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Experimental paper

Dose dependent neuroprotection of the noble gas argon after cardiac arrest in rats is not mediated by K_{ATP}—Channel opening☆

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ABSTRACT

Purpose: Argon at a dosage of 70% is neuroprotective when given 1 h after cardiac arrest (CA) in rats. In a rodent model, we investigated if the neuroprotective effects of argon are dose dependent and mediated by adenosine triphosphate dependent potassium (K_{ATP}) channels.
Methods: Forty-seven male Sprague-Dawley rats were subjected to 7 min of CA and 3 min of cardiopulmonary resuscitation (CPR). In protocol I animals were randomized to receive either 70% or 40% argon ventilation 1 h after successful CPR or no argon-treatment. Animals of the second protocol also received 1 h of 70% argon ventilation or no argon treatment but were randomized to a group receiving the K_{ATP} channel blocker 5-hydroxydecanoate (5-HD). For all animals a neurological deficit score (NDS) was calculated daily for seven days following the experiment before the animals were killed and the brains harvested for histopathological analyses.
Results: All animals survived. Control animals exhibited severe neurologic dysfunction at all points in time as measured with the NDS. Argon treated animals showed significant improvements in the NDS through all postoperative days in a dose dependent fashion. This was paralleled by a significant reduction in the neuronal damage index in the neocortex and the hippocampal CA 3/4 region. Administration of 5-HD neither abolished the positive effects on functional recovery nor on histopathologic changes observed in the argon group.
Conclusion: Our study demonstrates a dose dependent neuroprotective effect of argon administration in this rodent model, which is not mediated via ATP dependent potassium channels.

1. Introduction

Noble gas mediated neuroprotection has gained considerable attention in the last decade. Especially xenon has been extensively studied in various animal models of neurological injury including stroke, hypoxic-ischaemic encephalopathy and cardiac arrest.[1–7] Given the negligible side effects of the gas, which are well characterized from its use as an anaesthetic,[8] these remarkable results have translated in phase II clinical trials currently exploring the effects of xenon as an adjunct to mild therapeutic hypothermia (MTH) in neonates suffering from hypoxic ischaemic encephalopathy and in adults resuscitated from cardiac arrest. Pending the results of these investigations it has to be noted, that xenon delivery is cumbersome in the intensive care unit because specialized ventilators are needed to economically administer the gas. This is due to the rarity of the gas, which makes it costly and might preclude a widespread clinical use.

In contrast, argon is much more abundant in the atmosphere and therefore available at a significantly lower price. Interestingly, accumulating data from preclinical studies provide evidence that argon albeit the lack of an anaesthetic effect has also organ-protective properties.[9–12] Our own group has previously shown that rats exposed to a single one-hour administration of 70% argon demonstrated significant and persistent reductions in cardiac arrest induced neurological dysfunction. This was accompanied by a concomitant decrease in the number of damaged neurons in hippocampal and cortical regions of the brain.[13] Only few *in vitro* studies suggest argon to provide its neuroprotective properties in a dose dependent manner.[10,14] However, there is even less

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knowledge about argons most effective concentration *in vivo*.[15] Furthermore, the mechanisms involved in argon-induced neuroprotection are in contrast to xenon less well understood. Xenon, in part acts by opening adenosine triphosphate dependent potassium (K_{ATP}) channels.[16,17] The present study was therefore designed to address these issues in a rodent model of cardiac arrest induced neurological damage. We hypothesized that the beneficial effects of argon are dose dependent and mediated by K_{ATP} channels.

2. Methods

A total of 47 male Sprague-Dawley rats (Charles River, Germany) weighing between 400 and 500 g were investigated in two different protocols. In the first protocol we tested the neuroprotective effects of two different concentrations of argon (40% vs 70%). Argon gas was administered using prespecified gas cylinders containing the desired concentration (Linde Gas Therapeutics, Unterschleißheim, Germany). In the second protocol 5-hydroxydecanoate (5-HD; Product Number: H135, Sigma–Aldrich Chemie GmbH, Steinheim, Germany), a specific blocker of the mitochondrial K_{ATP} channel, was used to investigate whether K_{ATP} channel opening is involved in argon mediated neuroprotection. All animals were treated according to the following protocol unless otherwise mentioned.

Animals were housed in adequately spaced cages (60 cm × 40 cm; type 2000; Tecniplast; Buguggiate; Italy) with a 12-h light-dark cycle. Animals had free access to water and food prior to the study. The study protocol was approved by the appropriate institution (Landesamt für Natur, Umwelt und Verbraucherschutz Nordrhein-Westfalen; Recklinghausen; Germany). The experimental procedures were performed according to the Guide for the Care and Use of Laboratory Animals formulated by the National Research Council (National Academies Press, 1996) and the ARRIVE guidelines (National Centre for the Replacement, Refinement and Reduction of animals in research, 2010). In addition, all reported data and outcomes are in accordance with the "Utstein style guidelines for uniform reporting of laboratory CPR research.[18]

2.1. Animal preparation

We used a modified model of CA and CPR as previously described.[13] On the experimental day, rats were anaesthetized with an intraperitoneal injection of pentobarbital (45 mg kg^{-1}). Additional doses (10 mg kg^{-1}) of pentobarbital were administered if signs of animal discomfort were noted; i.e. sudden rise in heart rate or respiratory rate or movements of the tail or paws. The animals' chest and back were thoroughly shaven to allow for direct contact of the paddles used for defibrillation during CPR immediately after induction of anaesthesia.

After placing on a surgical board in the supine position the trachea was orally intubated using a modified 14G cannula (Abbocath-T, Abbott Hospital Division, North Chicago, IL, USA) as previously described.[19] Animals were mechanically ventilated (Servo Ventilator 900C, Siemens ELEMA, München, Deutschland) with an FiO_2 of 0.21. Respiratory frequency was adjusted to maintain end-tidal PCO_2 between 35 and 40 mmHg, which was continuously monitored using an infrared CO_2 analyzer (Cap Star 100, CWE Inc., Ardmore, PA, USA). A 3 lead electrocardiogram was continuously measured by monopolar needle electrodes (MLA1204 Needle Electrodes, ADinstruments, Oxford, UK). The right jugular vein and right femoral artery were surgically exposed and cannulated with polyethylene catheters (PE 50) and connected to high sensitivity transducers (Capto SP 844 Physiologic Pressure Transducer, Capto Inc., Skoppum, Norway) for the measurement of right atrial and mean arterial pressures (MAP), respectively. A thermocouple microprobe (IT-18, Physitemp Instruments, Clifton, NJ, USA) was placed into the abdominal aorta via the left femoral artery. Cardiac output (CO) was measured with the transpulmonary thermodilution technique using this microprobe. Blood temperature was monitored and maintained between 37 and 37.5 °C with the aid of a heating lamp. The left femoral vein was also cannulated with an additional PE 50 catheter to allow for administration of fluids and epinephrine during CPR. All catheters were flushed intermittently with saline solution containing 2 IU mL^{-1} of heparin.

2.2. Experimental procedure

Ventricular fibrillation (VF) was induced by transoesophageal electrical stimulation. After placing the electrode using fluoroscopy alternating current (10 V, 50 Hz) was delivered to the heart using a commercially available fibrillator (Fi 20 M, Stockert GmbH, Freiburg, Germany). CA was confirmed by an abrupt decrease in MAP to less than 20 mmHg. Simultaneously, ventilation was stopped. After 7 min of untreated CA, CPR was initiated including mechanical ventilation with an FiO_2 of 1.0 at a respiratory rate of 50 min^{-1} and chest compressions delivered by a custom made mechanical thumper at a stroke rate of 200 min^{-1}. An intravenous bolus of 0.02 mg kg^{-1} epinephrine was administered via the femoral access 30 s after starting chest compressions. After 3 min of CPR external defibrillation with 5 J (Zoll MSeries, Zoll Medical Corporation, Chelmsford, MA, USA) was attempted up to three times. If restoration of spontaneous circulation (ROSC) was not achieved administration of epinephrine at the same dosage and chest compressions were repeated for 1 min before additional direct current counter shocks (again up to three times) were delivered. ROSC was confirmed by spontaneous cardiac rhythm in conjunction with a rise in mean arterial pressure to greater than 50 mmHg. One hour after successful resuscitation, FiO_2 was reduced to 0.3 and the animals were randomly assigned into groups using the sealed envelope method. Animals in the first protocol received either 1 h of 70% argon in 30% oxygen ($n = 9$) or 40% argon in 30% oxygen and 30% nitrogen ($n = 9$) or no treatment ($n = 9$). In the second protocol a bolus of 9 mg kg^{-1} 5-HD ($n = 6$) or saline placebo ($n = 7$) was administered intravenously 50 min after ROSC, i.e. 10 min before argon ventilation was started at a concentration of 70%. Animals ventilated with 70% nitrogen and 30% oxygen served as controls ($n = 7$). In both protocols, animals were ventilated for a total of 5 h following ROSC. At the end of the experiment all animals received a single subcutaneous injection of 0.1 mg kg^{-1} buprenorphine for pain relief and were weaned from the ventilator. Following extubation animals were observed for approximately 30 min to ensure adequate spontaneous breathing before being returned to their cages.

2.3. Measurements

Ischaemia time was calculated as the sum of the duration of VF, CPR and the time needed to achieve ROSC. Heart rate, MAP, end-tidal CO_2 and blood temperature were continuously recorded on a multichannel recorder (Power Lab, AD Instruments, Spechbach, Germany). CO was calculated by bolus injections of 200 μL of cold saline (4 °C) into the right atrium. Two consecutive measurements were performed and the results averaged (Cardiac Output Pod, AD Instruments, Spechbach, Germany).

Arterial blood samples were drawn at baseline, 30 min and 4 h after ROSC. Arterial oxygen (PaO_2) and carbon dioxide ($PaCO_2$) partial pressures as well as glucose and lactate levels were measured using a conventional blood gas analyzer (ABL700, Radiometer Copenhagen, Denmark).