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Intravenous induction of anaesthesia in children

Aspects on propofol and etomidate

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“It’s going to hurt George,” she said, “but only for a moment.”

Abstract

Propofol is today the most commonly used drug for induction of anaesthesia and for short-term sedation. However, one specific problem is that intravenous injection of propofol often results in quite severe injection pain, which in the paediatric population is a serious clinical dilemma. Different methods have been tried in order to reduce injection pain, adding the local anaesthetic lidocaine being the most common, but the incidence of injection pain still remains 20-40%. Thus, there is an urgent need to find new alternatives to propofol that adequately will reduce injection pain during intravenous induction of anaesthesia. Furthermore, there is on-going debate whether exposure to general anaesthesia in early life may be harmful since a large number of animal studies have shown various signs of neurotoxicity after anaesthesia exposure at an early age. New induction agents aimed for use also in neonates and infants must therefore be investigated with regards to potential neurotoxicity.

In **Study I** the incidence and intensity of injection pain was compared between two different formulae of propofol; the former standard propofol solution (Diprivan®) with added lidocaine vs. a more novel plain formulation- Propofol-®Lipuro. Contrary to previous published adult results, the new formula was associated with a higher incidence of injection pain compared to Diprivan®+ lidocaine (66.7 % vs 39.0 %) (P = 0.016).

In **Study II** we compared Diprivan®+ lidocaine with an alternative hypnotic induction agent- etomidate- that now is available as a lipid preparation (Etomidate-®Lipuro). At an interim analysis demanded by the Ethics Committee, data showed that the pain incidence in the etomidate-group was significantly reduced compared to the propofol group (5.0 % vs 47.5 %; $p < 0.001$), and the study was subsequently stopped.

One of few side effects considering etomidate is a high incidence of myoclonic movements (MM) following induction of anaesthesia. In adults the incidence and intensity of MM is reduced if a small, non-sedative priming dose of etomidate is administered immediately before the main induction dose.

The aim of **Study III** was to investigate if this therapeutic modification was valid also in children. However, no evidence for a reduced incidence of MM was found following the use of a small priming dose in children (incidence of MM: priming dose: 75.0 % vs. placebo: 72.5 %). A post hoc analysis did identify children in the age group 5-10 years to display a higher incidence of MM compared to other ages groups (P=0.0021). In study I-III the incidence and severity of injection pain as well as the incidence and severity of MM were measured using 4-point assessment scores, which is in line with previously published paediatric studies.

In **Study IV** the effects on apoptosis and later behavioural alterations of varying doses of etomidate (0.3, 3, and 10 mg/kg) and two other commonly used anaesthetics (propofol 60 mg/kg and ketamine 50 mg/kg) were studied in infant mice (postnatal day 10). No evidence of enhanced apoptosis was found in any of the treatment groups when compared to placebo. In contrast to the other groups, ketamine exposed mice expressed altered motor behaviour when tested at an age corresponding to young human adults (P< 0.01). Enhanced apoptosis was measured by activated caspase-3 (ELISA) and behavioural alterations were assessed by measuring spontaneous activity in a new environment.

Conclusions

Etomidate-®Lipuro is associated with significantly less injection pain compared to traditional propofol (Diprivan®) with added lidocaine. Etomidate exposure in infant mice does not induce enhanced apoptosis or changes in long-term motor behaviour in an animal model.

List of publications

This thesis is based upon the following papers, referred to by the Roman numerals I–V.

I. Propofol injection pain in children: a prospective randomized double-blind trial of a new propofol formulation versus propofol with added lidocaine.
Nyman Y, von Hofsten K, Georgiadi A, Eksborg S, Lönnqvist PA.
Br J Anaesth. 2005; 95: 222-225.

II. Etomidate-Lipuro is associated with considerably less injection pain in children compared with propofol with added lidocaine.
Nyman Y, von Hofsten K, Palm C, Eksborg S, Lönnqvist PA.
Br J Anaesth. 2006; 97: 536-539.

III. Effect of a small priming dose on myoclonic movements after intravenous anaesthesia induction with Etomidate-Lipuro in children.
Nyman Y, von Hofsten K, Ritzmo C, Eksborg S, Lönnqvist PA.
Br J Anaesth. 2011; 107: 225-228.

IV. Etomidate exposure in early infant mice (P10) does not induce apoptosis or affect behaviour.
Nyman Y, Fredriksson A, Lönnqvist PA, Viberg H.
Acta Anaesthesiol Scand. 2016 Jan 13. doi: 10.1111/aas.12685. [Epub ahead of print]

Contents

List of Abbreviations

AC-3	Activated Caspase 3
ASA	American Society of Anesthesiologists
BGS	Brain Growth Spurt
CNS	Central Nervous System
CPMM	Cyclopropyl-methoxycarbonyl metomidate
DNT	Developmental Neurotoxicity
EMLA®	Eutectic Mixture of Local anaesthetics
GA	General Anaesthesia
GABA	γ-Aminobutyric Acid
ICD-9	International Classifications of Diseases- 9th revision
ICU	Intensive Care Unit
i.v.	Intravenous
LCT	Long Chain Triglyceride
MDD	Major Depressive Disorder
MGH	Massachusetts General Hospital
MCT	Medium Chain Triglyceride
MM	Myoclonic Movements
NMDA	N-methyl-d-aspartate
PICU	Paediatric Intensive Care Unit
PND	Post Natal Day
PRIS	Propofol Infusion Syndrome
RCT	Randomized Controlled Trial
SFAI	Swedish Society for Anaesthesiology and Intensive Care

1 Introduction

Every year several millions of surgeries and various diagnostic procedures are conducted on infants and children worldwide. Unlike in the adult population most of these events have to be performed under general anaesthesia.

Anaesthesia, from Greek an-, "without"; aisthēsis, "sensation"; is defined as loss of sensation and can be with or without loss of consciousness. When the loss of sensation is combined with a loss of consciousness it is described as general anaesthesia (GA). Regional anaesthesia on the other hand, can be described as a "nerve block", which anesthetizes a part of the body with no effect on consciousness. General and regional anaesthesia can also be combined.

GA is divided into three different phases: induction, maintenance and emergence. The induction of anaesthesia is accomplished either by inhaling a volatile anaesthetic gas with a face mask or by injecting an anaesthetic drug intravenously.

The intravenous (i.v.) or parenteral route is today often the preferred alternative, especially in the adult population, since this method is perceived as being both faster and smoother. It is also by many anaesthetists considered as a safer option. Furthermore, the intravenous route is favoured when a quick response is requested, e.g. rapid sequence induction (RSI). The maintenance of anaesthesia can be achieved either by continuing the i.v. drug used for induction as an infusion (total intravenous anaesthesia-TIVA) or by inhaling an anaesthetic gas.

The most frequently used i.v. induction agent today is propofol, a short-acting hypnotic and amnestic agent. The former most common i.v. induction agent thiopental is currently less used, one major reason being longer residual sedation compared to propofol.

One specific adverse effect of propofol is injection pain, a very undesirable disadvantage especially in infants and children. Studies performed both in children and adults have reported an incidence of injection pain of 30-90 %.[1-3] Various strategies, the most common being the addition of the local anaesthetic lidocaine, have been used to reduce the incidence but injection pain still remains 20-40%.[1-3]

A current concern of huge importance is the long-term safety following anaesthesia exposure in neonates and infants. Numerous studies in rodents and non-human primates have shown that almost all anaesthetic agents commonly used today are believed to be neurotoxic and cause long-lasting effects on the

brain. Because of the large number of infants and children that receive anaesthesia every year the clinical implications of this potential neurotoxicity related to anaesthesia at an early age may be very substantial. Until recently the published human paediatric studies have been retrospective and the results inconclusive. The interim neurodevelopmental results from a human randomized controlled trial (RCT) of regional vs general anaesthesia, the GAS study, show that any enhanced apoptosis induced by anaesthetic agents does not appear to be clinically relevant.[4] This and other ongoing prospective studies will hopefully contribute to answering the question whether anaesthesia to infants and children is harmful or not.

2 Background

Historical background of general anaesthesia

The birth of general anaesthesia is usually attributed to the first successful use of ether which was exhibited 16 October 1846. A public demonstration of a surgery carried out under ether anaesthesia was then performed at Massachusetts General Hospital (MGH) in Boston. The anaesthetic was delivered by a Boston dentist, William T.G. Morton, who during a chemistry lecture at Harvard Medical School some years earlier had learned that the easily acquired solvent sulfuric ether could turn a person unconscious. After experimenting with several different animals, and also testing on himself and friends, Morton started using ether as a pain relief for his dental patients. Convinced of the drug's safety and quality, Morton proceeded to demonstrate the favourable features of ether for an audience consisting of surgeons and medical students at MGH's operating theatre. The patient was a young man, Edward Gilbert Abbot, who had a tumour on his jaw removed. The surgeon was John Warren, who after the successful operation, during which the patient seemed to experience no pain at all, uttered the famous words "Gentlemen – this is no humbug". The operating theatre "The Ether Dome" is still preserved, open to the public and on October 16 every year "Ether day" is celebrated at MGH. A monument honouring the first use of ether as an anaesthetic is located in the Boston Public Garden. One of the four inscriptions on the monument says: "*To commemorate that the inhaling of ether causes insensibility to pain. First proved to the world at the Mass. General Hospital in Boston, October A.D. MDCCCXLVI*"

Induction of anaesthesia

Induction of anaesthesia can be accomplished either by inhalation of a volatile anaesthetic with a face mask as first demonstrated by Morton (e.g. ether, halothane or currently sevoflurane) or by intravenous injection of an anaesthetic induction agent (see below). Inhalational induction of anaesthesia in children is even today still quite commonly used in large parts of the world since establishing intravenous access may be difficult in paediatric patients. However, following the introduction of EMLA® (Eutectic Mixture of Local Anaesthetics), a Swedish innovation that provide cutaneous anaesthesia, intravenous induction of anaesthesia has become popular also in children, not only in Scandinavia but from a

global perspective as well.

The current thesis has a focus on intravenous induction of anaesthesia in children and will not further discuss inhalational induction.

Intravenous induction of anaesthesia

Prior to the early 1930's general anaesthesia was solely provided by either ether or chloroform. However, at this time the drug class barbiturates was discovered and was initially used to treat anxiety and sleeping disorders as oral preparations. This was soon followed by the development of intravenous preparations that were found capable of inducing anaesthesia. The first successful induction of anaesthesia by intravenous injection of a barbiturate is often attributed to John S. Lundy at the Mayo Clinic (Rochester, USA). Lundy introduced the i.v. barbiturates sodium amobarbital (1929) and sodium pentobarbital (1930) for clinical use in anaesthesia. Furthermore, the concept of "balanced anaesthesia" was introduced by Lundy already in 1926.[5, 6]

The short acting i.v. barbiturate sodium thiopental (Pentothal®) was clinically introduced in 1934 by Ralph M. Waters at the University of Wisconsin Medical School (Madison, USA). Waters was the world's very first professor in anaesthesiology and also became the first honorary member of the Swedish Society of Anaesthesiologists. In appreciation for his contributions to the development of anaesthesiology in Sweden, Waters received the Order of Wasa from King Gustav V of Sweden in 1947.

Sodium thiopental totally dominated the market for the next 50 years. Despite the dominance of thiopental a number of different intravenous induction agents was developed but fell into disuse for various reasons, e.g. Nembutal®, Brietal®, Epontol® and Althesin®.

With the introduction of new intravenous induction agents (e.g. propofol) that possessed better characteristics than thiopental, this classic intravenous induction agent is much less used today. An example of this is that only a few drug companies still produce this drug and at times it is difficult to get thiopental delivered in a reliable fashion. Furthermore, thiopental has come to get a questionable reputation since it in the USA has been used as a component of the drug mix used for intravenous executions. However, thiopental may still represent a useful alternative for induction of anaesthesia in small babies since the more modern alternative propofol may cause pronounced and quite prolonged hypotension in this patient category. [7-10]

Here follows information about three different i.v. anaesthesia agents commonly used in clinical practice today and that are part of the different studies included in this thesis.

Propofol

Propofol (2, 6 – Di-isopropylphenol) is now the dominating i.v. agent throughout the world due to its favourable properties being a smooth induction and fast recovery. The anaesthetic effect of propofol is believed to be mediated mainly via actions at the γ-aminobutyric acid type A ($GABA_A$) receptor.[11, 12]

Propofol is not only used for induction but also for maintenance of anaesthesia either alone or combined with other drugs. It is especially suitable for day-surgery because of the quick recovery- this in contrast to the former most used induction agent thiopental. The use of propofol has expanded from being merely an anaesthetic agent used for induction and maintenance to also include sedation in intensive care units and out-patients procedures.

Propofol is not (especially in children) recommended for long-term sedation because of the risk for propofol infusion syndrome (PRIS) that is potentially fatal. PRIS was initially described in the paediatric population [13, 14] but PRIS has also been reported to occur when critically ill adults have been sedated with propofol.[15, 16]

The syndrome was defined by Bray in 1998 as: "(1) the sudden, or relatively sudden, onset of a marked bradycardia which was resistant to treatment and which progressed to asystole; (2) the presence of lipaemic plasma; (3) a clinically enlarged liver or one which was found to be infiltrated with fat at autopsy; (4) the presence of a metabolic acidosis to the extent of at least one arterial blood sample with a base deficit greater than 10; (5) the presence of muscle involvement with evidence of rhabdomyolysis or myoglobinuria". According to Bray bradycardia (1) and at least one of (2), (3), (4), or (5) have to be present in order to define the condition as PRIS. Bray's recommendation was to limit the duration to < 48 hours and use dose of less that 4mg/kg/h.

Even with shorter duration and when propofol have been used exclusively during anaesthesia PRIS is suspected to have occurred, both in children and adults.[17-20]

A normal induction dose of propofol produces a 20–40 % reduction in blood pressure during induction which is caused by arterial vasodilation due to reduced vascular sympathetic tone, but also by a direct effect on myocardial contractility.[21-24]

Another known adverse effect is a dose-dependent depression on ventilation and apnoea is often seen during induction.[1, 21, 25]

Propofol is a phenol and like other phenols it irritates skin, mucous membranes as well as the venous intima. Initially propofol was solved in Cremophor EL – a castor oil - but due to a high incidence of anaphylaxis and very high frequency of pain on injection the product (by name ICI 35868) was withdrawn

from the market. The emulsion eventually chosen as a solvent was the one with the same components as the parenteral fat formulae, Intralipid® that contains soybean oil (long chain triglyceride-LCT), egg yolk lecithin and glycerol. Even with this lipid-formula the incidence of pain still remained unacceptable high- around 60-70 %.[2, 3, 26]

The mechanism of the propofol-induced injection pain is not fully understood but is believed to be caused either by a direct stimulation of pain receptors in the venous wall or by an indirect stimulation which leads to release of different mediators (kinin cascade) that stimulates nerve endings and causes late onset of pain.[27]

Since the introduction of propofol countless attempts have been made in effort to minimize both the incidence and the intensity of propofol-induced injection pain. Various drugs, such as antiemetics, non-steroidal anti-inflammatory drugs, β blockers, 5-HT3 receptor antagonists, lidocaine and opioids have been used more or less successfully.[2, 3, 28] Propofol has been given slow, fast, cooled or warmed without any proven pain-reducing effect.

The introduction of a novel lipid-formula (Propofol-®Lipuro), which contains medium chain triglycerides (MCT) together with soya bean oil, have in some studies shown a reduction in pain incidence[29-33] while other studies have not been able to confirm these results.[34, 35]

Of all the numerous interventions and agents that have been tested in the attempt to reduce propofol-induced injection pain, the local anaesthetic lidocaine (Xylocaine®) is the most commonly used option in clinical practice. Its effect is believed to be caused by a direct action but may also be due to a decreased pH of the lipid emulsion, which in turn decrease the free propofol concentration in the aqueous phase.[36]

Today the recommendation for reducing injection pain is to choose an as big vein as possible, e.g. the antecubital vein that has a larger diameter than the veins on the dorsum of the hand. If the hand is chosen pre-treatment with lidocaine in conjunction with venous occlusion is advocated.[3] Lidocaine can also be given as a pre-treatment without venous occlusion before the induction with propofol or mixed (admixture) with the propofol in the same syringe directly prior to injection. These interventions have however been observed to be somewhat less efficacious compared to pre-treatment with lidocaine combined with venous occlusion.[2, 3]

Another current advice is, in addition to the pre-treatment or admixture with lidocaine, to also include an opioid before the induction of the anaesthesia (if not contraindicated).[3]

A recently published review focuses on the effect of lidocaine in reducing

high intensity pain caused by propofol, including 87 studies and a total of 10.460 adult subjects. Six sub-groups from the different studies were identified: (1) low dose lidocaine admixture - ≤ 20mg or ≤ 0,2 mg/kg, (2) high dose lidocaine admixture - >20 mg or > 0,2 mg/kg, (3) low dose lidocaine pre-treatment alone - ≤ 20mg or ≤ 0,2 mg/kg, (4) high dose lidocaine pre-treatment alone - >20 mg or > 0,2 mg/kg, (5) low dose lidocaine with venous occlusion-≤ 20mg or ≤ 0,2 mg/kg, (6) high dose lidocaine with venous occlusion- >20 mg or > 0,2 mg/kg. The main results from this meta-analysis are that lidocaine is capable of significantly reducing high intensity pain and also to reduce the overall incidence of pain. The least efficient method appears to be pre-treatment with low dose lidocaine alone. A subgroup analysis of the dose of lidocaine suggests that a higher dose is more effective for reducing and preventing propofol-induced pain than a lower dose in both the admixture and the pre-treatment groups.[28] The authors conclude that all methods are effective and the preferred option should therefore be used according to the circumstances.

It is well known that the incidence of propofol-induced pain is higher in children.[1, 26] One obvious reason for this is that veins have smaller dimensions in paediatric subjects. The recommended antecubital vein is not always easy to access in children and is less favoured since extravasation more easily can be overlooked. Another drawback is that the antecubital vein easily occludes when the elbow is flexed. To apply a venous occlusion is seldom possible in the paediatric population since it can be uncomfortable for the child and also more time-consuming. Instead the most common procedure and the only option proven to be effective is to mix the propofol with the local anaesthetic lidocaine prior to injection. Doses of lidocaine required to have effect is somewhat higher compared to adult data: 0.2 mg/kg instead of 0.1 mg/kg.[26] A more dilute propofol preparation, consisting of 0.5% propofol instead of 1 % in a lipid solution, has been shown to reduce the pain-incidence in children.[37] However, it still remains an unacceptable high 23 %.

Etomidate

Etomidate (D-Ethyl-1-(α-methylbenzyl)-imidazole-5-carboxylate) is an imidazole derivate with the same favourable properties as propofol: fast onset and quick recovery. Etomidate's anaesthetic effects are considered to be mediated mainly via actions at $GABA_A$ receptors.[11, 38, 39]

Etomidate does not affect myocardial function or sympathetic tone which makes it a haemodynamically very stable drug. After a normal induction dose only a minimal change in heart-rate and blood-pressure is usually seen.[21, 24, 40] Furthermore, unlike propofol and barbiturates very minimal respiratory depression is noted.[21]

Etomidate is hydrophobic at physiologic pH so to increase solubility it is either solved in propylene glycol (Amidate®, Hypnomidate®) or in a lipid emulsion (Etomidate-®Lipuro). The solvent based on propylene glycol is known to induce injection pain in 60-80 % if no adjuvant is given and there is also a high associated risk for thrombophlebitis and thrombosis.[41, 42] However, when solved in soybean oil and MCT the pain incidence is virtually zero and no venous irritation is noted in adults.[43, 44]

One side effect of etomidate is the occurrence of myoclonic movements (MM) that occur in 50-80 % of patients during induction.[45] The incidence and intensity in adults is related to the dose and can be attenuated by premedication with benzodiazepines, dexmedetomidine, opioids, ketamine or a split-dose induction.[45-51]

The MM are not connected to seizure-like EEG (electroencephalogram) activity, instead it is postulated that the myoclonus is a phenomenon of subcortical disinhibition, much like the phenomenon of restless legs during normal human sleep.[45] It is worth noting that myoclonic activity is also seen after propofol induction with an incidence of approximately 6 -15 %.[52-54]

Another side effect that has raised concern about the safety of etomidate is the inhibition of corticosteroid synthesis. When etomidate is used as an infusion to maintain anaesthesia, the concentrations of cortisol, cortisone, and aldosterone decrease in plasma and furthermore the concentrations of 11-deoxycorticosterone, 11-deoxycortisol, progesterone, and 17-hydroxyprogesterone increase. It is believed that etomidate inhibits adrenal steroid synthesis principally by blocking the activity of 11β-hydroxylase.[55, 56] This mitochondrial cytochrome enzyme converts 11-deoxycortisol to cortisol and 11-deoxycorticosterone to corticosterone. Contrary, the effect of a single induction dose of etomidate does not appear to cause clinically relevant interference with corticosteroid synthesis.[57-59]

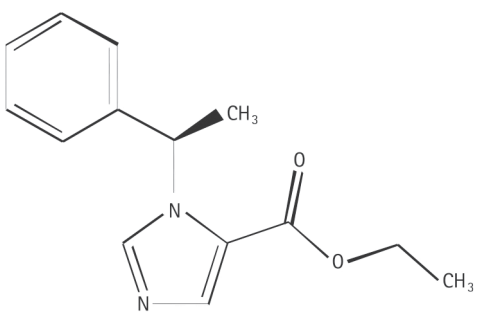

Figure 1
Structure of etomidate

Ketamine

Ketamine (2-(o-Chlorophenyl)-2-(methylamino)-cyclohexanone hydrochloride), represents another well-known anaesthetic agent that is assessed in study IV. Effects of ketamine are believed to be mediated mainly by inhibition of the N-methyl-d-aspartate (NMDA) receptor.[11, 39]

There are two optical isomers of ketamine: S (+) ketamine and R (-) ketamine. S (+) Ketamine (Ketanest®) is about twice as potent compared to racemic ketamine and possibly cause less adverse effects.[60-62]

Ketamine can be used for both induction and maintenance of anaesthesia and is especially popular in prehospital and trauma settings as it maintains circulation and respiration.[63, 64] Since ketamine has analgesic properties at sub-anaesthetic blood concentrations it also makes it suitable for postoperative analgesia and sedation.[65]

The intravenous administration of ketamine is not known to be associated with any noticeable injection pain.[66]

Emergence delirium and psychotomimetic side effects are not uncommon when using ketamine and benzodiazepines has traditionally been recommended in order to reduce these problems.[67] More recent paediatric studies contradict this previous custom and instead suggest that benzodiazepines do not reduce emergence reactions and postoperative reactions.[68, 69]

In some countries ketamine is used as a recreational drug known as "Special K", "Kit-Kat" and "Cat Valium".[70] Apart from the obvious concerns with drug addiction and its related problems (e.g. acute toxicity and chronic psychological effects) a prolonged use of ketamine has been noted to cause urinary bladder inflammation with ulcerative cystitis. The reason why ketamine cause cystitis is unclear.[71]

Recent studies have shown that ketamine and other NMDA receptor modulators possibly can possess effects in treating therapy resistance depression (major depressive disorder, MDD).[72, 73] However, a review report including 25 studies found limited evidence for ketamine's efficacy over placebo and the authors concluded that further RCTs with adequate blinding and longer follow up are needed in order to evaluate the effects of ketamine on MDD.[74]

Regarding potential neurotoxicity ketamine is one of the most thoroughly studied anaesthetic agents.

Here follows a brief description of the mechanisms of general anaesthesia.

Molecular mechanisms of anaesthesia

Anaesthetics agents are believed to produce their main target-effect via interaction with $GABA_A$ and/or NMDA receptors.[11, 39, 75, 76]

Gamma-Amino Butyric acid (GABA) is an amino acid that acts as an inhibitory neurotransmitter in the central nervous system (CNS) and $GABA_A$ receptors are the major inhibitory neurotransmitter-gated ion channels in the human brain. The $GABA_A$ receptor, which belongs to a superfamily of ligand-gated ion channels, is a pentameric structure around a central pore, located partly extracellularly, partly intracellularly. So far 19 different subunits have been identified and in different combinations they include about 50 different $GABA_A$ sub-receptors. When GABA binds to the $GABA_A$ receptor it produces an influx of chloride into the cell that makes it slightly more negative and causes an inhibitory postsynaptic effect.

Drugs that mainly act on the $GABA_A$ receptor are (apart from endogen GABA) the positive allosteric modulators (agonists) benzodiazepines, barbiturates, propofol, etomidate, volatile agents and ethanol. By increasing the activity of inhibitory $GABA_A$ receptors the neuronal excitability is reduced and the effects are amnesic and hypnotic. A negative allosteric modulator is flumazenil (Flumazenil®, Lanexate®), a drug that can be used in treating benzodiazepine overdose.

While GABA is an inhibitory transmitter in the mature brain, its actions are primarily excitatory in the developing brain.[77] The intracellular chloride levels are higher in the immature neurons than in adult neurons but the mechanism and implications for this is unknown. The fact that the incidence of seizures is highest early in life could possibly be related to that GABA excites the immature neurons.[78]

The major excitatory neurotransmitter-gated ion channels in the human brain are the glutamate receptors. To this family the NMDA-receptor belong which unlike the pentameric $GABA_A$ receptor is composed only by four subunits. The NMDA receptors are activated by endogenous glutamate and aspartate. When glutamate is released from the presynaptic neuron by an action potential this leads to a flow of calcium and sodium into the cell and potassium out of the cell which produces an excitatory postsynaptic potential.

Agents that work mainly through NMDA receptor antagonism are ketamine, nitrous oxide, ethanol, xenon, diethyl ether and cyclopropane.[39, 75]

Both molecular (in-vitro) and animal studies involving transgenic mice have shown that different anaesthetics have different degrees of target selectivity. Anaesthetic drugs can according to some authors be divided into three different groups. Group 1 drugs (propofol, etomidate and barbiturates) act primarily through specific $GABA_A$ receptors associated with different subunit types. Group 2 drugs (ketamine, nitrous oxide, xenon and cyclopropane) seem to act on a small number of targets, including glutamate receptors and two-pore potassium channels. Group 2 have in clinical concentrations minimal effect on $GABA_A$ recep-

tors. Group 3 (volatile anaesthetics) are the least selective group which affects many possible molecular targets such as $GABA_A$ and two-pore receptors. Studies show that hypnosis and amnesia seem to be linked to enhancement of $GABA_A$ receptors for both group 1 and 3 anaesthetics. Analgesia correlates strongly with NMDA receptor inhibition, a quality possessed especially by anaesthetics in group 2.[39] Recent studies have indicated that other receptors for example HCN1 (hyperpolarization activated cyclic nucleotide gated potassium channel 1) can be targets for both volatile anaesthetics and ketamine.[79, 80] Due to these new studies the targets and effects of anaesthesia are somewhat better understood and undoubtedly more specific and improved agents with less unwanted side-effects will hopefully be developed.[39]

As alluded in the Introduction, virtually every commonly used anaesthetic, whether volatile or intravenous, have been shown to produce neurotoxicity in preclinical studies on rodents and non-human primates.

Neurotoxicity

Developmental neurotoxicity (DNT) is defined as: "any adverse effect on the chemistry, structure and function of the nervous system during development or at maturity, induced by chemical or physical influences".[81] The definition of DNT also includes changes in behaviour, brain morphology and neurochemistry after gestational and/or lactational exposure. It is today widely accepted that the developing brain is more sensitive to the effects of different chemicals than the adult brain.[82, 83] The incidence of human neurodevelopmental diseases such as attention deficit, hyperactivity disorders, autism and others is increasing.[84] The reason for this is not fully understood, but is believed to be caused by different genetic and social components in addition to chemicals in the environment.[85] Since many years it has been known that several compounds that can be found in our environment, e.g. lead, methylmercury and polychlorinated biphenyls are harmful to the child's development.[86]

Worries about the potentially negative effects from GA emerged in the 1950's when personality changes were observed following anaesthesia.[87] Some decades later a concern arose regarding the possible health hazard for pregnant health care workers and anaesthetic waste gases (halothane). In a study by Quimby and co-workers where rats exposed to subclinical doses of halothane (10 ppm, which was then an accepted threshold value in an operating room) 8 hours/day for 5 consecutive days, were later found to display impaired behaviour and histopathological changes in the brain.[88]

A ground-breaking study in 1999 from Ikonomidou and colleagues, where the NMDA-blocking agent dizocilpine (MK801) was found to cause widespread

neurodegeneration in neonatal rats, raised the question of potential deleterious effects of other NMDA receptor blocking anaesthetic drugs.[89] A few years later a study by Jevtovic-Todorovic and co-workers found that a combination of the GABA-agonist midazolam, the combined GABA-agonist and NMDA-antagonist isoflurane and the NMDA-antagonist nitrous oxide given to neonatal rodents not only caused substantial loss of brain-cells by enhanced apoptosis but also caused persistent memory and learning impairments.[90]

After these initial studies a large number of related experiments have been performed. Several research groups have in animal studies reported various expressions of DNT after anaesthesia exposure– such as acute cell-death, changes in dendritic architecture, reduced synaptic density, decreased levels of neurotrophic factors, mitochondrial degeneration, destabilization of the cytoskeleton, cell cycle abnormalities and altered behaviour.[91-112]

To understand the mechanisms which could potentially lead to these aberrations some basic knowledge about normal development of the central nervous system (CNS) is advantageous.

Brain development

The sequence of brain development does not vary significantly between different mammalian species, but the time-span is different depending on animal specie. A way to compare diverse neural events between species is to access www.translatingtime.org. 18 various mammalian species, including humans, are part of this model which makes it easier for researchers to compare data obtained in studies performed on different laboratory animals.[113]

In the first embryonic part of CNS development the brain obtains its form by proliferation, migration and differentiation. The embryonic brain development is followed by the foetal developmental period when functional circuits in the brain are formed and matured. A particularly vulnerable period during this period is called the "brain growth spurt" (BGS). During the BGS the brain rapidly expands in weight and size mainly caused by an increase of glia cells. Other processes occurring during BGS are growing and maturing of neurites, establishment of neural connections, synaptogenesis and myelination.[83]

Early studies in the 1970's by Dobbing and Sand found differences in brain development between species by simply weighing the brains post-mortem. The definition of BGS was then defined by the total brain weight gain as a percentage of its adult weight.[82, 114] Today it is commonly accepted that the BGS period starts to appear in humans from the last trimester to the first 2-3 years after birth. In rodents BGS is solely neonatal with a peak around postnatal day (PND) 7-10 and continues during the first month in life.[83] Synaptic pruning, the process by

which unwanted or unused synapses are eliminated, continues in humans up until adolescence. By then nearly 50 % of the synapses that were present at 2 years of age are abolished.

Before the introduction of magnetic resonance imaging (MRI) it was generally believed that the brain was fully developed by the age of six but today we know that the function and morphology of the brain is a continuous lifelong process. This development depends on the genetic set-up, the internal and external environment, and of course on experience. At adulthood the mature brain consists of >100.000 billion neurons. Each neuron can be connected (synapse) with 1000's of other neurons which results in an endless number of neuronal connections or neuronal circuits. All this taken together makes the brain by far the most refined and complex organ in the human body.[83]

Apoptosis

A requirement for normal development is apoptosis which is also known as programmed cell death. This is a naturally occurring cellular "suicide" where redundant or possibly harmful cells are eliminated. Apoptosis, which can affect individual or smaller groups of cells, is an energy-consuming process with no inflammatory response. The process involves the activation of a group of cysteine proteases: "caspases" (proteases= enzymes that degrade proteins). It is an extremely complex process and is believed to occur mostly through two main pathways, the extrinsic and the intrinsic pathway. Each pathway requires certain triggering signals to start a cascade of molecular events. The extrinsic pathway involves binding of ligands to death receptors [members of the tumour necrosis factor (TNF) receptor gene super family] in the transmembrane, which leads to activation of Caspase-8 which in turn activates the final step: the executioner or activated caspase-3 (AC-3). The intrinsic pathway or the mitochondrial pathway is non-receptor mediated and is initiated by stimulus such as radiation, hyperthermia, infections, toxins as anaesthetics, etc. All these stimuli initiates an increase in the mitochondrial membrane permeability which leads to a release of pro-apoptotic proteins (cytochrome c most essential) into the cytosol. This results in an activation of caspase-9 which subsequently activates caspase -3. Both the extrinsic and intrinsic pathways end at the point where capase-3 is activated. Here the "execution pathway" starts which results in fragmentation of DNA, degradation of nuclear/cytoskeletal proteins and formation of apoptotic bodies which are subsequently engulfed by macrophages. AC-3 is considered to be the most important of the executioner caspases and has frequently been used as a marker to quantify neuroapotosis caused by anaesthesia.[115, 116]

Necrosis is the other major death process. In contrast to apoptosis, necrosis is

a passive, non-energy consuming process that usually affects larger fields of cells and also activates inflammation.[115]

Methods to measure apoptosis and neurodegeneration

There are various methods to measure apoptosis and neurodegeneration, all with their pros and cons. One traditional method is to evaluate cytomorphological alterations detected by staining and then assessed by microscopy. When the effects of anaesthetics are investigated the Fluoro-Jade method often has traditionally been used. These techniques have, as several others, difficulties to differentiate apoptosis from necrosis.[117]

Another method to estimate apoptosis is to measure DNA fragmentation by the TUNEL (terminal dUTP nick end-labeling) method. Array kits are commercially available but the method is regarded as expensive, time-consuming and often show false positive results.[117] The activation of different caspases can be detected in a variety of ways (western blot, immunoprecipitation and immunohistochemistry). However, one disadvantage is that caspase-activation may occur but does not necessarily result in enhanced apoptosis.[115,117]

Most animal studies concerning anaesthesia and neurotoxicology focus on different ways to measure apoptosis and various other structural signs of neurodegeneration. Some studies in addition to this also measure neurodevelopmental outcome after anaesthetic exposure to support the potential importance of enhanced apoptosis. (See below).

Behavioural animal tests

In addition to different in-vitro measurements the potentially deleterious effects of anaesthesia have also been assessed by various animal behavioural tests. One method to evaluate normal behaviour in rodents is to measure spontaneous motor activity in a novel home environment. The decrease in activity over time is a normal profile of spontaneous behaviour and referred as habituation. This test can be said to represent a crude measurement of cognitive function.[118] Other behavioural tests commonly used in rodents are e.g. Radial Arm Maze, Morris Water Maze (two tests that assess learning capability, specifically working memory and spatial memory) or Elevated Plus Maze test (an exploration- and interaction-based anxiety test).[119]

In non-human primates (rhesus monkeys) other more refined test as Operant Test Battery (OTB) have been performed to determine the possible negative effect of anaesthesia on the developing brain.[103, 120]

Epidemiological studies

The numbers of human studies are rather limited compared to the vast amount of animal studies. For obvious reasons it is not possible to examine brain tissue in healthy children so all the human studies depend on assessing neurological functions. Some epidemiological studies have demonstrated an association between early-life exposure to anaesthetics and altered long-term neurocognitive outcome while others have not. Thus, so far the results are inconclusive.

A series of retrospective epidemiological cohort studies have been published by the Mayo group. The studies are based on the same birth cohort consisting of 5.357 children that were born between 1976 and 1982 in Olmsted County, Minnesota, USA. In these studies Wilder et.al used the fact that the children had already been evaluated in terms of learning difficulties.

In their first study 593 children that had anaesthesia (predominately halothane and nitrous oxide) exposure before the age of 4 were compared with 4.764 children with no exposure.[121] One exposure did not affect the outcome but children who had 2, 3 or more suffered 1.59–2.6 times more often from learning disabilities compared to the un-exposed group.

Using the same cohort Wilder et al. compared 359 children who had anaesthesia and surgery before the age of 2 with 5.007 children who did not.[122] Again one exposure did not increase the risk for learning disabilities but children who had more than two exposures were 1.95 times more likely to be diagnosed with ADHD (attention-deficit hyperactivity disorder) compared with the non-exposed group.

The Mayo group studies do have some weaknesses. One concern is that almost one third of the original cohort moved from the county during the study period and data from those children were lost to follow up. Furthermore, a selection bias probably occurred since families with sick children were possibly less likely to move from the area and instead preferred to stay close to the hospital. Additionally, children suffering from different chronic diseases are more prone to require frequent anaesthetics and may also have various degrees of learning disabilities.

To correct for the latter sicker children with American Society of Anesthesiologists (ASA) physical status 3 and 4 were excluded in a subsequent study but even then the anaesthesia group scored worse.[123] In this study very few children <1 year were included. Furthermore, all types of surgeries were included and known confounding factors were not taken into consideration.

In two retrospective cohort studies DiMaggio et al. analysed children born between 1999 and 2002 that were enrolled in the New York State Medicaid program. In their first study 383 children undergoing surgical hernia repair before

the age of 3 were compared with 5.050 with no surgery.[124] Even after adjusting for known confounding factors developmental or behavioural disorders were twice as common in the hernia repair group as in the control group. Type of anaesthesia, frequency or the duration was not accounted for. The second retrospective study performed by DiMaggio involved siblings born between 1999 and 2005 (twins of unknown zygocity) where 304 children of a cohort consisting of 10.450 had anaesthesia younger than 3 years and 10.146 had not. A 60 % greater risk to be diagnosed with developmental and/or behavioural disorders was found in the exposed group.[125]

The studies by DiMaggio et al. used data obtained from a health insurance organization program that relates to the poorest population in New York State where the frequency of important diagnoses e.g. mental illness could be higher. ICD-9 codes (International Classifications of Diseases- 9th revision) were used to define both enrolment and outcome which also could influence the results.

In the Netherlands Bartels et al. used the national twin registry to study 1.143 monozygotic twin pairs (born 1986-1995) where both, one or none of the twins had surgery before 3 year of age. At the age of 12 when the children were tested by teachers' ratings and standardized tests the exposed group was found to perform worse. However, 15 % (71 twin pair) were discordant for anaesthesia, i.e. one twin had undergone anaesthesia, and the co-twin had not. An interesting finding was that the unexposed twin in a discordant pair did not differ from its exposed sibling which could indicate that factors other than anaesthesia are responsible for the developmental impairment.[126]

In Denmark Hansen et al. performed a follow-up study of the Danish birth cohort from 1986 to 1990 in order to investigate the association between anaesthesia and surgery for inguinal hernia repair in infancy and later academic achievements. During these years 2.689 children in Denmark had inguinal repair surgery before the age of 1. The age-matched control group was a 5 % population sample (14.575 children) randomly selected within the cohort. The performance of the exposed children was inferior when tested in 9th grade but after adjusting for known confounders no difference was found between the groups.[127]

A similar study of the same cohort (1986–1990) included 779 neonates who were less than 3 months when undergoing pyloric stenosis repair. They had similar academic achievements when tested in adolescence compared to the 14.665 controls.[128] However, the non-attainment rate when tested in 9th grade was somewhat higher for the exposed group in both the hernia and pyloric study.

The Hansen group has also recently shown that when comparing 228 children from the same cohort (1986-1990) who had undergone neurosurgery in infancy, the exposed group had a higher mortality rate and also performed signifi-

cantly less well when tested at adolescence. The key implication of this study is not to merge all different surgical procedures together when studying the effect of anaesthesia on the developing brain.[129]

In Western Australia Ing et al. performed an analysis on an established pregnancy cohort (the Raine study – initially created to evaluate long-term effects of pre-natal ultrasound). Of 2.608 children (born from 1989 to 1992) assessed, 321 were exposed to anaesthesia before the age of 3. When tested at 10 years of age the anaesthesia group had displayed deficits in abstract reasoning and language compared to the control group. In contrast motor-function and behaviour were not affected.[130] All types of surgeries were included, also myringotomies, a circumstance that is known to be associated with language and learning problems and appears as a plausible explanation for these findings.[131]

Another study from Ing et al. used the same cohort. In this study 781 children were included and of these 112 had been exposed to anaesthesia before 3 years of age. Three outcomes were investigated: academic achievement, ICD-9 codes, and direct neuropsychological testing. Deficits were found in the exposed group when direct neuropsychological testing and ICD-9 codes were compared but no difference in the academic achievement was found.[132] Both studies have interrelated outcomes and use multiple tests that could influence the results.[133]

Since complete cognitive testing is extremely time-consuming and expensive this often leads to studies with very small study populations. An example of this is a study by Stratmann et al.[134] where only 28 children between 6-11 years of age, who had various surgeries before age 1 were included. The age and gender matched control group also consisted of only 28 children. After extensive testing the children in the anaesthesia group were found to have significantly lower recollections scores and performed worse regarding recollection of associative information while IQ and child behaviour checklist scores were unaffected.

In summary it can be concluded that epidemiological retrospective studies are limited by numerous confounders. The results are therefore unfortunately inconclusive and in order to try to answer the question if early exposure to anaesthesia may be neurotoxic or not, additional prospective randomized studies are necessary.

Ongoing prospective randomized studies

The GAS study (general anaesthesia vs regional anaesthesia) is a multicentre trial with 28 participating hospitals in Australia, Italy, the USA, the UK, Canada, the Netherlands and New Zealand where infants < 60 gestational age, born > 26 weeks of gestation scheduled for inguinal hernia repair were randomly assigned to either general anaesthesia (sevoflurane based) or regional anaesthesia

(spinal or caudal). Between 2007-2013 a total of 722 infants were enrolled; 363 in the awake group and 359 in the general anaesthesia group. The primary outcome of the trial will be the Wechsler Preschool and Primary Scale of Intelligence Third Edition (WPPSI-III) Full Scale Intelligence Quotient score at age 5 years. The secondary outcome, with results recently reported, was assessed at 2 year by the composite cognitive score of the Bayley Scales of Infant and Toddler Development III (Bayley III). At this interim analysis no evidence was found that 1 h of sevoflurane anaesthesia increases the risk of adverse neurodevelopmental outcome at 2 years of age compared with awake-regional anaesthesia.[4] These results are reassuring but the final results of the GAS study are not expected until 2017.

A further ongoing study is the Pediatric Anesthesia and Neuro-Development Assessment (PANDA) study, a multicentre study based at Columbia University with 4 participating centres in the USA. The study start was in May 2009 and the estimated completion date December 2016. In this study 500 ASA 1-2 (healthy or mild systemic disease) children who undergo inguinal hernia repair under GA before the age of 3 are compared with an unexposed sibling. Data are collected from a retrospective database. At the age between 8 and 15 the children will be tested extensively with various neurocognitive tests. No results have up till now been released.[135]

A third ongoing study-the MASK study (Mayo Safety in Kids study) is led by researchers at the Mayo Clinic in collaboration with the National Center for Toxicological Research (NCTR). This study will compare performance of children exposed to anaesthesia on single or multiple occasions before the age of 3 with children never exposed. The testing consists of an extensive series of different neurocognitive tests, including the Operant Battery Test (OBT), which has been used to test cognitive performance in non-human primates.[103, 120] The project will sample 1.000 children from a cohort of all children born in Olmsted County, Minnesota, between the years 1994 and 2007 and the tests will be performed at the ages 8-12 or 15-19.[136]

3 Aims

Rationale for the thesis

As alluded to in the Introduction and Background sections, injection pain represents a substantial clinical dilemma in the context of intravenous induction of anaesthesia in children when propofol is used. Thus, there is an urgent need to find new alternatives how to reduce injection pain to acceptably low levels in this setting. Furthermore, new alternatives must be investigated with regards to the timely issue of potential neurotoxicity following exposure to anaesthetics in early life.

Specific Aims

- To compare the incidence of injection pain between the former standard LCT propofol + lidocaine with the new MCT-LCT propofol formulae as plain solution (study I)
- To evaluate the incidence of injection pain between LCT propofol + lidocaine and etomidate dissolved in a fat emulsion (study II)
- To investigate if a small priming dose of etomidate can reduce the incidence of myoclonic movements (study III)
- To study the effects of neonatal etomidate exposure on cerebral apoptosis and adult behavioural effects in an established mouse model (study IV)

4 Material and methods

STUDY I-III

Full details can be found in the original articles.

General

Studies I-III were performed in accordance with the declaration of Helsinki and approved by the regional ethics review board in Stockholm. Parental and/or individual consent were obtained in all cases. The investigations were performed at Astrid Lindgren Children's Hospital.

Demographic data

In study I the computer generated randomization resulted in a significant difference regarding gender distribution between the two study groups. (22 girls in the propofol+lidocaine group compared to only 11 girls in the propofol-lipuro group) Demographic data in the study groups were similar in both study II and III.

Patient population

Study I

83 paediatric patients ASA 1-2 (healthy or mild systemic disease), age range 2-18 years and scheduled for elective day surgery were enrolled. The children were randomized to receive either propofol (Diprivan®) with added lidocaine (previous standard) or a new propofol formulation (Propofol-®Lipuro).

Study II

A total of 110 children ASA 1-2 (age range 2-16 years) scheduled for elective day surgery were planned to be included in the study. The patients were randomized to receive propofol with added lidocaine or the new lipid formulation of etomidate (Etomidate-®Lipuro). The Ethics Committee requested an interim analysis which was performed after 80 patients. Due to a clearly significant result in favour of etomidate the study was then stopped at the interim analysis point.

Study III

80 children ASA 1-2 (age range 1-15 years) scheduled for elective day surgery were randomized to receive either a small priming dose of etomidate or a lipid emulsion placebo. After the priming dose a standardized induction dose of etomidate was administered.

Anaesthetic protocol

Premedication

Following the application of an EMLA® patch (eutectic lidocaine/prilocaine 5 % patch) all patients had an intravenous cannula inserted on the dorsum of the hand. Before transfer from the day-care unit to the operating room intravenous midazolam (0. 05 mg/kg) was given as premedication. In study I the patients also received paracetamol (40 mg/kg) rectally (previous standard).

Anaesthetic methods

Study I

After standard monitoring was applied the anaesthetic induction was either by propofol with added lidocaine or plain propofol-lipuro. The coded syringes contained 10 ml propofol 10mg/ml with 1 ml lidocaine 10mg/ml added or propofol-lipuro 10 mg/ml as plain solution. The speed of the injection was similar for both study drugs, approximately 0.4 ml/sec, and the total dose given to the patients was 3 mg/kg.

Study II

The study drugs in study II were either propofol with added lidocaine or Etomidate-®Lipuro. The coded syringes contained either 10 ml propofol 20 mg/ml with added 1 ml lidocaine 20 mg/ml or Etomidate-®Lipuro 2mg/ml.
After application of non-invasive monitoring anaesthesia was induced by the injection of 0. 15 ml/kg of the study drug (propofol 3 mg/kg or etomidate 0. 3 mg/kg).

Study III

Before the patients received a standard induction dose of etomidate they had been randomized to either receive a priming dose of etomidate (0. 03 mg/kg = 0. 015 ml/kg) or a lipid placebo (0.015ml/kg) from a prefilled coded syringe. Sixty seconds later the anaesthesia was induced with etomidate (0. 3 mg/kg = 0. 15 ml/kg).

Assessment of Injection Pain

Studies I and II

One specially trained nurse anaesthetist (KvH) assessed injection pain according to a four-point scale: 1=no pain (no reaction to injection), 2=slight pain (minor verbal/facial response or motor reaction to injection), 3=moderate pain (clear verbal/facial response or motor reaction to injection) and 4=severe pain (the patient both complained of pain and withdrew the arm).[26] The assessment was made from the start of the injection to the point when the patient lost consciousness. In study II special care was taken to note the pain assessment immediately after the patient lost consciousness, thus, before possible myoclonic activity would poten-

tially occur. This was done in order to preserve proper blinding. After the assessments were performed, the study was terminated, and the anaesthetic was continued according to the planned surgical intervention.

Assessment of Myoclonic Movements

Studies II and III

In study II the myoclonic assessment was done immediately after the pain-scoring and during the following 1-2 minutes. In study III the occurrence and degree of myoclonic movements was recorded during a 2 min period after the induction dose was injected. In both study II and III a 0-3 point scale was used: 0= no myoclonic movements, 1=minor myoclonic movements, 2=moderate myoclonic movements, 3= major myoclonic activity.[41, 45] When the assessment of myoclonic activity had been performed, the study was stopped and anaesthesia was thereafter handled as appropriate for the scheduled surgery.

STUDY IV

A more detailed description of material and methods can be found in the original article.

General

Study IV was conducted in accordance with the European Communities Council Directive of 24th November 1986 (86/609/EEC) after approval from the local ethics committee (Uppsala University and Agricultural Research Council) and by the Swedish Committee for Ethical Experiments on Laboratory Animals.

Animals

Thirty-seven pregnant Naval Medical Research Institute (NMRI) mice were purchased from Scanbur, Sollentuna, Sweden. Within 48 hours the litters were adjusted to 10-14 pups to contain offspring of both sexes in approximately equal numbers. On postnatal day 10 (PND 10) the treatment drugs or control (saline) were administered by subcutaneous injection.

The study population consisted of six different treatment groups and each treatment group was derived from 3-6 different litters.

From the six different study groups six animals were randomly assigned for analysis of apoptosis.

The animals intended for neurochemical measurement of AC-3 were sacrificed by decapitation 24 hours after exposure and the brains were then dissected on an ice-cold glass plate. Cortex and hippocampus were collected and instantly frozen in liquid nitrogen. The brain regions were separately stored at -80° C until the final analyses were carried out.

Ten animals from each of the six treatment groups were randomly chosen for spontaneous behaviour testing at an age of two months. These animals were weaned at the age of 4 weeks and then raised in groups of 6-7 animals in a room for male mice only.

To compare with previous studies only male offspring were used for the neurochemical and behavioural recordings.

Treatment and drugs

The male pups were on PND 10 injected with 0.3, 3 or 10 mg/kg etomidate, 60 mg/kg propofol, 50 mg/kg ketamine or placebo (saline) by subcutaneous injection in the neck in a volume of 5 ml/kg. The doses of propofol and ketamine were chosen in order to allow comparison with earlier studies. The drugs were given subcutaneously and not intravenously due to the small size of the animals. The pups weigh at PND 10 approximately 4-6 grams.

Apoptosis analysis

Cerebral cortex and hippocampus were homogenized and centrifuged. In order to relate the concentration of AC-3 to the total protein concentration in the samples a Bicinchoninic Acid Protein (BCA) assay was done and the total protein content was calculated. Then AC-3 was measured with a sandwich enzyme immunoassay (ELISA) kit according to the instructions from the manufacturer.

Behaviour analysis

The spontaneous behaviour in a novel home environment test was performed at the age of 2 months. The animals were tested between 8 a.m. and 12 p.m. under the same light and temperature conditions as their housing conditions. A total of 10 mice were randomly picked from 3-4 different litters in each treatment group. Motor activity was measured for a 60-min period, divided into 3 × 20 min periods (0-20, 20-40 and 40-60 min), in an automated device consisting of 12 cages (40 × 25 ×15 cm) placed within two series of low and high infrared beams (Rat-O-Matic, ADEA Elektronik AB, Uppsala, Sweden).[137]

Three behavioural variables were measured:

Locomotion: Counting took place when the mouse moved horizontally through the low-level (10 mm above the bedding material) grid of infrared beams.

Rearing: Movement in vertical plane was registered when the high-level (80 mm above the bedding material) grid of infrared beams was interrupted.

Total activity: All types of vibration within the cage, i.e. those caused by the animal movements, shaking (tremors) and grooming, were registered by a sensor (a needle mounted on a lever with a counter weight), connected to the test cage.

Statistical procedures

Power calculation

Study I

Based on our initial clinical experience and the opportunity to detect a 25% difference in the primary endpoint between groups with an alpha-value <0.05 and a beta-value of 80%, a sample size of 40 patients was generated for each group.

Study II

The primary end point of the study was the presence (score 1–3) or absence (score 0) of injection pain. In accordance with our previous findings, the power calculation was based on a conservative 25% incidence of pain in the propofol– lidocaine group and an expected pain incidence of 5% in the etomidate-lipuro group. The alpha- and beta-values were set at 0.05 and 90%, respectively. With compensation for a limited number of potential drop-out cases the total number of patients was estimated at 110 (55 patients in each study group). However, the Ethics Committee demanded an interim analysis so that patients would not be subjected to unnecessary injection pain if there was a difference in pain incidence between the two study groups. Thus, it was decided to include an interim analysis following 80 patients and if a statistical difference at P<0.02 was present between the two study groups at this interim point the study should be stopped.

Study III

A power calculation was performed based on data from study II[54] and adult data reported by Doenicke and colleagues.[45] The primary goal of the study was to decrease the incidence of MM from 80% to 40% with alpha- and beta -values set at 0.05 and 90%, respectively. On the basis of these parameters, a total study size of 80 patients (40 patients per group) was suggested.

Statistical analysis

Study I-II-III

Non-parametric statistical procedures were used in all the analyses. The 95% confidence intervals (95% CIs) for proportions were calculated as given in Ott and Mendenhall.[138] Classified data from two independent populations were compared by Fisher's exact test. Classified data from several independent populations were compared by the x chi2 test for independence. The Wilcoxon matched-pairs signed-ranks test was used for comparison of two independent data sets. Several independent data sets were evaluated by the Kruskal–Wallis test with Dunn's post-test. Correlations were assessed by the Spearman rank correlation test. The tests were two-tailed and P-values of < 0.05 are described as statistically significant.

Statistical analysis

Study IV

Evaluation of the AC-3 analysis of apoptosis was made using one-way ANOVA (analysis of variance), pairwise testing with Tukey's HSD (honestly significant difference) post hoc test.

The data from the spontaneous behaviour tests in a novel home environment were subjected to a split-plot ANOVA design and pairwise testing was performed using Tukey's HSD post hoc test.

5 Results

Results from Study I

A significantly higher number of patients in group pD (LCT-propofol+ lidocaine) were pain free (pain score=0) during the injection compared with group pL (MCT-LCT propofol) (61.0% vs 33.3%) (P=0.016). *(Figure 2)*

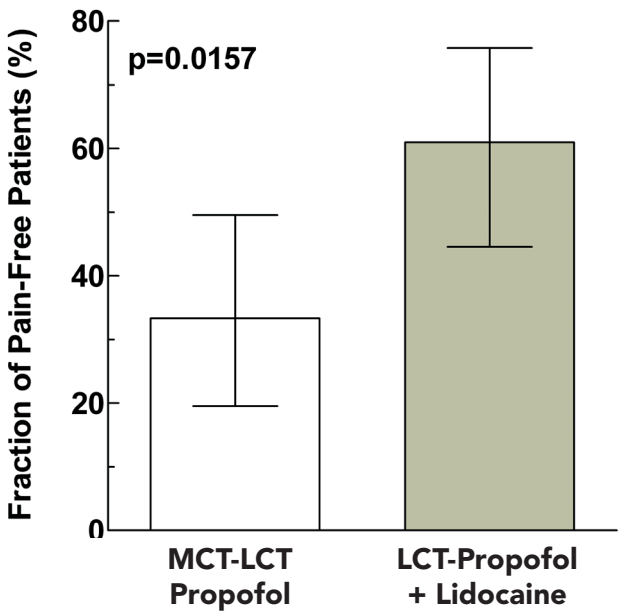

Figure 2
Children with pain-free injection of propofol

Results from Study II

The incidence of injection pain, the primary end point of the study, was significantly lower in the Etomidate-®Lipuro group (5.0%; 95 % CI 0.61–16.9%) compared with the LCT- propofol+lidocaine group (47.5%; 95 % CI 31.5 - 63.9%) (P<0.001). *(The distribution of pain scores is shown in Figure3)*

A higher incidence of myoclonic activity was seen in the Etomidate-®Lipuro group (85.0%; 95 % CI 70.2–94.3%) compared with the propofol+lidocaine group (15.0%; 95 % C 5.7–29.9%) (P<0.001).

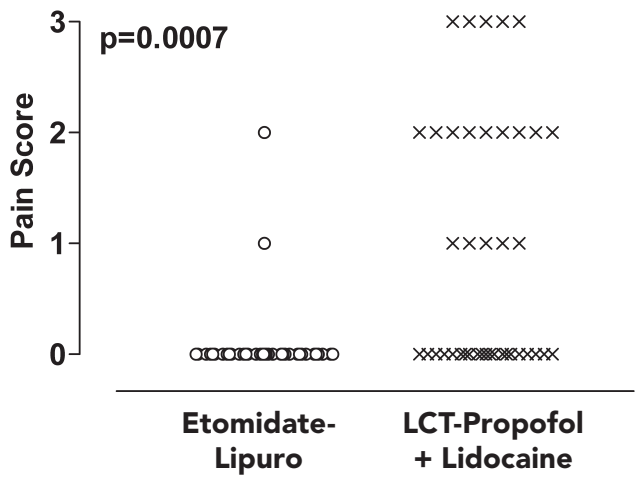

Figure 3
Incidence of injection pain, Etomidate-®Lipuro vs LCT-propofol with lidocaine

Results from Study III

Neither the total incidence nor the degree of MM was found to differ between the two study groups. Incidences of MM (score> 0) were observed in 75.0% and 72.5% of the patients in the etomidate and placebo groups, respectively. The total incidence of MM being 73.8% (95 % CI: 62.7–83.0%). Thus, no effect of the priming dose was seen.

The incidence of MM (score > 0) differed significantly between the age groups (P=0.0014) and was found to be significantly higher in the age group 5–10 yrs. (90.2%; 95 % CI: 76.8–97.3%) compared with both younger (<5 yrs., 47.1%; 95 % CI: 23.0–72.2%; P=0.0008) and older children (>10 yrs., 63.6%; 95% CI: 40.7–82.8%; P=0.01730). *(Figure 4)*

The MM scores were highest in patients aged 5-10 years (P=0.0021).

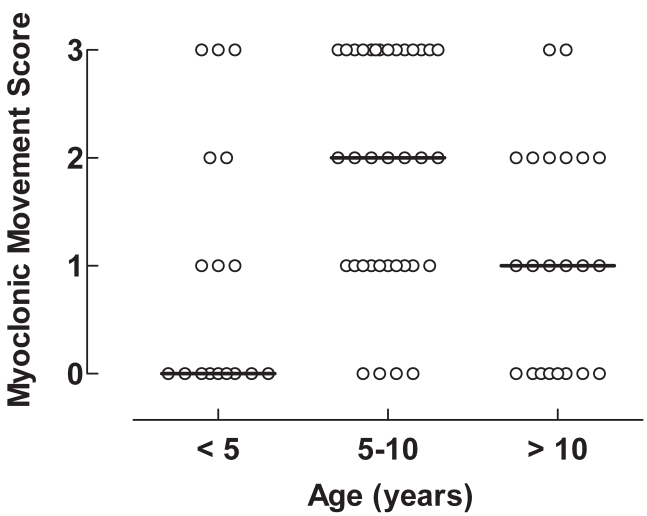

Figure 4
Distribution of myoclonic movements in three age groups of patients.

Results from Study IV

Neonatal exposure to etomidate, propofol or ketamine did not affect the concentration of AC-3 in neonatal cortex or hippocampus, compared to the control group.

In the control group there was a distinct decrease in activity in all the three behavioural variables over the three consecutive 20-min. periods (as expected). The three etomidate groups (0.3, 3 or 10 mg/kg) and the propofol group did not differ from the control group during any of the three consecutive 20-min periods. Mice exposed to ketamine showed a significantly reduced activity for all the three behavioural variables, locomotion, rearing and total activity, during the first 20-min period (0–20) compared to the control animals and all the other treatment groups. During the third (40–60) 20-min period, the ketamine exposed animals displayed a significantly higher activity for all the three test variables, compared to the control group and the other treatment groups. *(Figure 5)*

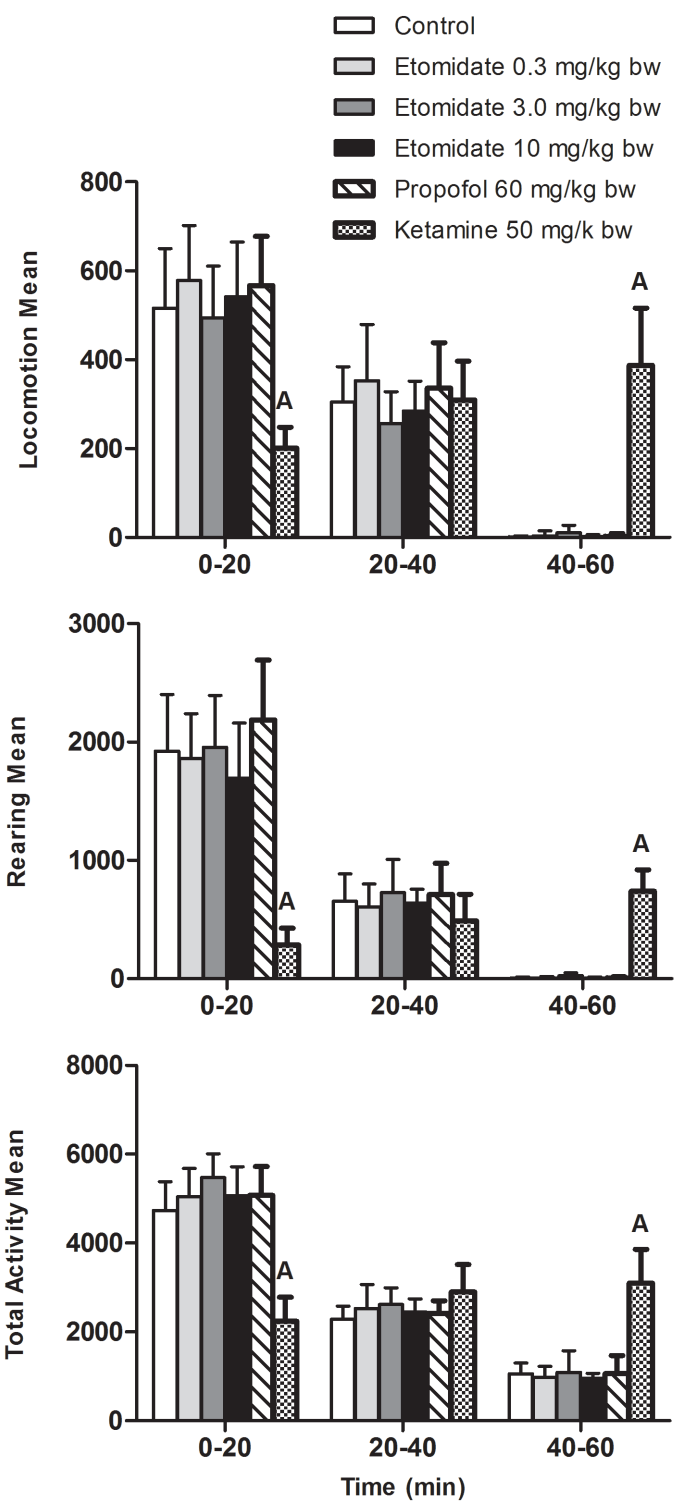

Figure 5
Spontaneous motor activity of young adult mice exposed to either saline (control), etomidate 0.3. 3 or 10 mg/kg, propofol 50 mg/kg or ketamine 50 mg/kg on postnatal day 10. Statistical significance is indicated by A ($p<0.01$) vs all other treatment groups.

6 Discussion

Aspects on the use of propofol vs. etomidate in paediatric anaesthesia and paediatric intensive care

Due to the many desirable characteristics of propofol compared to previously existing alternatives this compound has become the "gold standard" for intravenous induction of anaesthesia. However, from a paediatric perspective the routine use of propofol is associated with some limitations. Below a comparative discussion regarding the use of propofol and etomidate, essentially from a paediatric perspective, is provided.

General considerations

Prolonged intravenous infusions

Propofol

Propofol Infusion Syndrome (PRIS): The exact mechanism of PRIS is still unknown but is believed to be associated with failure of mitochondrial respiration and defects of fatty acid oxidation.[139] A recently published rodent study shows that propofol interrupts the electron flow in the respiratory chain mainly at the site of coenzyme Q.[140] There is no diagnostic test for PRIS which is always a presumed diagnosis and when other causes to the symptoms are ruled out. The treatment is rapid recognition and removal of the agent. Otherwise, the treatment is mainly supportive by the use of inotropic and vasopressor agents and aggressive electrolyte control. Haemodialysis, haemofiltration, extracorporeal membrane oxygenation (ECMO)[141] and blood exchange transfusion[142] have in case reports been described to be effective.

The mortality from PRIS is high – according to earlier reports and studies ranging from 64% [143] to 73%.[144] Intially PRIS was assumed to only appear in the paediatric intensive care unit (PICU). A typical patient was a mechanically ventilated child with a respiratory infection who received a high dose and a prolonged infusion of propofol.[13,14] Currently it is believed that PRIS is more often seen in the adult patient who during intensive care is sedated by a normal dose of propofol. Mortality rate among reported adult cases of PRIS seem to have decreased over time and is now reported to be approximately 50%.[145]

Although rare, but potentially lethal, the risk for this very serious complication has prompted the American and English health authorities to ban the use of continuous propofol infusions for PICU sedation.[146, 147]

The current recommendation by Swedish Society for Anaesthesiology and Intensive Care (SFAI) is that propofol-sedations longer than 24 hours and doses > 3 mg/kg/h should be performed only in "exceptional cases".[148]

Most often PRIS have been described to occur after prolonged sedation with high doses. However, PRIS may not be exclusively associated with extended PICU or ICU sedation since there have been case reports indicating that PRIS may also be associated with more time limited infusions of propofol.[19,145,149-152] This may be a concern even when using propofol in a strict paediatric anaesthesia context.

Etomidate

Inhibition of corticosteroid synthesis during prolonged etomidate infusions: Soon after the clinical introduction of etomidate it became popular not only for induction of anaesthesia but also for continuous ICU sedation. However, a seminal publication in 1983 by Ledingham indicated that prolonged infusions of etomidate appeared to be associated with increased mortality compared to previous sedation regimens.[153] Soon thereafter it was shown that etomidate does interfere with a specific step in the synthesis of corticosteroids in the adrenal cortex.[55, 154] Due to these findings and the reports of a higher mortality rate among intensive patients that had received etomidate, the clinical use of the drug was substantially reduced. However, due to the excellent haemodynamic stability associated with the use of etomidate, this compound remained the drug of choice in certain centres for induction of anaesthesia and endotracheal intubation in haemodynamically compromised patients as well as trauma victims.[155, 156]

A single induction dose of etomidate is generally believed not to interfere with corticosteroid levels in any relevant manner,[157-159] although there does exist an on-going debate if etomidate can be safely used as an induction agent for endotracheal intubation in adults with pronounced sepsis.[160-164] A multicentre randomized controlled trial that compared etomidate and ketamine for intubation in critically ill patients found no difference in mortality.[159] A recently published meta-analysis reported data from 18 different studies (2 RCTs, 16 observational studies), with over 5.000 patients included.[59] The major finding was that a single dose of etomidate did not increase mortality in patients with sepsis.

Inhibition of corticosteroid synthesis after continuous infusion of etomidate has also been shown to occur in children. Murat and co-workers found clearly reduced levels of cortisol and aldosterone following an average infusion time of 2.8 hours (mean total dose of etomidate 4.6 mg/kg). There was furthermore a prolonged rise of the precursors to cortisol and aldosterone; 11-deoxycortisol and

11-deoxycorticosterone.[165] Although never properly studied (c.f. future perspectives), a single induction dose of etomidate is most likely of no clinical relevance regarding cortisol levels in the vast majority of paediatric patients. In a study published 2015 cortisol levels after a single dose propofol or etomidate were measured in paediatric patients undergoing urologic surgery.[58] The cortisol levels, which were assessed by serial salivary samples, were suppressed in the etomidate group and lasted approximately 24 hours. However, no changes in clinical outcome were observed.

In clinical practice today it is quite common to administer a dose of dexamethasone in order to prevent postoperative nausea and vomiting (PONV). This procedure is commonly regarded as totally harmless and leads to a similar suppression of the hypothalamic-pituitary-adrenal axis as when etomidate is given as a single dose.[166]

Several etomidate analogues are being developed in the attempt to minimize the unwanted side effects of etomidate. The currently most promising is cyclopropyl-methoxycarbonyl metomidate (CPMM) which in animal studies (dogs) have shown to maintain the favourable properties but with less adrenocortical suppression compared to etomidate.[167] Perhaps this drug will be an interesting alternative in the future.

Haemodynamic stability

Propofol

In healthy adults (ASA 1-2) the haemodynamic effects of a normal induction dose of propofol is usually well tolerated. However, in haemodynamically compromised patients or in individuals with significant co-morbidities (ASA 3-4) propofol may have very severe haemodynamic consequences.[168-171]

The mechanism for the decrease in blood pressure is mainly due to arterial vasodilatation caused by a reduced vascular sympathetic tone but is also due to a direct effect on myocardial contractility.[172, 173]

The reduction in systemic vascular resistance may be an important effect in children with congenital heart disease (CHD). In children with a cyanotic cardiac shunt the reduction in systemic vascular resistance can lead to a fall in the ratio of pulmonary to systemic blood flow, causing desaturation.[174] It is therefore advisable to use propofol with great caution when anaesthetizing paediatric cardiac patients.[175]

The general paediatric patient population usually does not suffer from any major haemodynamic side effects following the use of propofol but if used in premature or new-born babies quite dramatic hypotension may result.[7, 8, 10, 176] The duration of such hypotension can be rather prolonged (60 min) and appears to outlast the anaesthetic effect of a normal bolus dose of propofol.[9]

Thus, the use of propofol as an induction agent in these patient categories seems unwise.

Etomidate

This drug is commonly regarded as being associated with the highest degree of cardiovascular stability among the anaesthesia induction agents,[177] causing even less effects on the cardiovascular system than ketamine. For this reason etomidate is often selected when anesthetizing haemodynamically compromised patients, including certain paediatric patients with CHD.[175, 178, 179] This group of patients may require anaesthesia for various procedures, non-cardiac or cardiac surgery and in this context etomidate is a preferred option compared to propofol.[175]

Furthermore, etomidate is also often the preferred drug in paediatric emergency departments when a rapid intubation is required.[180, 181]

The haemodynamic effects have so far not been properly studied in premature babies and neonates (c.f. future perspectives). However, to date there has not been any published data indicating that the use of etomidate in these patient categories is associated with any negative haemodynamic consequences.

More thesis-specific considerations

Pain on injection

The cause of propofol-induced injection pain is not fully understood. It has traditionally been believed to be caused by a direct stimulation of pain receptors and/or an indirect stimulation leading to release of different mediators causing a late onset of pain.[27] More recent findings suggest that the TRPA1 receptor (transient receptor potential cation channel subfamily A member 1, also known as the "wasabi-receptor") is a main mediator of propofol-induced injection pain. TRPA1 resides in the cellular membrane of sensory nerve cells and is triggered by exogenous agents (e.g. wasabi or poison ivy) or by endogenous signals from tissue damage and inflammation.[182-184]

Whatever the origin; the problem is still there. Several hundreds of various studies have been performed and are still carried out in an attempt to diminish or hopefully abolish propofol injection pain. (See background) The only proven way to reduce the incidence and intensity of the pain is to add the local anaesthetic lidocaine – either as a pre-treatment with venous occlusion or as an admixture.[2, 3, 28]

In the paediatric context injection pain is of course especially troublesome. It is a well-known fact that children are often frightened by needles and cannulas. In this setting it appears illogical to use EMLA® and various distraction techniques to make the insertion of the intravenous cannula as pain-free as possible if you subsequently will inject "fire" into it.

During the induction of anaesthesia it is not possible for children to adequately express their degree of pain and therefore most of the paediatric studies performed have an investigator-based pain evaluation assessing parameters e.g. arm withdrawal, grimacing, crying or screaming. Since the paediatric studies use various methods to assess and record the incidence of pain and also have different study designs it is difficult to compare these studies with each other.

Adding the local anaesthetic lidocaine to propofol reduces the injection pain but the need for mixing drugs is always a hazard. Apart from the time and cost aspects there are risks for drug mixing errors but also interactions. The addition of lidocaine to propofol is reported to cause a time and dose-dependent emulsion droplet enlargement that may cause pulmonary embolism.[185, 186] Furthermore, mixing drugs will increase the risk for bacterial contamination, something that may be especially problematic regarding lipid solutions since they are prone to bacterial growth.[187]

Since some of the adverse effects are ascribed to the lipid emulsion, (e.g. hyperlipidaemia, allergy risks, possible bacterial growth, embolism) efforts have been made to find other solvents for propofol. Fospropofol disodium is a water soluble prodrug of propofol which was approved by the United States Food and Drug Administration (FDA) in 2008 and marketed as Lusedra. No injection pain is reported but other adverse effects registered in a phase III trial were paraesthesia (47.6%) and pruritus (14.7%).[188] The onset time compared to propofol is 3-4 longer which makes it less suitable for anaesthesia induction. Lusedra has recently been withdrawn from the US market and results from a Chinese fospropofol phase II trial lend further support for this. This study showed a slow onset of the drug and the occurrence of pruritus and or paraesthesia in 95 % of the subjects[189] which clearly makes it inferior to propofol.

Different micro-emulsions of propofol such as Aquafol have also been developed in the attempt to eliminate adverse effects of the lipid emulsions,[190, 191] but these drugs cause even more frequent and severe injection pain compared to traditional propofol.[190]

Incidence of involuntary myoclonic movements

Many intravenous induction agents, including etomidate and propofol, may cause the occurrence of involuntary myoclonic activity within the first minutes following injection. The actual mechanism responsible for this is not fully elucidated but is generally believed to be associated with interference with inhibitory neurons in the brain stem. The phenomenon is called disinhibition – a suppression of cortical activity before depression of subcortical activity which leads to abnormal movements.[45, 52, 192]

Another closely related subcortical phenomenon is the nocturnal myoclonus

(periodic movements in sleep) that occurs in almost every healthy human as well as in other mammals.[193]

There is currently no indication that myoclonic activity is associated with any negative effects for the already anesthetized patient, but it may somewhat interfere with the assessment of anaesthetic depth and the occurrence of MM may be perceived as unpleasant by the accompanying parent. Thus, despite not providing any added patient benefit, it may still be desirable to reduce the occurrence of myoclonic activity following induction of anaesthesia.

The use of etomidate appears to be associated with the highest incidence of myoclonic activity of the commonly used induction agents.[52] The incidence of MM at induction of anaesthesia in adults who have not received premedication has been reported to be within the 50-80 % range.[41, 45]

In adults various attempts have been made to reduce the incidence of MM. Administration of opioids, benzodiazepines and the α2-adrenoceptoragonist dexmedetomidine prior to the injection of etomidate has been reported to reduce but not eliminate the occurrence of MM.[41, 48, 50] Pre-treatment with magnesium sulfate[194] and lidocaine[195] have also been shown to reduce the incidence and intensity of the myoclonic activity. To administer a small "priming" dose of etomidate a few minutes before the induction dose has been found effective in adults, reducing the incidence of MM from 75 % - 87 % (placebo) to only 26 %.[45, 49]

The incidence of MM was assessed as a secondary end point in Study II. In line with the findings in adults a considerably higher incidence of MM was observed in children induced with etomidate (85 %) compared to propofol (15 %).[54] Thus, the incidence of MM following induction of anaesthesia with etomidate does not appear to differ from what is observed in adults.[41, 45, 49]

In Study III we investigated whether the concept of using a priming dose of etomidate would be effective in reducing the incidence of MM also in paediatric patients. Contrary to the findings in adults we were unable to find any beneficial effect of a priming dose when compared to Intralipid® placebo. Why the effect of a priming dose of etomidate is not useful in children but has been found effective in adults is difficult to explain. However, despite using the same relative amount for the priming dose in children as in adults (10 % of the normal induction dose) it may be speculated that the dose used in Study III was too small to influence the incidence of myoclonic movements (for further argument regarding this issue please see Discussion section Study III).

As an interesting post-hoc finding in Study III we could identify a specific age range (5-10 years of age) that appeared especially prone to the development of MM. The underlying reason for the higher incidence of MM in this age group is currently unknown. Maybe a disparity in body composition, neurologic development or pharmacokinetics can be responsible for this observation.

Apoptosis and long term behavioural and cognitive effects in animal studies

Since the ground-breaking studies by Ikonomidou et al. in 1999, followed by Jevtovic-Todorovic et al. in 2003, countless animal studies have been performed and published. A variety of research groups have documented different deleterious effects from anaesthetic exposure in several animal species ranging from nematodes to rhesus monkeys. It is generally believed that the longer the exposure the more severe will the damage be. Furthermore, when several anaesthetics that act on different targets are combined the toxic effects seem to be intensified.[90, 110] Despite these observations the key question remains: Is the measured cell-death found in all these preclinical animal studies of any clinical relevance?

If you consider the total cell number of the immature brain, anaesthetic exposure will only produce a very limited enhancement of apoptotic cells compared to the normal on-going programmed cell death. In a report from Istaphanous et al.[100] they found that 2 % of the cells in layer II/III of the cortical cortex expressed apoptotic markers after a 6 hour isoflurane anaesthetic. If extrapolated to the whole cortex this represents only a very small total number of cells. Brambrink et al. counted 30-40 apoptotic cells out of 100 000 in the neonatal rhesus monkey brain after a 5 hour long isoflurane exposure.[196] Thus, the magnitude of enhanced apoptosis is small and has been estimated to less than 0.1 % of the total number of cerebral neurons.[197]

Some of the animal studies examining cellular degeneration have also assessed neurocognitive outcome but here the results are conflicting. No definitive causal relation between anaesthesia-induced neuroapoptosis and subsequently altered neurocognitive development has yet been demonstrated. A recent animal study in fact does demonstrate the opposite. In an investigation by Lee et al.[198] isoflurane exposure to new-born rats caused a huge difference in post-anaesthesia behaviour between males and females despite no sex differences in apoptosis.

The results from animal studies can of course not be directly translated to humans. Even when accounting for body size and different metabolic rates among species, the doses or the duration of the exposure in animal studies tend to be higher than what is normally used for humans. For obvious reasons the small size of the neonatal rodents preclude continuous haemodynamic monitoring and repeated blood tests, which is standard in clinical anaesthesia for human. Therefore, some of the adverse effects that are attributed to the anaesthesia can be an effect of hypoxia, hypotension, hypocapnia or hypoglycemia. In a study by Wu et al. 14 day old rats were anesthetized with sevoflurane or isoflurane and were then either mechanically ventilated or left spontaneously breathing.[199] The spontaneous breathing rats had significantly higher mortality, neuroapoptosis and impaired neurocognitive outcome compared to the mechanically ventilated rats, findings that highlight the im-

portance of the above mentioned issues.

In animal studies the histological brain injury thought to be induced by anaesthetics can be assessed by histological methods, which for obvious reasons is not possible in children. Thus, to address the question if anaesthesia possess neurotoxic effects, which may be harmful to the paediatric population, needs to be addressed by prospective randomized clinical trials (please see Background).

Strengths and limitations of the dissertation studies

In Studies I-III a similar prospective, double blind, randomized controlled design, based on realistic power calculations, was used. In our mind this should be viewed as a considerable strength. The fact that these studies all were performed in a general paediatric ambulatory surgical setting, including the typical patient populations that represent a majority of everyday paediatric surgery, make our results easy to generalize for paediatric anaesthesia practice in general.

A certain limitation regarding Studies I-III is that the evaluation scores used have not undergone proper validation. In contrast to other assessment scores, it is not really realistic to accomplish this in the context of anaesthesia induction. However, we have used assessment scores that have been used in previously published studies.[26, 41, 45] (for details please see individual Studies I-III)

In Study IV a very well-established and robust animal model was used that should be considered to be advantageous. To investigate potential apoptosis in larger parts of the brain did appear more relevant to us than focusing on small and exceptionally vulnerable areas that probably do not have any effects on long-term outcome. Furthermore, to expose the mice at PND 10 does much better translate to exposure in human early infancy when the majority of major surgical interventions for birth defects and malformations are performed. On the contrary, exposure at PND 7 which has been the norm in a large part of the published literature is equal to an extremely premature human baby and, does not mirror the correct time for major neonatal surgery in human babies.[83, 200]

One limitation regarding study IV is that some proteins, such as caspases, are expressed only transiently. The time from initiation of apoptosis to completion can occur as quickly as 2–3 hours. Therefore a false negative result can occur if the assay is done too early or too late. It is then reassuring that the behavioural testing did not show any difference between the etomidate-exposed group and the control group.

As is the case with all studies within this field that has been performed in neonatal rodents, there are a number of limitations that should be recognised, e.g. species differences, dosage issues, and lack of proper monitoring of physiologic parameters. For a more detailed discussion relating to this, please see the Discussion section of Study IV.

Future perspectives/Further studies

The current thesis indicates that etomidate is a valid alternative for intravenous induction of anaesthesia in the paediatric population. Although etomidate can be used in clinical practice without any major hesitation there are still a number of issues that need to be clarified.

First, the effect of a single induction dose of etomidate on corticosteroid synthesis needs to be further elucidated. Initially the effect in healthy children (ASA 1-2) should be investigated followed by studies performed in sicker patients, e.g. ASA 3-4 individuals, trauma victims and septic paediatric patients. If any clinically relevant suppression of cortisol synthesis should emerge in the more vulnerable patient population it may be recommended to follow plasma cortisol after the use of etomidate. If low cortisol levels is detected it can be treated with relevant substitution by i.v. cortisone.

Second, to hopefully verify that etomidate is associated with better haemodynamic stability compared to propofol also in the vulnerable group of premature babies. This would be welcomed since cardiovascular instability in these patients will affect morbidity, e.g. the incidence of intraventricular haemorrhage and leukomalacia.[201]

Third, further studies of interventions that may reduce the incidence of myoclonic movements are warranted.

The current debate questions the overall validity of animal studies regarding potential neurotoxicity of exposure to anaesthetics in early life.[200, 202, 203] However, further animal studies are needed to identify various approaches that may ameliorate or even inhibit the negative effects that have been shown for most anaesthetics. The use of alpha-2 adrenoceptor agonists has been identified as one possibility in this regard[112, 204] but the study of other possible therapeutic options would be much welcomed. Also, the further development of new alternatives to the currently available agents, e.g. the etomidate analogue CPMM which seem to lack the negative effect on the steroid synthesis is another option.[167]

All in all, we believe that etomidate can establish itself as a useful alternative for intravenous induction of anaesthesia in children.

7 Conclusions

From the studies of which this thesis consists the following conclusions are drawn.

Study I

A new MCT-LCT propofol formulation as a plain solution is associated with a higher incidence of injection pain compared to LCT propofol with added lidocaine when used for induction of anaesthesia in children.

Study II

The use of a new lipid formulation of etomidate is associated with significantly less injection pain than propofol with added lidocaine when used for induction of anaesthesia in children.

Study III

The use of a small, non-sedative, priming dose does not influence the incidence of involuntary myoclonic movements after i.v. induction of anaesthesia with etomidate in children.

Children in the age range of 5–10 years appear to be more prone to react with involuntary myoclonic movements after i.v. induction of anaesthesia with etomidate than other paediatric age groups.

Study IV

A single dose of etomidate in early infant mice at PND 10 does not produce evidence of enhanced cerebral apoptosis or impaired adult motor behaviour.

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