The energy metabolism of cerebral mitochondria of hypertensive rats to study the action of drugs on this major risk factor for the prevention of ischaemic strok

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Hypertension is a fundamental risk factor for acute ischemic stroke (AIS), increasing the risk of cerebral oedema and haemorrhagic transformation: on hospital admission, blood pressure (BP) >140/>90 mmHg is present in over 60% of AIS patients (Qureshi, 2008). However, although anti-hypertensive treatments prevent stroke, the benefit of BP-lowering in AIS is controversial, because of the possible impaired collateral perfusion of ischaemic *penumbra* (Moretti et al., 2015). Also metabolic alterations are strongly related to AIS (Villa et al., 2013) and hypertension (Girouard & Iadecola, 2006), because (i) energy availability is required for molecular and cellular functions and (ii) the neurovascular coupling is impaired in both pathological conditions.

For these considerations, the aim of this on-going study is to assess the effects of hypertension on brain energy metabolism evaluating the maximal rate (V_{max}) of representative and regulatory mitochondrial enzyme activities (*Functional Proteomics*) related to Krebs' cycle, electron transport chain (ETC), glutamate and related amino acids metabolism as a measure of tissue energy transduction (Ferrari et al., 2015). Because of the heterogeneity of brain mitochondria (Villa et al., 2013, 2016), this research was performed (i) on somatic mitochondria (FM) located *in vivo* in neuronal perikaryon and (ii) on synaptic light (LM) and (iii) heavy (HM) ones. These 3 types of mitochondria were purified from the cerebral cortex of (i) male Wistar Kyoto (Cobs, CR) controls and of (ii) spontaneously hypertensive rats (SHRs), at start aged 6 months (young animals).

In controls, enzyme activities possess different V_{max} respect to the type of mitochondria. This micro-heterogeneity is of fundamental metabolic importance; in fact, these mitochondrial enzyme catalytic properties have been shown to be responsible for metabolic modifications of physiopathological significance in brain *in vivo*, affecting tissue responsiveness to noxious stimuli, in particular discriminating pre- or post-synaptic location of drugs actions (Villa et al., 2012, 2013, 2016).

This metabolic micro-heterogeneity of brain mitochondria was evidenced also in SHRs, where hypertension selectively modified enzyme activities in all mitochondrial types respect to controls: (i) in FM, NADH-cytochrome *c* reductase activity (Complex I-III) is lower and glutamate dehydrogenase higher; (ii) in LM, cytochrome oxidase (Complex IV), glutamate dehydrogenase and glutamate-oxaloacetate transaminase V_{max} are higher; (iii) in HM, succinate dehydrogenase (Complex II) and cytochrome oxidase are lower and higher, respectively.

Thus, mitochondria of SHRs are characterized by: (i) a decreased entry of reducing equivalents into ETC through Complex I (FM) and II (HM), (ii) an increased energy metabolism of synaptic mitochondria, as indicated by the greather Complex IV activity, a marker of neuronal activation (Wong-Riley, 2012), and (iii) an increased glutamate metabolism in both pre- and post-synaptic compartments. This *in fieri* experimental project on brain energy metabolism will allow to study: (i) the effect of hypertension during ageing at 6, 12, 18 and 24 months of age; the action of old/new drugs (ii) on the prevention of cerebral ischaemia and (iii) on its metabolic outcomes following the recovery of post-ischaemic phase, in the perspective of developing new therapeutic agents.

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