMP monitoring system, used in the current study, can add valuable information regarding the metabolic state of the brain during focal ischemia.

1. Introduction

Ischemic stroke is a major cause of death and adult disability in many countries (Zhan and Yang, 2006). Focal ischemia is characterized by reduced cerebral blood flow (CBF) in the cortex, which leads to disruption in oxygen balance (Lipton, 1999; Sims and Muyderman, 2010), therefore leading to interruption of extra-cellular ion balance, increased lactate levels, decrease in extra-cellular pH, accumulation of glutamate and free radicals and suppression of spontaneous electrical activity (Brouns and De Deyn, 2009; Ginsberg, 2003; Hossmann, 2009; Lipton, 1999).

Focal ischemia in the brain creates a gradual injury, which spreads from severe injury in the center of the lesion, known as the core, towards moderate damage in periphery regions, known as the penumbra (Lipton, 1999; Murphy and Corbett,

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2009). Severity of injury is also known to be influenced by the duration of blood shortage, affecting the damage occurring during the ischemic period, as well as reperfusion injury after restoration of blood supply (Hossmann, 2009).

Decrease in blood supply leads to shortage of oxygen in the brain, affecting the intracellular respiratory chain activity in the mitochondria. As a result, ATP synthesis is reduced and mitochondrial NADH levels increase (Mayevsky, 1984; Sims and Muyderman, 2010). Due to its high sensitivity to intracellular oxygenation, NADH levels can serve as an indicator to mitochondrial function and tissue metabolic state (Mayevsky, 1984; Mayevsky and Chance, 2007).

The aim of the current work was to investigate mitochondrial function and blood supply in the core and the penumbra of the ischemic tissue in different ischemic durations. In order to assess the vitality of the tissue during ischemia, a unique multi-site–multi-parametric (MSMP) monitoring system which enables real-time, in vivo, simultaneous and continuous monitoring of mitochondrial NADH and CBF was used. The use of real-time monitoring of NADH, in addition to CBF, is rarely used tool in the study of stroke (Sims and Muyderman, 2010), which may shed light on the function of the mitochondria during ischemia. In addition, the use of a short session of anoxia before the induction of focal ischemia, as well as a second session of anoxia following reperfusion, may give indication for the ability of the ischemic tissue to respond to metabolic challenges.

2. Results

2.1. *Ischemia for 10 min*

Fig. 1 presents an average response of the brain to right MCAO for 10 min, following by 60 min of reperfusion, as recorded from the core and the penumbra on the right hemisphere.

At the onset of ischemia, CBF levels decreased to 12±3% in the core ($p<0.001$ compared to baseline), while in the penumbra CBF dropped to 38±7%, and afterwards stabilized at 57±11% ($p<0.001$), resulting in significant differences between the two sites ($F=38.984$, $df=1$, $p<0.001$) during the entire ischemic period ($p<0.05$).

Reflectance levels showed a decrease to 89±3% in both sites ($p<0.001$), followed by a secondary increase only in the core 3 min later (114±11%), and then gradually returned to baseline. Fluorescence increased significantly to a level of 140±5% in both sites ($p<0.001$), afterwards another increase occurred in the core (150±15%), while a decrease down to 106±3% occurred in the penumbra, leading to significant differences between the two sites ($F=25.15$, $df=1$, $p<0.001$) 3 min following ischemia until reperfusion ($p<0.05$).

NADH levels increased to 135±6% in the core ($p<0.001$) and to 132±3% in the penumbra ($p<0.001$), following a decrease to 117±4% only in the penumbra, which lead to significant differences between the two sites ($F=13.175$, $df=1$, $p<0.001$) 5 min from the onset of the occlusion until its removal ($p<0.05$).

At reperfusion, a rapid increase in CBF was observed, to peak levels of 310±40% in the core ($p<0.01$ compared to baseline) and 228±53% in the penumbra, leading to significant differences between both measuring sites ($F=108.158$, $df=1$, $p<0.001$) 15 min following reperfusion.

Reflectance and fluorescence levels decreased to 75±5% in the core ($p<0.001$) and to 85±5% in the penumbra ($p<0.01$) and returned to baseline 30 min after reperfusion. NADH returned to baseline immediately after reperfusion.

2.2. *Ischemia for 30 min*

Fig. 2 presents the response in the core and the penumbra to right MCAO for 30 min, following by 60 min of reperfusion. Immediately following MCAO, CBF levels decreased at both sites and remained low during the entire ischemic period.

In the core, CBF decreased to a minimal level of 10±3% and then moderately increased up to levels of 20±7% ($p<0.001$ compared to baseline), while in the penumbra CBF decreased to a minimal level of 28±7% and then increased up to 60±12% ($p<0.001$ compared to baseline), resulting in significant differences between both measuring sites ($F=14.019$, $df=1$, $p<0.001$), which lasted 3 min from the onset of the occlusion until its removal ($p<0.05$).

At the beginning of ischemia, reflectance level decreased by ~10% at both sites ($p<0.05$). An insignificant trend of increase was marked only at the core, up to levels of 130±10%, with stabilization at levels of 117±10%, resulting significant differences between both sites ($F=14.227$, $df=1$, $p<0.001$) 3 min from the onset of the occlusion until reperfusion ($p<0.001$). At the onset of the occlusion fluorescence levels increased toward 127±4% in the core ($p<0.001$) and 117±4% in the penumbra ($p<0.01$), and 3 min later another increased occurred, toward levels of 180±18% in the core ($p<0.001$) and 143±14% in the penumbra ($p<0.01$). From that point until the removal of the ligation, a moderate decrease was observed at both sites, down to levels of 150±9% in the core ($p<0.01$) and 115±8% in the penumbra. The difference between both measuring sites were significant ($F=14.019$, $df=1$, $p<0.001$) from the 3rd minute following the occlusion until its removal ($p<0.001$).

NADH levels increased and then moderately decreased at both sites. The maximal increase was to 140±4% in the core ($p<0.001$), and toward 127±4% in the penumbra ($p<0.001$) following with a decrease down to 130±4% and 110±4% in the core and penumbra, respectively ($p<0.001$). Significant differences between both sites ($F=59.618$, $df=1$, $p<0.001$) were detected 3 min following the occlusion until the reperfusion phase ($p<0.01$).

At reperfusion a significant increase in CBF toward levels of 320±70% was seen in the core ($p<0.05$), while in the penumbra only a trend of increase toward 235±55% was observed. Reflectance levels returned to baseline following reperfusion, while in fluorescence a significant decrease down to 70±8% was marked only in the core ($p<0.05$), resulting in significant differences between both sites ($F=8.99$, $df=1$, $p<0.01$) 30 min following reperfusion ($p<0.05$). NADH levels returned to baseline at both sites immediately following reperfusion, but a moderate increase in the penumbra ($p<0.05$ compared to baseline) resulted in significant differences between both sites ($F=15.68$, $df=1$, $p<0.001$) starting from 20 min after reperfusion and lasting until the end of the experiment ($p<0.05$). All the other parameters returned to baseline 30 min after the beginning of reperfusion.