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EXPERIMENTAL NEUROSCIENCES

Paper No: 16.00

Volatile Anesthetic Preconditioning Present in the Invertebrate Caenorhabditis elegans

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Introduction: Volatile anesthetics (VAs) have been found to induce a delayed protective response called preconditioning to subsequent hypoxic/ischemic injury. VA preconditioning has been primarily studied in canine and rodent heart.
Objective: A more genetically tractable model of VA preconditioning would be extremely useful. Here, the authors report the development of the nematode Caenorhabditis elegans as a model of VA preconditioning.
Methods: Wild-type and mutant C. elegans were exposed to isoflurane, halothane, or air under otherwise identical conditions. After varying recovery periods, the animals were challenged with hypoxic, azide, or hyperthermic incubations. After recovery from these incubations, mortality was scored.
Results: Isoflurane- and halothane-preconditioned animal shad significantly reduced mortality to all three types of injuries compared with air controls. Concentrations as low as 1 vol% isoflurane (0.64 mM) and halothane (0.71 mM) induced significant protection. The onset and duration of protection after anesthetic were 6 and 9 h, respectively. A mutation that blocks inhibition of neurotransmitter release by isoflurane did not attenuate the preconditioning effect. A loss-of-function mutation of the Apaf-1 homolog CED-4 blocked the preconditioning effect of isoflurane, but mutation of the downstream caspaseCED-3 did not.
Conclusions: Volatile anesthetic preconditioning extends beyond the vertebrate subphylum. This markedly broadens the scope of VA preconditioning and suggests that its mechanisms are widespread across species and is a fundamental and evolutionarily conserved cellular response. C. elegans offers a means to dissect genetically the mechanism for VA preconditioning as illustrated by the novel finding of the requirement for the Apaf-1 homolog CED-4.

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Paper No: 251.00

Ketamine induces apoptosis in human neurons differentiated from embryonic stem cells via reactive oxygen species

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Introduction: Ketamine has been shown to cause neurotoxicity in developing animal models (1-2), leading to a serious concern regarding the safety of pediatric anesthesia. Some epidemiological studies suggest that anesthesia administration early in life is associated with learning outcomes later in life (3-4), while others found no significant differences (5-6). Thus, it is imperative to find a good model to study anesthetic-induced developmental toxicity in human neurons.
Objectives: We investigated toxic effect of ketamine on neurons differentiated from human embryonic stem cells (hESCs).
Methods: Differentiated neurons were identified by the expression of neuron-specific markers using immunofluorescence staining. Two-week-old neurons were then treated with different doses and durations of ketamine with or without reactive oxygen species (ROS) inhibitor Trolox. Cell

viability, apoptosis, and ROS production were evaluated by MTT assay, activate caspase-3 analysis, and CM-H2DCFDA staining, respectively.

Results: Differentiated neurons expressed neuron-specific markers. Three millimolar ketamine time-dependently decreased cell viability after 6, 12, and 24 hr incubation. In addition, higher dosage of ketamine resulted in more cell death and ROS production as well as stronger caspase-3 activity. Furthermore, Trolox significantly prevented ketamine-induced cell death and decreased caspase-3 activity and ROS formation in a dose-dependent manner.

Conclusions: This study illustrates for the first time that 1) Ketamine induces a time- and concentration-dependent induction of apoptosis in human neurons via ROS-mediated pathway; 2) Importantly, ketamine-induced neurotoxicity can be attenuated by Trolox; and 3) Neurons differentiated from hESCs might be a promising in vitro model for studying anesthetic-induced neurotoxicity and its underlying mechanisms.

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Paper No: 285.00

Isofluorane postconditioning in stroke: a possibility for patients?

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Strategies to limit lethal reperfusion and ischemic injury in patients with ischemic stroke are of major clinical interest. Halogenated anesthetics, have demonstrated a positive effect for treatment by preconditioning in brain ischemia and cardiac surgery. As a new idea, postconditioning in brain ischemia may be very attractive for clinical application, especially during re perfusion after stroke. In vitro studies demonstrated that isofluorane was strongly neuroprotective after oxygen-glucose-deprivation (OGD). We evaluated in vivo in a mouse stroke model the impact of isofluorane on infarct size and neurological outcome. Outbred CD1 mice were subjected to 30 min middle cerebral artery occlusion (MCAo). One group of mice (postconditioning group) received isofluorane at 1.0, 1.5 and 2.0 minimum alveolar concentrations (MAC), administered over 30 minutes in the re perfusion period immediately after occlusion. A second group (no postconditioning group) of mice did not receive isofluorane and were woken in the first ten minutes after re-perfusion. The infarct volume and neurological deficit scores were evaluated at 48 hours. Lesion size at 48 h was significantly reduced in the postconditioning groups, from 21.73 ± 3.59 mm3 ($n=6$, no postconditioning group) to 11.15 ± 1.02 mm3 in the group treated with 2.0 MAC ($n = 6$) ($p = 0.01$), 11.52 ± 0.67 mm3 in the group treated with 1.0 MAC ($n = 6$) ($p = 0.03$) and 11.88 ± 1.46 mm3 in the group treated with 1.5 MAC ($n = 6$) ($p = 0.044$). Neurological scores were better in the 1.0 MAC group compared to the no postconditioning group ($p = 0.04$). Together our results show that postconditioning with isofluorane (1.0 MAC, 1.5 MAC and 2.0 MAC) not only reduces infarct size but also results in a better neurological performance. This is an important result because it is the first time that protection by postconditioning using isofluorane is demonstrated in vivo. This will be relevant for clinical application in that isofluorane is widely available and commonly used in clinical practice with good tolerance and therefore clinical trials may be easily developed leading to improved patient treatment.

Paper No: 951.00

Effect of propofol on acetylcholine release evoked by veratridine in rat hippocampal synaptosomes

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Introduction: General anesthetics are widely used to induce general anesthesia but their molecular mechanisms of action remain obscure. Acetylcholine (ACh) is a common distributed excitatory neurotransmitter in mammals' brain, that is involved in the regulation of consciousness, awakening, cognitive, memory and sleep (Perry, 1999). Propofol is an intravenous anesthetic commonly used for induction and maintenance of anesthesia and for sedation in intensive care. This agent is capable to modify synaptic transmission by altering the release of neurotransmitters in the presynaptic region and also modulating the response in the region postsynaptic (Hui Zhang et al, 2009). In the present study we investigated the effect of propofol on the ACh release

induced by veratridine, a voltage-dependent Na+ channel opening agent, in rat hippocampal synaptosomes.
Objectives: Evaluate the effect of propofol on the release of ACh induced by veratridine in rat hippocampal synaptosomes.
Methods: Synaptosomes from Wistar rat cerebral hippocampus were prepared by sucrose gradient method as described previously by Westphalen and Hemmings (2003). The final pellet was incubated with [methyl-3H] choline chloride for 15 min at 37°C. Prelabeled synaptosomes were confined between Whatman GF/B filter discs and superfused using an apparatus set to collect 2-min fractions. The results were obtained and analyzed in DPM (decays per minute) of [3H]-ACh release per fraction and represented the average of samples done in triplicate, repeated at least five times on different days. The results were analyzed by ANOVA followed by Newman-Keuls. $p<0.05$ was considered statistically different.
Results: Veratridine (10, 50 and 100 ?M) increased the [3H]-ACh release in rat hippocampal synaptosomes. The release of ACh induced by 50 and 100 ?M of veratridine was similar ($p>0.05$) and the release of ACh induced by veratridine 50 ?M was higher than the 10 ?M ($p<0.05$). Propofol (1, 3, 10, 30, 100 and 300 ?M) decreased the release of ACh evoked by 50 ?M veratridine.
Discussion and Conclusion: The results demonstrated that veratridine evoked ACh release in rat hippocampal synaptosomes. Propofol, at clinical and supraclinical concentrations, reduced veratridine-evoked ACh release in rat hippocampal synaptosomes in a dose dependent manner.

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Paper No: 992.00

Influence of ageing and male gonads in pain perception in rats

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Introduction: Pain is complex experience influenced by many factors. Among these factors, there are ageing process and gonadal hormones. The use of animal pain models allows the isolation of variables, facilitating the comprehension of the roles each factor has on pain.
Objectives: Two experimental pain models were used, pain induced by formalin paw injection and plantar incision, to study the influence of ageing and male gonads on pain perception.
Methods: Adult rats (6 months), aged rats (22 months), young non orchiectomized and orchiectomized rats were evaluated for the number of flinches after paw formalin injection and mechanical withdrawal threshold after plantar incision.
Results: The results showed aged rats presented decreased number of flinches than adult rats during phase II of formalin induced behavior at 25, 30 and 35 minutes time points. Aged rats presented lower paw withdrawal threshold after mechanical stimulus in the plantar incision model, at 2nd, 3rd, 4th, 7th and 10th postoperative days, the return to baseline levels occurred after the 18th day in aged rats and after the 10th day in adult rats. Orchiectomized rats presented decreased number of flinches compared to non orchiectomized rats in the initial section of phase I, 5 minutes time point, and in phase II, at 25, 30, 35, 40, 45, 50, 55 and 60 minutes time points. In orchiectomized rats, paw withdrawal threshold was lower when compared to non orchiectomized rats on the 1st, 2nd, 4th and 7th postoperative days. Return to baseline values occurred after the 18th day in orchiectomized rats and after the 14th day in non orchiectomized rats.
Conclusions: Data showed that pain perception changes with the ageing process according to the type of stimulus and that male gonads present analgesic effect on both pain models evaluated.

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Sildenafil and morphine coadministration: analgesia enhancement and antiedematogenic effect

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Introduction: Drug combinations have been used to treat pain aiming to have satisfactory analgesia with few side-effects. Laboratory studies have shown that phosphodiesterase-5 inhibitors, like sildenafil, when combined with morphine are able to enhance analgesia (1,2) or influence in edematogenic events (3). However, no study has shown the phenomena simultaneously, making it impossible to plan a future clinical application.
Objectives: Our aim was to investigate the effects of sildenafil and morphine coadministration in a model of articular incapacitation, edema and plasma leakage induced by formalin in rat knee joints.
Methods: After the formalin injection (1.5%; i.art.) articular incapacitation was measured by counting the paw elevation time (PET; s) during 1 min period of forced walk, each 5 min throughout a 60-min experimental session. Edema was

evaluated by the articular diameter (AD; cm) increase, and plasma leakage was measured by the amount of evans blue (25 mg/kg; i.v.; 30 min before the test) in synovial fluid (PL; μg/mL) 1 hour after formalin injection. Under this protocol, sildenafil (1, 2.5, 5 mg/kg; i.p; 30 min before the test) and morphine (1, 2.5, 5 mg/kg; s.c; 30 min before the test) were performed, then the subeffective doses were coadministered (1/1 mg/kg; i.p./s.c; 30 min before the test).

Results: Sildenafil could not modify nociception or edema, although the higher dose increased the plasma leakage (5 mg/kg; $p < 0.05$). Only the higher dose of morphine significantly reduced the formalin nociception (5 mg/kg; $p < 0.05$) but there was no modification in edema or plasma leakage. The coadministration of subeffective doses of sildenafil and morphine caused a stronger hyponociceptive effect ($p < 0.01$) than morphine alone, and still reduced the plasma leakage ($p < 0.05$).

Conclusions: These data support the notion that sildenafil can improve the analgesic morphine response with concomitant antiedematogenic effect. Probably, the NO/cGMP pathway is an important mediator in this event. We believe that this drug combination might be a useful implement to manage pain with potentially fewer undesirable reactions.

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