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► **To cite this version:**

Matthias Kohlhauer, Mathieu Panel, Marine Vermot Des Roches, Estelle Faucher, Yara Abi Zeid Daou, et al.. Brain and myocardial mitochondria follow different patterns of dysfunction after cardiac arrest. Shock, 2021, Online ahead of print. 10.1097/SHK.0000000000001793 . inserm-03279846

HAL Id: inserm-03279846

<https://inserm.hal.science/inserm-03279846v1>

Submitted on 6 Jul 2021

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Brain and myocardial mitochondria follow different patterns of dysfunction after cardiac arrest

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Running head: Mitochondria and cardiac arrest

Conflicts of Interest: M Kohlhauer and R Tissier are shareholders of a start-up company dedicated to the clinical research on therapeutic hypothermia through total liquid ventilation (Orixha).

Funding source: This study was supported by the Agence Nationale pour la Recherche (COOLIVENT Grant).

28 **Abstract**

29 Mitochondria is often considered as the common nexus of cardiac and cerebral dysfunction
30 after cardiac arrest. Here, our goal was to determine whether the time course of cardiac and
31 cerebral mitochondrial dysfunction is similar after shockable vs non-shockable cardiac arrest
32 in rabbits. Anesthetized rabbits were submitted to 10 min of no-flow by ventricular fibrillation
33 (VF group) or asphyxia (non-shockable group). They were euthanized at the end of the no-
34 flow period or 30 min, 120 min or 24h after resuscitation for *in vitro* evaluation of oxygen
35 consumption and calcium retention capacity. In the brain (cortex and hippocampus), moderate
36 mitochondrial dysfunction was evidenced at the end of the no-flow period after both causes of
37 cardiac arrest vs baseline. It partly recovered at 30 and 120 min after cardiac arrest, with lower
38 calcium retention capacity and higher substrate-dependant oxygen consumption after VF vs
39 non-shockable cardiac arrest. However, after 24h of follow-up, mitochondrial dysfunction
40 dramatically increased after both VF and non-shockable cardiac arrest, despite greater
41 neurological dysfunction after the latter one. In the heart, mitochondrial dysfunction was also
42 maximal after 24h following resuscitation, with no significant difference among the causes of
43 the cardiac arrest. During the earlier timing of evaluation, calcium retention capacity and ADP-
44 dependant oxygen consumption were lower and higher, respectively, after non-shockable
45 cardiac arrest vs VF. In conclusion, the kinetics of cardiac and cerebral mitochondrial
46 dysfunction suggests that mitochondrial function do not play a major role in the early phase of
47 the post-resuscitation process but is only involved in the longer pathophysiological events.

48

49 **Key words:** Mitochondria; Cardiac arrest; Ischaemia; Reperfusion

50

51 **Introduction**

52 Out-of-hospital cardiac arrest is a major cause of mortality in western countries.
53 Following resumption of spontaneous circulation (ROSC), a “post-cardiac arrest syndrome” is
54 characterized by neurological, cardiac and multi-visceral failure. The severity of this syndrome
55 depends on the initial cardiac rhythm identified during cardiopulmonary resuscitation (CPR),
56 with worst outcome after non-shockable vs shockable rhythms **(1, 2)**. Neurological dysfunction
57 is responsible for 65% of the overall mortality while acute heart failure is the predominant cause
58 of death during the first 3 days following resuscitation **(3)**.

59 It is often assumed that the pathophysiology of ischemic disorders shares common
60 mechanisms in the brain and heart after cardiac arrest, considering that the mitochondria is a
61 common nexus for both organs **(4–6)**. In the absence of oxygen, the mitochondrial respiratory
62 chain is indeed a major source of reactive oxygen species (ROS), which leads to massive
63 oxidation and cellular death **(7)**. For instance, ischaemia induces a reversion of the succinate
64 dehydrogenase (complex II) leading to alteration of tricarboxylic acid cycle with dramatic
65 reduction of fumarate into succinate **(8, 9)**. At reperfusion, succinate dehydrogenase resumes
66 its normal function and converts back the succinate into fumarate. However, the large stock of
67 succinate accumulated during ischaemia leads to an electron overflow in the mitochondrial
68 respiratory chain and induces a reverse electronic flow from complex II to complex I. This
69 contributes to a massive production of superoxide anion at reperfusion **(8)**. Additionally, the
70 cytosolic calcium overload induced after ischaemia-reperfusion promotes the formation of a
71 mitochondrial Permeability Transition Pore (mPTP), which is responsible for mitochondrial
72 swelling and release of pro-apoptotic factors **(10)**. Experimentally, its pharmacological
73 inhibition has been widely shown to provide cardioprotection after focal or generalized
74 ischaemia **(11, 12)**. However, the pathophysiology of post-cardiac arrest disorders probably
75 differs between brain and heart, and may depends upon the cause of cardiac arrest (shockable
76 vs non shockable rhythms).

77

78 Accordingly, the goal of the present study was to compare the time course of
79 mitochondrial dysfunction in brain and heart after cardiac arrest. We used two different
80 conditions of cardiac arrest in rabbits, using ventricular fibrillation vs asphyxia models of
81 cardiac arrest. In both conditions, we investigated the time course of the mPTP formation and
82 mitochondrial respiration dysfunction. We also compared the neurological and cardiovascular
83 outcomes in both conditions in order to assess putative links between mitochondrial kinetics
84 disorders and related end-organ injuries.

85 **Materials and Methods**

86 **Experimental procedure**

87 Experiments were approved by the institutional review board for animal research
88 (ComEth AnSES/ENVA/UPEC n°16; Project 18-069), in accordance with French regulations
89 for animal use for research purpose. Male New-Zealand rabbits (2.7 ± 0.2 kg) were anaesthetised
90 with tiletamine and zolazepam (25 mg/kg i.m. each). After intubation and mechanical
91 ventilation, anaesthesia was maintained with sodium pentobarbital (5–10 mg/kg/h i.v.).
92 Methadone (0.5 µg/kg i.m.) was administered for analgesia. An arterial pressure probe was
93 inserted into the medial ear artery for mean arterial pressure (MAP) continuous recording.
94 Electrocardiogram was evaluated throughout the experiment. After paralysis with vecuronium
95 bromide (0.1 mg/kg i.v.), mechanical ventilation was stopped and animals were randomly
96 submitted to 10 min of no-flow induced by either ventricular fibrillation (VF group) or asphyxia
97 (Non-shockable CA group).

98 In the VF group, cardiac arrest was induced by applying an alternative current (10 V,
99 0.4 mA) between two electrodes applied upon the chest and inserted into the oesophagus,
100 respectively. In the non-shockable group, cardiac arrest was induced by asphyxia through
101 endotracheal tube clamping. Blood pressure rapidly decreased and no-flow was defined as a
102 mean blood pressure drop below 20 mmHg.

103 In both groups, cardiopulmonary resuscitation (CPR) was performed after 10 min of no-
104 flow by continuous chest compression and adrenaline administration every two minutes (15
105 µg/kg i.v.). Cardiac massage was performed manually at the rate of 200 beat per min. In the
106 VF group, electric shocks were also delivered every two minutes (10 J/kg in VF group) until
107 electrical conversion of the heart rhythm. Resumption of spontaneous circulation (ROSC) was
108 considered as a mean blood pressure remaining above 60 mmHg during at least one minute
109 without chest compression. After ROSC, rabbits remained under mechanical ventilation
110 ($FiO_2=30\%$) with adrenaline infusion at a dose adjusted to achieve a mean blood pressure of
111 70 mmHg.

112 Animals were euthanized at the end of the no-flow period or 30 min, 120 min or 24 h
113 after ROSC. We also isolated mitochondria from additional healthy animals with no cardiac
114 arrest for baseline values determination. In the group followed during 24h, animals were
115 awoken 6 h after ROSC. Before euthanasia, the neurological status was assessed according
116 to a specific score, as previously described (13). This scoring method is evaluating the normal
117 physiological function of the rabbits (feeding and grooming) as well as impairment of locomotor
118 function and conscious state.

119 **Isolation of mitochondria**

120 In order to assess mitochondrial dysfunction at different time points, animals were
121 euthanized at the end of the no-flow period or 30 min, 120 min or 24 h after ROSC, using a
122 lethal dose of pentobarbital (60 mg/kg i.v.). We also isolated mitochondria from additional
123 healthy animal only submitted to anaesthesia, mechanical ventilation and surgical preparation
124 for baseline values. After euthanasia, we rapidly sampled 1 g of the left ventricle, right brain
125 cortex and hippocampus.

126 **Isolation and purification of mitochondrion**

127 Cardiac samples were minced and homogenized with an Ultra-Turrax T25 (set at
128 position 5; IKA-Werke GmbH & Co. KG, Staufen, Germany) for 5 s in 15 mL of buffer containing
129 300 mM sucrose, 10 mM HEPES and 2 mM EGTA, pH 7.4, at 4°C. Cortex and hippocampus
130 were simply minced and placed in 10 mL of the same 4°C buffer. Preparations were repeatedly
131 passed in a Potter-Elvehjem glass homogenizer (motor-driven Teflon pestle set at 1500 rpm)
132 with additional 25 µL of Bovine Serum Albumin. Preparations were centrifuged at 3000 g during
133 5 min at 4°C and supernatant containing the mitochondria were recovered and centrifuged at
134 10 000 g for 10 min. After the second centrifugation, we discarded the supernatant and
135 resuspended the pellet in 200 µL of buffer for the heart and 600 µL of buffer for the brain
136 tissues. In each solution, we determined the protein concentrations by the Advanced Protein
137 Assay Reagent (57697, Sigma-Aldrich, St. Louis, MO, USA) in order to quantify the extraction
138 yield.

139 **Mitochondrial permeability transition pore**

140 The formation of mPTP was assessed by monitoring mitochondrial calcium retention
141 capacity. Mitochondrion were submitted to increasing concentrations of calcium (10 μ M) until
142 reaching a threshold at which mitochondria release calcium. This calcium release signs the
143 formation of mPTP as previously described (14). Accordingly, mitochondria (1 mg/ml) were
144 energized with 5 mM pyruvate/malate and incubated in the respiration buffer supplemented
145 with 1mM Calcium Green-5N fluorescent probe (C3737, Invitrogen, Eugene, OR, USA). The
146 concentration of calcium in the extramitochondrial medium was monitored by means of a
147 JascoFP-6300 spectrofluorimeter (Jasco, Bouguenais, France) at excitation and emission
148 wavelengths of 506 and 532 nm, respectively. The calcium signal was calibrated with addition
149 of known calcium amount.

150 **Mitochondrial oxygen consumption**

151 Mitochondrial oxygen consumption was measured at 30°C with a Clark-type electrode
152 (Hansatech Instruments Ltd, Norfolk,UK). Mitochondria (0.4 mg protein/ml) were incubated in
153 a respiration buffer containing 100 mM KCl, 50 mM sucrose, 10 mM HEPES and 5 mM
154 KH_2PO_4 , pH 7.4 at 30°C. Respiration was initiated by addition of 2.5 mM pyruvate/2.5 mM
155 malate (state 2 respiration rate). After 1 min, ATP synthesis was induced by addition of 1 mM
156 ADP (state 3 respiration rate) and 1 mmol/L carboxyatractyloside was added to measure the
157 substrate-dependent respiration rate (state 4). The respiratory control ratio (state 3/state 4)
158 was then calculated. Finally, uncoupling state was measured by administrating 1 μ M of
159 carbonyl cyanide 4-(trifluoromethoxy)phenylhydrazone in the incubation solution (state 3').

160 **Statistical analysis**

161 All results were expressed as mean \pm SEM. Individual values are illustrated in
162 Supplemental Figures 1, 2 and 3. Data normality was evaluated using a Shapiro-Wilk test.
163 Normality was confirmed for all parameters except state 3 oxygen consumption in the cortex
164 and state 4 oxygen consumption in both cortex and hippocampus. Data distribution were
165 therefore normalized for the latter parameters prior to statistical analysis. Then, respiratory

166 control and mitochondrial retention capacity were analysed using a two-way ANOVA followed
167 by Holm-Sidak test, with investigation of the group effect (VF vs non-shockable cardiac arrest)
168 and the time effect (baseline, *i.e.*, baseline conditions, end of no-flow, 30 min, 120 min and 24
169 h after CPR initiation). Neurological function was compared using a Kruskal-Wallis tests,
170 respectively. Statistical significance was set at $p < 0.05$.

171 **Results**

172 Fifty animals successfully underwent the entire protocol before mitochondrial isolation,
173 *i.e.*, 6 rabbits for each type of cardiac arrest with sampling at baseline, end of the no-flow period
174 or 30 or 120 min after CPR, and 4 rabbits for each type of cardiac arrest with sampling at 24 h
175 after neurological evaluation. In the non-shockable cardiac arrest group, MAP was dropping
176 below the value of 20 mmHg in 5.5 ± 0.2 min after disconnecting the ventilator. ROSC was
177 achieved in 2.1 ± 0.3 and 1.9 ± 0.7 min in VF and non-shockable groups, respectively. The total
178 dose of adrenaline administered during CPR to obtain ROSC was 23.3 ± 4.6 and 25.1 ± 8.1 $\mu\text{g}/\text{kg}$
179 in the non-shockable cardiac arrest and VF groups, respectively. In the VF group, the total
180 number of electrical defibrillation shocks was 3.2 ± 1.3 per animal. During the follow-up of the
181 animals, hemodynamic parameters and blood gases values were not different among animals
182 submitted to VF or non-shockable cardiac arrest (Table 1).

183 In the animals followed during 24 h, the amount of epinephrine administered after
184 ROSC to prevent hypotension was higher after VF vs non-shockable cardiac arrest, with no
185 significant difference (Figure 1, upper panel). At the timing of neurological dysfunction
186 evaluation, corresponding dysfunction scores were significantly higher after non-shockable
187 cardiac arrest vs VF (87 % [81 – 100] vs 66 % [52 – 87], respectively; Figure 1, lower panel).

188 **Mitochondrial extraction yield**

189 As illustrated by Figure 2, mitochondrial extraction yields from brain cortex and
190 hippocampus were reduced at 24 h after cardiac arrest as compared to baseline, but not at the
191 end of the no-flow period and 30 or 120 min after CPR. At each time point, extraction ratio
192 remained similar among both causes of cardiac arrest. In the heart, a significant decrease in
193 the mitochondria extraction ratio was conversely observed in all conditions after cardiac arrest
194 as compared to baseline, suggesting an increased rate of damaged mitochondria after cardiac
195 arrest. However, extraction ratio again remained similar among both causes of cardiac arrest.

196 **Mitochondrial calcium retention capacity**

197 Mitochondrial calcium retention capacity was evaluated by the calcium concentrations
198 inducing mPTP formation. As illustrated by Figure 3 (left panel), those concentrations were
199 significantly decreased in the hippocampus and cortex at the end of the no-flow period vs
200 baseline after both VF and non-shockable cardiac arrest. After 120 min following CPR, those
201 concentrations were lower in animals submitted to VF as compared to non-shockable cardiac
202 arrest. At the later time-point of the follow-up (24 h), a maximal dysfunction was observed after
203 both causes of cardiac arrest as compared to baseline, with no difference among VF and non-
204 shockable cardiac arrest.

205 In the heart, the calcium retention capacity was significantly altered since the end of
206 the no-flow period in animals submitted to non-shockable cardiac arrest as compared to
207 baseline, but not in animals submitted to VF. In the latter group, such alteration was only
208 observed at the later time-point of evaluation (24 h after cardiac arrest).

209 **Mitochondrial respiration**

210 As illustrated by Figure 3 (right panel), a strong dysfunction in the mitochondrial
211 respiration assessed by the respiratory control ratio was evidenced in the cortex at the end of
212 the no-flow period as compared to baseline. After a transient recovery at 30 and 120 min after
213 CPR, a strong dysfunction was again observed 24 h after cardiac arrest. In the heart,
214 respiratory control ratio was significantly decreased since the end of the no-flow period in
215 animals submitted to VF but not in those submitted to non-shockable cardiac arrest. However,
216 a maximal and similar decrease in respiratory control ratio was observed 24 h after both causes
217 of cardiac arrest as compared to baseline.

218 As illustrated by Figure 4, oxygen consumption at state 3 (ATP synthesis in the
219 presence of ADP), state 4 (substrate-dependant oxygen consumption independently from ATP
220 synthesis) and state 3' (oxygen consumption during mitochondrial uncoupling) progressively
221 increased from the end of the no-flow period to 120 min after CPR in the cortex and
222 hippocampus of the animals submitted to VF. It was not the case in the animals submitted to

223 non-shockable cardiac arrest, with lower oxygen consumption in the brain as compared to VF
224 after 120 following CPR. This demonstrates an over-consumption of oxygen after VF but not
225 non-shockable cardiac arrest at this early time-point. However, after 24 h following cardiac
226 arrest, a strong increase in state 4 oxygen consumption was observed vs baseline after both
227 causes of cardiac arrest, along with state 3 and state 3' oxygen consumption decrease. The
228 difference was not significant among the cause of the cardiac arrest, despite a trend toward
229 lower state 3 and state 3' oxygen consumption after VF vs non-shockable cardiac arrest.
230 Conversely, in the heart, states 3 and 3' oxygen consumption were reduced after VF as
231 compared to baseline and non-shockable cardiac arrest, before a strong increase in states 3,
232 4 and 3' oxygen consumption after both causes of cardiac arrest after 24 h.

233 Discussion

234 In our study, mitochondrial dysfunction demonstrated different patterns between the
235 brain and the heart. In both organs, a severe mitochondrial dysfunction was observed at 24 h
236 after cardiac arrest and was similar between the two models of cardiac arrest. In comparison,
237 mitochondrial dysfunction was rather mild during the first 2 hours following cardiac arrest, with
238 different time courses between organs. To date, the implication of mitochondria in post-cardiac
239 arrest physiopathology has often been extrapolated from results obtained in selective and
240 longer period of ischaemia than cardiac arrest, including stroke or myocardial infarction (15,
241 16). In those conditions, mitochondrial dysfunction is expected to be very severe and early
242 after reperfusion. In the context of prolonged cardiac arrest (i.e. > 15 min of no-flow), it has
243 also been shown that mitochondrial dysfunction is immediately very severe and stable over
244 time (17, 18). To our knowledge, our study is the first to demonstrate an early phase with
245 relatively mild mitochondrial dysfunction followed by a late phase (after 24 h) with dramatic
246 impairment of mitochondrial dysfunction with different profiles between brain and myocardium.

247 Regarding the early phase of dysfunction in the heart, we observed an absence of
248 mitochondrial dysfunction at the end of the no-flow period whereas early reperfusion was
249 associated with impairment of the mitochondrial respiration and calcium retention capacity.
250 This underlines the effect of reperfusion in the promotion of mitochondrial dysfunction, which
251 is consistent with the literature on regional myocardial ischaemia showing that reactive oxygen
252 species produced at reperfusion are responsible for mPTP opening (8, 19). This has also been
253 demonstrated in a mouse model of cardiac arrest with a progressive alteration of myocardial
254 electronic flow between 30 and 60 min of reperfusion but not during ischaemia (20). This is
255 thought to be due to the specific sensitivity of complex I and IV of the mitochondrial respiratory
256 chain, whereas other complex remain quite insensitive to ischaemia-reperfusion during post-
257 cardiac arrest syndrome (20). However, a recent study investigated the mitochondrial function
258 in post-cardiac arrest patients and demonstrated that succinate-driven respiration is actually
259 more impaired than glutamate/malate-driven respiration in skeletal muscle after cardiac arrest

260 (21). Yet, in our model, we did not specifically assess the complex II activity. Different
261 subpopulation of cardiac mitochondria could also be associated with differential response to
262 myocardial ischemic-reperfusion injury. It is indeed well demonstrated that mitochondrial
263 mechanical isolation is associated with over representation of subsarcolemmal as compared
264 to interfibrillar mitochondrial population (22). The modest cardiac dysfunction that we observed
265 here at the early time-points after cardiac arrest could then be related to this phenomenon, as
266 interfibrillar mitochondria are thought to be responsible for providing most of the energy of the
267 contractile apparatus.

268 Regarding the time course of dysfunction in the brain, mitochondrial function appeared
269 to be strongly impaired during no-flow period with a transient reversion after CPR and then a
270 delayed deterioration the day after cardiac arrest. In the literature, data obtained after 10 min
271 of VF in rats demonstrates that 5 min after ROSC are sufficient to partially restore the
272 mitochondrial function as compared to the one measured during ischaemia (23). Similarly,
273 after 10 min of VF in dogs, a study demonstrated that the decrease in mitochondrial respiratory
274 control and calcium retention capacity were reversed within 100 min of reperfusion (24). To
275 our knowledge, there is only one study showing that mitochondrial dysfunction is severe and
276 stable after ROSC (25). However, in this study, the authors used a very long duration of cardiac
277 arrest (15 min) and no vasopressor were administered during follow-up (25). In another study
278 in rats, it was demonstrated that cardiac arrest was associated with sustained mitochondrial
279 dysfunction up to 1 h after cardiac arrest (26). In this study, the authors used a prolonged no-
280 flow period of 8 min of CPR. Both study then used a more severe model of cardiac arrest than
281 the one used in our study. Species difference could also explain some discrepancies since
282 rabbits have a slower heart rate than rodents. The relatively modest mitochondrial dysfunction
283 that we evidenced in the brain as compared to the heart at the early stage after cardiac arrest
284 could also be related to a mechanism of energetic preservation in the brain. For example, the
285 so-called “Astrocyte-Neuron Lactate Shuttle” could protect the brain by neurons fuelling with
286 lactates by astrocytes in ischemic conditions (27). Additionally, the main source of energy in
287 the brain is glucose whereas heart is mostly using fatty acid as a primary metabolic substrate.

288 This could explain the differences between organs in our study. However, literature on brain
289 mitochondrial dysfunction after cardiac arrest is still sparse and sometimes contradictory,
290 which could be explained by different experimental conditions.

291 Importantly, we also observed that the early cerebral mitochondrial dysfunction (from
292 no-flow to 120 min after CPR) was more severe after VF than non-shockable cardiac arrest,
293 despite the fact that the ultimate neurological function was worse after non-shockable cardiac
294 arrest. Importantly, the latter observation is consistent with clinical data showing that non-
295 shockable cardiac arrest is usually associated with more severe neurological dysfunction than
296 shockable cardiac arrest (2). Additionally, we demonstrated that mitochondrial dysfunction
297 during the early phase is not predictive of the mitochondrial dysfunction observed after 24h.
298 Altogether, those results suggest that early brain mitochondrial dysfunction is not correlated to
299 the severity of the ultimate neurological injury and that delayed mitochondrial dysfunction is
300 more probably secondary to other pathophysiological events such as brain oedema or post-
301 cardiac arrest myocardial dysfunction rather than direct ischemic injury during the initial no-
302 flow. This also suggests that ideal mitochondrial protective strategies should not be
303 administered immediately after ROSC but rather be adapted to the windows of susceptibility
304 of mitochondria. However, the very large majority of clinical or experimental strategy to protect
305 mitochondria target the firsts minutes of mitochondrial dysfunction. As an illustration,
306 administration of inhibitor of the succinate dehydrogenase during CPR in rats, was not
307 associated with any improvement of the survival rate after 3 days (28). Another example is the
308 recent CYRUS clinical trial, which also failed to demonstrate any benefit following the selective
309 inhibition of mPTP formation by cyclosporine when administered during CPR to prevent
310 reperfusion-induced injury in cardiac arrest patients (10).

311

312 **Conclusion**

313 Differences between early and late mitochondrial dysfunction after VF or non-
314 shockable cardiac arrest suggest that mitochondrial function might not play a unique role in
315 the pathophysiology of the post-cardiac arrest syndrome but could rather be a secondary
316 pathophysiological event.

317

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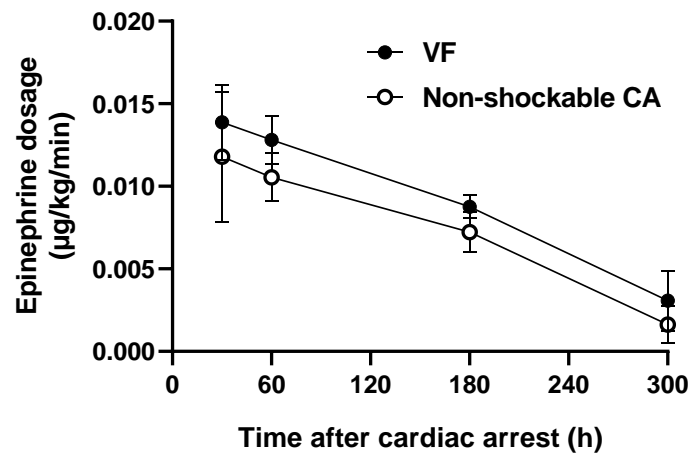
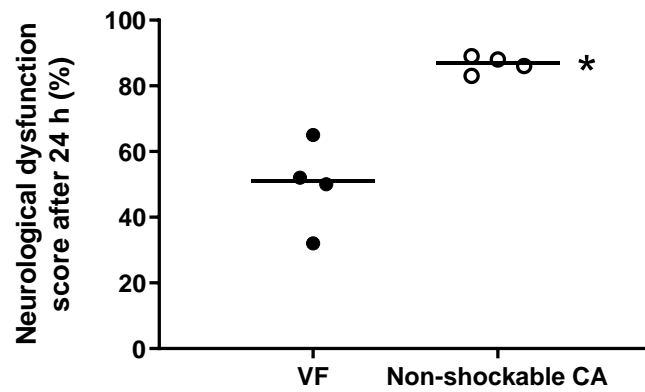
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420 **Legend to figures**

421 **Figure 1: Dose of epinephrine administered after resuscitation and neurological**
422 **dysfunction scores in rabbits followed during 24 h shockable (VF, n=4) or non-**
423 **shockable cardiac arrest (CA, n=4).**

424 **; p<0.05 between groups*



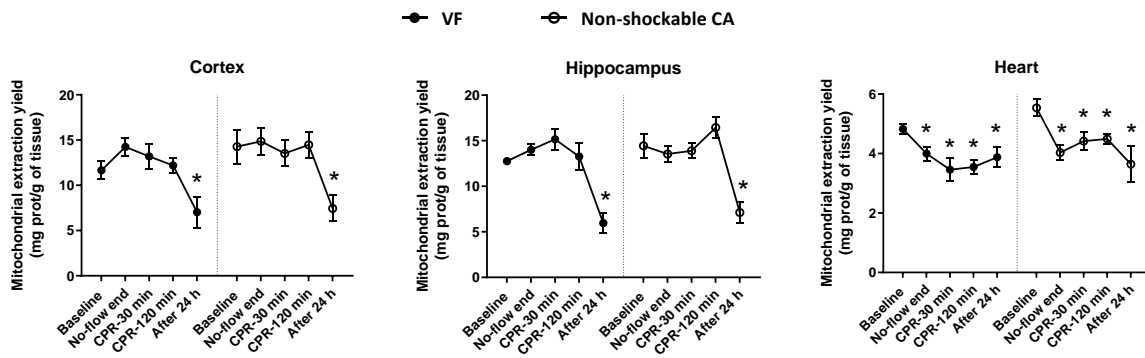
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427 **Figure 2: Mitochondrial extraction ratio assessed by mitochondrial protein dosage in**
 428 **hippocampus, brain cortex or heart samples of rabbits submitted to ventricular**
 429 **fibrillation (VF) or non-shockable cardiac arrest (CA) followed by cardiopulmonary**
 430 **resuscitation (CPR).**

431 *Values are expressed as mean±SEM; *, p<0.05 vs corresponding baseline value*

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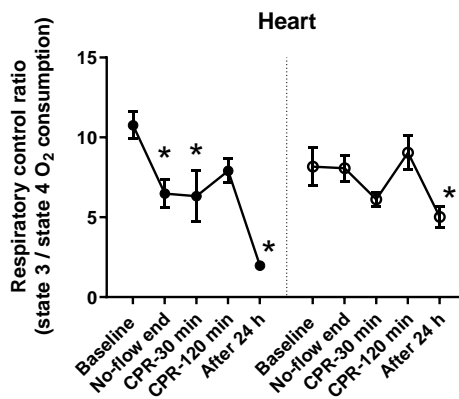
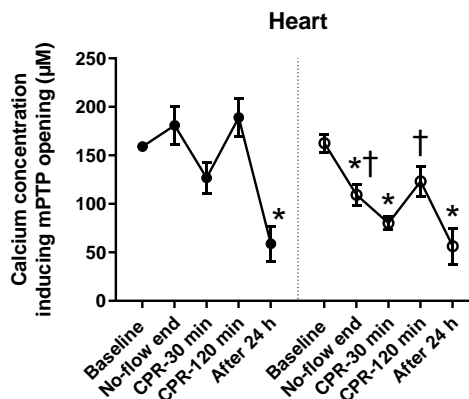
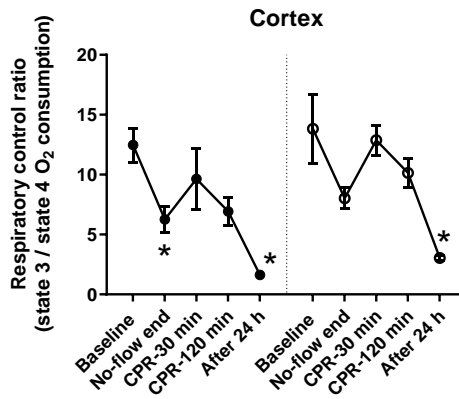
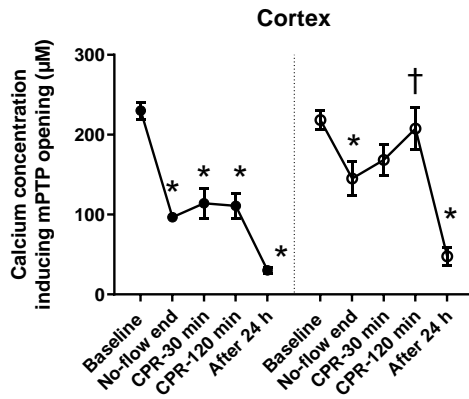
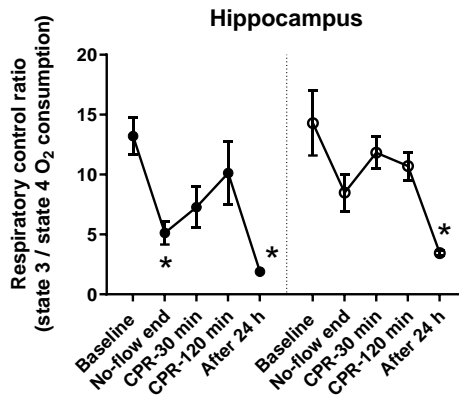
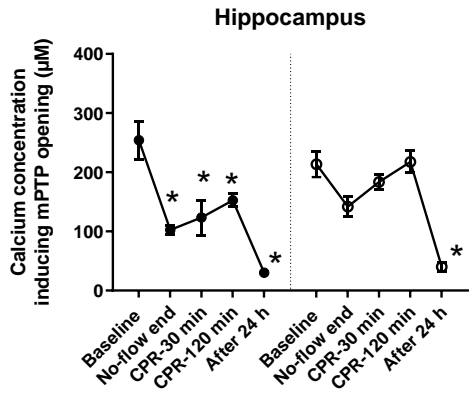
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434 **Figure 3: Calcium concentration leading to the formation of the mitochondrial**
435 **permeability transition pore (mPTP, left panel) and respiratory control ratio (right panel)**
436 **of mitochondria isolated in the hippocampus, brain cortex or heart of rabbits submitted**
437 **to ventricular fibrillation (VF) or non-shockable cardiac arrest (CA) and subsequent**
438 **resuscitation. Mitochondria were isolated from tissues withdrawn in either baseline**
439 **conditions or at the end of the no-flow period or after 30 min, 120 min or 24 h following**
440 **cardiopulmonary resuscitation (CPR). Control ratio has been calculated as the ratio**
441 **between state 3 and state 4 oxygen consumptions, i.e., ADP- and substrate-dependent**
442 **respiration rates, respectively.**

443 *Values are expressed as mean±SEM; *, p<0.05 vs corresponding baseline value; †, p<0.05*
444 *vs corresponding VF value; mPTP, mitochondrial permeability transition pore.*

● VF

○ Non-shockable CA



446 **Figure 4: Respiratory activity in mitochondria isolated from the hippocampus, brain**
447 **cortex or heart of rabbits submitted to ventricular fibrillation (VF) or non-shockable**
448 **cardiac (CA) with subsequent resuscitation. Mitochondria were isolated from tissues**
449 **withdrawn in either baseline conditions or at the end-of the no-flow period or after 30**
450 **min, 120 min or 24 h following cardiopulmonary resuscitation (CPR). States 3, 4 and 3'**
451 **oxygen consumptions represent ADP-, substrate-dependent (pyruvate/malate) and**
452 **uncoupled respiration rates, respectively.**

453 *Values are expressed as mean±SEM; *, p<0.05 vs corresponding baseline value; †, p<0.05*
454 *vs corresponding VF value.*

455

● VF ○ Non-shockable CA

