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Systemic physiology and neuroapoptotic profiles in young and adult rats exposed to surgery: A randomized controlled study comprising four different anaesthetic techniques

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ABSTRACT

Background: Experimental evidence indicates that general anaesthetics can induce apoptotic neurodegeneration in the developing brain. The majority of these studies have been performed in the absence of surgery and it currently remains unclear how the presence of surgical stimuli would influence neuroapoptosis as well as systemic homeostasis. Here we explored this possibility by performing dorsal skin flap surgery in young and adult rats under four distinct currently used anaesthesia regimens.
Methods: Young (21-days) and adult (2 months) male Sprague–Dawley rats were randomized to 150 min exposure to one of four anaesthetics regimens: (i) sevoflurane/dexmedetomidine, (ii) sevoflurane/fentanyl; (iii) propofol/dexmedetomidine, and (iv) propofol/fentanyl. Animals underwent a dorsal skin flap procedure while physiologic, metabolic and biochemical parameters were closely monitored. Neuroapoptotic profiles were evaluated in the cortex, thalamus and hippocampus (CA1 and CA3) at the end of the procedure in each experimental group.
Results: Significant perturbations of systemic homeostasis were found under all anaesthetic regimens. Hyperglycemia and decreased heart rate were particularly relevant in experimental groups receiving dexmedetomidine, while propofol administration was associated with increased systemic lactate levels and metabolic acidosis. A substantial difference in anaesthesia/surgery-induced neuroapoptosis was found between young and adult rats in several brain regions. Combination of sevoflurane and dexmedetomidine resulted in the highest number of caspase-3 positive cells, although the extent of cell death remained relatively low in all experimental groups.
Conclusion: Combination of anaesthesia and surgery induces significant perturbations of physiological parameters in both young and adult spontaneously breathing rats undergoing surgery. These observations further enlighten the need for detailed physiological monitoring under these experimental conditions. Although some statistically significant differences in activated caspase-3 profiles were detected between experimental groups, the overall extent of neuronal cell death remained very low under all conditions questioning, thereby, the physiological significance of apoptotic neurodegeneration in the context of anaesthesia and surgery.

1. Introduction

A multitude of studies performed during the past two decades have demonstrated that virtually all anaesthetic agents, when administered in high doses, can induce increased neuroapoptosis in neonatal laboratory animals with subsequent learning deficits (Vutskits et al., 2012). Initially these studies comprised mainly

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rodents but recently they have been confirmed in non-human primates (Brambrink et al., 2012; Rizzi et al., 2008; Slikker et al., 2007). Whether this has human implications is currently unknown (Hansen et al., 2011; Wilder et al., 2009).

Recurrent potential criticisms regarding these experimental studies concern their translational relevance to human paediatric anaesthesia practice in terms of drug doses, duration of exposure, the non-use of multi-parameter monitoring and airway management as well as lack of surgical procedures. The morpho-functional correlation in terms of developmental stage between experimental animals and humans infants is also highly debated (Clancy et al., 2001). Last but not least, very few animal studies have reported on detailed physiological, metabolic and biochemical parameters. This latter issue may be important, because specific anaesthesia- and surgery-associated factors, including hypotension, acidosis, hypoxia/hyperoxia, hypocarbia/hypercarbia and hypoglycemia/hyperglycemia, may by themselves independently contribute to neurocognitive outcome (Anand and Soriano, 2004; Todd, 2004; Vutskits et al., 2012).

The primary aim of this randomized experimental trial was to investigate how four different anaesthetic techniques used for a well-defined surgical procedure (dorsal skin flap) and the associated perioperative physiologic, metabolic and biochemical changes influenced neuroapoptosis in young (21-day-old) and adult (2-month-old) rats. Since recent experimental data suggest that the α2-receptor agonist dexmedetomidine may be less neurotoxic than other general anaesthetics or even may possess neuroprotective properties and thus minimize the detrimental effects caused by other anaesthetics (Ma et al., 2004; Panzer et al., 2009; Sanders et al., 2008, 2009), the secondary aim was to explore any potential protective effects of dexmedetomidine.

2. Material and methods

2.1. Animals and housing

21-day-old male Sprague–Dawley rats (young) (Taconic, Ejby, Denmark) weighing 96 ± 8 g ($n = 27$) and two-month-old male Sprague–Dawley rats (adult) (Taconic, Ejby, Denmark) weighing 402 ± 32 g ($n = 30$) were used in this study. Animals were maintained on a normal 12 h day/night cycle at 21 °C with a relative humidity of 45–55%. The rats were fed with standard diet and water ad libitum.

This study was approved from and carried out according to the recommendations of the Animal Experiments Inspectorate (2012-15-2934-00129). The internationally accepted principles in the care and the use of experimental animals were followed.

2.2. Randomization

Rats were block-randomized using computer software (GraphPad Software, Inc., California, USA) into 4 groups for both young (suffix "y") and adult rats (suffix "a"): receiving sevoflurane/dexmedetomidine (SD), sevoflurane/fentanyl (SF), propofol/dexmedetomidine (PD), or propofol/fentanyl (PF) (Fig. 1). A naïve young control group ($n = 3$) was also included. Rats that did not complete the full procedure or had incomplete dataset were replaced in order to ensure at least six rats per group. Investigators were blinded as to whether the rats received fentanyl or dexmedetomidine.

2.3. Anaesthetic procedure

Initially, all rats received 4% of sevoflurane in a chamber until properly anaesthetized and then changed to inhalation via a facemask on a heating pad. Rats randomized to propofol were kept anaesthetized with sevoflurane until an intravenous (IV) tail catheter was placed. An IV bolus of propofol (4 mg/kg) was administered and a continuous infusion (40 mg/kg/h) started. A 50/50% mixture of air and oxygen with a fresh gas flow of 1.2 l/min was used in all rats during the whole procedure. A subcutaneous (SC) bolus dose of normal saline (10 ml/kg) was administered indicating T0. After initial measurements of monitored parameters at T0 either intraperitoneal (ip) dexmedetomidine (3 μg/kg) or fentanyl (4 μg/kg) were administered. The anaesthetic duration was set to a total of 150 min in all animals to ensure enough time for preparation, monitoring, insertion of lines, surgery, etc.

The depth of anaesthesia was assessed by observation of voluntary movements, toe and paw withdrawal reflexes and corneal reflexes. Adequate depth of anaesthesia was defined as no voluntary movements and lack of reflexes to surgical stimuli.

2.4. Physiologic, metabolic and biochemical monitoring

Following anaesthesia induction, a pulse oximeter [Model 8500 V] (Nonin Medical Inc Plymouth, Minnesota, United States) was attached to the hind limb, and a rectal thermometer [Model BAT-12] (Physitemp Instruments, Inc., Clifton, New Jersey, United States) inserted. Peripheral oxygen saturation (SpO_2), heart rate (HR) and temperature (tp) were monitored continuously and recorded every 15 min during surgery.

A 0.4 mm femoral catheter (Gradko International, Winchester, England) was placed with the tip in the abdominal aorta in order to monitor invasive mean arterial blood pressure (MAP) and for arterial blood gas samples. Arterial blood gas samples were collected after the catheter was inserted (T60), after surgery (T105) and just prior to euthanization (T150) and analysed for: pH, pCO_2, pO_2, HCO_3^-, base excess (BE), lactate, Na^+, K^+, Ca^{2+}, and glucose. [Gem Premier 3000] (Instrumentation Laboratory SpA, Milan, Italy).

2.5. Surgery

All rats underwent the same well-defined surgical procedure at T80 comprising a 2 × 7 cm modified distally based rectangular McFarlane flap on the dorsum (McFarlane et al., 1965). The flap extended 7 cm cranially from the point where the paired gluteal muscles met and was elevated including the panniculus carnosus.

2.6. Brain harvesting

At the end of anaesthesia/surgery (T150), rats were perfused through the left ventricle with 200 mL of 4% paraformaldehyde (PFA) in 0.1 M buffer. Brains were harvested and stored for two days at 4 °C in PFA, after which they were transferred to Sorensen buffer (Phosphate buffer, stock solution, 0.01 M, pH 7.4) and kept at 4 °C. Brains were then cut in 2 mm coronal slices in a rat brain matrix (PlaticsOne Virginia, USA), followed by paraffin embedment.

2.7. Immunohistochemistry

Paraffin sections were cut at a level −3,6 mm from the Bregma followed by immuno-histochemical staining using a Dako Autostainer Universal Staining System (Dako, Glostrup, Denmark). The sections were deparaffinized and endogenous peroxidase activity was quenched by immersion in 1.5% hydrogen peroxide followed by heat-induced epitope retrieval in Tris ethylene glycol tetra-acetic acid (TEG) buffer solution (10 mmol/L Trisbase and 0.5 mmol/L EGTA, pH 9). The sections were subsequently incubated for 60 min with a cleaved caspase-3 monoclonal antibody (ASP175, Cell Signal, 1:400). The detection of the antigen–antibody complex was performed using a anti-rabbit EnVision kit, followed by visualization with diaminobenzidine (DAB) as chromogen. Finally, the