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STUDIES ON NEUROMUSCULAR BLOCKING AGENTS AND THEIR ANTAGONISTS DURING ANAESTHESIA

by

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I see no good reasons why the views given in this volume should shock the religious sensibilities of anyone

Charles Darwin 1869

ABSTRACT

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STUDIES ON NEUROMUSCULAR BLOCKING AGENTS AND THEIR ANTAGONISTS DURING ANAESTHESIA

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Neuromuscular blocking agents (NMBAs) are widely used in clinical anaesthesia and emergency medicine. Main objectives are to facilitate endotracheal intubation and to allow surgery by reducing muscle tone and eliminating sudden movements, which may otherwise lead to trauma and complications. The most commonly used NMBAs are non-depolarizing agents with a medium duration of action, such as rocuronium and cisatracurium. They bind to the acetylcholine receptors in the neuromuscular junction, thus inhibiting the depolarization of the postsynaptic (muscular) membrane, which is a prerequisite for muscle contraction to take place.

Previously, it has been assumed that nitrous oxide (N_2O), which is commonly used in combination with volatile or intravenous anaesthetics during general anaesthesia, has no effect on NMBAs. Several studies have since claimed that N_2O in fact does increase the effect of NMBAs when using bolus administration of the relaxant. The effect of N_2O on the infusion requirements of two NMBAs (rocuronium and cisatracurium) with completely different molecular structure and pharmacological properties was assessed. A closed-loop feedback controlled infusion of NMBA with duration of at least 90 minutes at a 90% level of neuromuscular block was used. All patients received total intravenous anaesthesia (TIVA) with propofol and remifentanil. In both studies the study group (n=35) received N_2O/Oxygen and the control group (n=35) Air/Oxygen. There were no significant differences in the mean steady state infusion requirements of NMBA (rocuronium in Study I; cisatracurium in Study II) between the groups in either study.

In Study III the duration of *the unsafe period of recovery* after reversal of rocuronium-induced neuromuscular block by using neostigmine or sugammadex as a reversal agent was analyzed. *The unsafe period of recov*ery was defined as the time elapsed from the moment of no clinical (visual) fade in the train-of-four (TOF) sequence until an objectively measured TOF-ratio of 0.90 was achieved. The duration of these periods were 10.3 ± 5.5 and 0.3 ± 0.3 min after neostigmine and sugammadex, respectively ($P < 0.001$). Study IV investigated the possible effect of reversal of a rocuronium NMB by sugammadex on depth of anaesthesia as indicated by the bispectral index and entropy levels in thirty patients. Sugammadex did not affect the level of anaesthesia as determined by EEG-derived indices of anaesthetic depth such as the bispectral index and entropy.

Key words: rocuronium, sugammadex, cisatracurium, nitrous oxide, reversal

TIIVISTELMÄ

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LIHASRELAKSANTIT JA NIIDEN ANTAGONISTIT YLEISANESTESIAN AIKANA

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Lihasrelaksantteja käytetään yleisesti yleisanestesian aikana ja ensihoidossa sekä helpottamaan intubaatiota, että kirurgian läpiviemistä. Lihasrelaksaation avulla pystytään vähentämään lihastonusta ja estämään äkkinäisiä liikkeitä, joista saattaisi muuten aiheutua leikkaus-komplikaatioita. Tavallisimmin käytetään ei-depolarisoivia, keskipitkävaikutteisia aineita, kuten rokuronia tai sisatrakuuria. Nämä lääkeaineet sitoutuvat hermolihasliitoksen asetyylikoliini-reseptoriin, jonka seurauksena postsynaptisen kalvon depolarisaatio estyy. Tämä puolestaan johtaa lihaksen supistumisen estoon.

Typpioksiduulia eli ilokaasua (N_2O) käytetään yleisesti yleisanestesian aikana yhdessä laskimonsisäisten tai haihtuvien anestesia-aineiden kanssa. Typpioksiduulilla ei ole aikaisemmin luultu olevan vaikutusta lihasrelaksaatioon. Muutaman viime vuosina tehdyn tutkimuksen mukaan typpioksiduuli kuitenkin lisää boluksena annostellun lihasrelaksantin vaikutuksia. Tämän väitöskirjatutkimuksen kahdessa ensimmäisessä osatyössä selvitettiin typpioksiduulin vaikutusta kahden molekyylirakenteeltaan erilaisen lihasrelaksantin (rokuronin ja sisatrakuurin) annostarpeeseen. Lihasrelaksantin annosteluun käytettiin tietokoneohjattua infuusiota vähintään 90 minuutin ajan. Lihasrelaksaatio pidettiin infuusion avulla 90 %:n tasolla. Kaikki potilaat saivat laskimonsisäisen anestesian (TIVA) propofolilla ja remifentaniililla. Molemmissa tutkimuksissa tutkimusryhmä (n=35) sai typpioksiduulia hapen kanssa ja kontrolliryhmä (n=35) sai happi-ilmaseosta. Kummassakaan tutkimuksessa ei todettu ryhmien välillä merkitseviä eroja niin sanotussa steady state vaiheessa infusoidun relaksantin (osatyössä I rokuroni; osatyössä II sisatrakuuri) tarpeessa.

Osatyössä III selvitettiin ns. *unsafe period of recovery* -ajanjakson pituutta neostigmiinilla tai sugammadeksilla kumotun rokuronirelaksaation yhteydessä. Väitöskirjatyössä käytetyn määritelmän mukaan termi "*unsafe period of recovery"* kuvaa aikaa, joka kuluu siitä hetkestä, kun visuaalisesti arvioituna neljän sarja -vasteen heikkenemistä ei ole enää havaittavissa siihen hetkeen, jolloin objektiivisella lihasvoiman mittarilla mitataan TOF-suhde 0.90. Neostigmiinin jälkeen tähän kului aikaa 10.3 ± 5.5 ja sugammadeksin jälkeen 0.3 ± 0.3 min ($P < 0.001$). Osatyössä IV tutkittiin, vaikuttaako rokuronilla aikaansaadun lihasrelaksaation nopea kumoaminen sugammadeksilla anestesian syvyyteen. Anestesiasyvyyttä arvioitiin käyttämällä bispektraali-indeksiä ja entropiaa. Tämän tutkimuksen mukaan sugammadeksilla ei ole vaikutusta näiden mittarien avulla mitattuun anestesian riittävyyteen.

Avainsanat: rokuroni, sugammadeksi, sisatrakuuri, typpioksiduuli, lihasrelaksaation kumoaminen

CONTENTS

ABBREVIATIONS

ACh	Acetylcholine
AChE	Acetylcholinesterase
AChR	Acetylcholine receptor
AEP	Auditory evoked potentials
AMG	Acceleromyography
AP	Action potential
ASA	American Society of Anesthesiologists
α-unit	Alfa-unit; the specific binding site for ACh at the AChR
BIS	Bispectral index
BMI	Body Mass Index
CICV	'Cannot intubate, cannot ventilate'
CNS	Central nervous system
DBS	Double burst stimulation
ED_{50}	The 50% effective dose
ED_{95}	The 95% effective dose
EEG	Electroencephalogram
EMG	Electromyography
FDA	United States Food and Drug Administration
GABA	γ-aminobutyric acid
Hz	Hertz; SI unit of frequency (cycles per second)
IBW	Ideal body weight
I_{ss}	Asymptotic steady state rate (of an infusion)
L	Litre(s)
mA	milliampere
MAP	Mean arterial pressure
min	Minutes

ms	Milliseconds
Na^+	Sodium ion
NMB	Neuromuscular block
NMBA	Neuromuscular blocking agent
NMDA	N-methyl-D-aspartic acid
N_2O	Nitrous Oxide
pCO_2	Partial pressure of carbon dioxide (in a gas mixture)
PNS	Peripheral Nerve Stimulator
PORC	Postoperative residual curarization
PTC	Post tetanic count
QTc	The QT interval (of the ECG) corrected for heart rate
RE	Response entropy
s	Second
SD	Standard deviation
SE	State entropy
$T_{1/2}$	Half-life
TCI	Target controlled infusion
TIVA	Total intravenous anaesthesia
TOF	Train-of-four
TR	Train-of-four ratio
V1	The central compartment
V2	The second (fast) compartment
V3	The third (slow) compartment

LIST OF ORIGINAL PUBLICATONS

This thesis is based on the following original publications, which are referred to in the text by Roman numerals I-IV.

I. Illman H, Antila H, Olkkola K. Quantitation of the Effect of Nitrous Oxide on Rocuronium Infusion Requirements Using Closed-loop Feedback Control. Anesthesiology 2008;108: 388-91

II. Illman H, Antila H, Olkkola K. Effect of Nitrous Oxide on Cisatracurium Infusion Demands: a Randomized Controlled Trial. BMC Anesthesiology 2010;10:14

III. Illman H, Laurila P, Antila H, Meretoja O, Alahuhta S, Olkkola K. Duration of Residual Neuromuscular Block after Administration of Neostigmine or Sugammadex at Two Visible Twitches During Train of Four Monitoring. Anesth Analg 2011;112:63-8

IV. Illman H, Antila H, Olkkola K. Reversal of Neuromuscular Blockade by Sugammadex Does not Affect EEG Derived Indices of Depth of Anesthesia. J Clin Monit Comput 2010;24:371–6

1. INTRODUCTION

Neuromuscular blocking drugs (NMBAs) are usually studied during intravenous anaesthesia supplemented with nitrous oxide (N_2O), as volatile anaesthetics have been documented to affect the pharmacodynamics of muscle relaxants (Olkkola and Tammisto 1994, Hemmerling et al. 2001). It has generally been presumed that N_2O does not affect neuromuscular blockade (NMB).

However, during recent years, several studies have claimed that following bolus administration of NMBAs, N_2O may actually potentiate their action (Plaud et al. 2002, Kopman et al. 2005a, Fiset et al. 1991). Because single doses of muscle relaxants are seldom sufficient for the entire duration of surgery, additional doses are usually needed. From a clinical point of view it might therefore be more appropriate to study the interaction between N_2O and NMBAs at steady state during their continuous administration. Computer-controlled infusion of NMBAs is a powerful clinical pharmacological tool in quantifying the drug interactions (Olkkola and Schwilden 1990, Kansanaho and Olkkola 1996a and b). It was therefore considered important to study the possible interaction of rocuronium (Study I) and cisatracurium (Study II) with N_2O using computer-controlled closed-loop administration of rocuronium and cisatracurium in patients undergoing surgery.

Several objective, i.e. quantitative, methods for assessing and monitoring the degree of NMB have been developed over time. Nevertheless, worldwide most clinicians use subjective assessment of the train-of-four response by using a simple peripheral nerve stimulator (PNS) or even rely merely on clinical signs when assessing neuromuscular recovery (Naguib et al. 2010, Donati 2010). These non-objective monitoring methods have been proven unreliable in numerous studies (Viby-Mogensen et al. 1985, Kopman et al. 2005b, Kirkegaard et al. 2002) and relying on such methods can be associated with an elevated risk for postoperative residual curarization (PORC). It has also been repeatedly shown that PORC (as defined by a train-of-four ratio < 0.9) is a common and widespread problem in postoperative care units (PACUs) around the world (Debaene at al. 2003, Cammu et al. 2006). PORC is associated with numerous adverse physiological effects that may increase the risk for postoperative morbidity. Despite this, routine pharmacological reversal of a NMB is still not used in many hospitals (Grayling and Sweeney 2007).

The novel reversal agent sugammadex has a completely different mechanism of action as compared to traditional reversal agents, such as the cholinesterase inhibitors, and is capable of rapid and complete reversal of a rocuronium NMB in 1-3 minutes. Neostigmine and other cholinesterase inhibitors have several limitations and are in practice incapable of reversing deeper levels of NMB sufficiently (Kopman et al. 2005b). It seems fair to assume that relying on subjective (visual or tactile) evaluation of a NMB by using a PNS and using neostigmine for reversal of an intermediate rocuronium block may be a dangerous combination, leading to an overestimation of the degree of neuromuscular recovery in some patients and putting them at risk for PORC- related complications. Although clinical studies have demonstrated that the recovery of neuromuscular function is significantly faster after sugammadex than neostigmine, there is no evidence that when relying on

PNS, the use of sugammadex in fact shortens the period when the patients are at risk for PORC. Further studies are obviously needed.

The data on the possible relationship between NMB and depth of anaesthesia is contradictory. A decrease in bispectral index (BIS), state entropy and auditory evoked potentials have been seen during NMB. According to the afferentation theory NMB prevents signals arising in muscle stretch receptors from accessing the brain and causing arousal (Lanier et al. 1994). Others (Bruhn et al. 2000a, Messner et al. 2003) state that NMB prevents disturbing EMG-signals arising from muscles of the forehead from falsely elevating BIS-levels. Neostigmine reversal is also linked to arousal in some patients (Vasella et al. 2005). However, several studies (Greif et al. 2002, Richmond et al. 1996, Vasella et al. 2005) have shown opposite results, i.e. no effect of neuromuscular blockade on the BIS- and AEP-levels. Because the information on the relationship between NMB and depth of anaesthesia as quantified by several EEG-derived indices is conflicting, it was considered timely and relevant to study the possible effect of sugammadex reversal of a rocuronium NMB on depth of anaesthesia as measured by the bispectral index and spectral entropy.

2. REVIEW OF THE LITERATURE

2.1. Neuromuscular physiology

2.1.1. Neuromuscular transmission

The neurons or nerve cells, i.e. the basic functional units of the nervous system, produce and conduct electrochemical impulses between various organs and the central nervous system (CNS). Afferent pathways conduct impulses from sensory receptors into the CNS, while the efferent motor neurons conduct impulses from the CNS to the skeletal muscles, smooth muscle and glands. Both reflex and voluntary control of skeletal muscles is dependent on the efferent pathways.

A nerve consists of a bundle of neurons. Most nerves are composed of both sensory and motor fibres. The fibres are called axons and are generally shielded by myelin. Axons end in close proximity to another cell (neuron or other cell) and the functional connection between the neuron and the cell innervated by that neuron is called a synapse. The junction between a motor neuron and a skeletal muscle cell, i.e. the neuron-muscle synapse, is called the neuromuscular junction. The presynaptic part of the neuromuscular junction consists of the terminal part of the nerve. The terminal part of one axon is divided into several branches and each branch forms a synapse with one muscle cell. Thus one neuron innervates many muscle cells and these form a unit called the motor end plate (Fox 1993).

The nerve ending contains a neurotransmitter called acetylcholine (ACh), which is synthesized from choline and acetyl-coenzyme A. Small amounts of ACh is released continuously from the nerve ending into the neuromuscular junction, causing miniature end-plate potentials. The ACh is stored in vesicles that, following the arrival of an electric impulse through the axon, conjugate with the nerve cell membrane to release its content. The postsynaptic membrane of the muscle cell is heavily folded forming numerous clefts. Postjunctional ACh-receptors are situated in these clefts and these receptors are essential for the transfer of the nerve impulse to the muscle cells, i.e. they mediate synaptic excitation at the neuromuscular junction (Fox 1993). ACh-receptors are also present on the presynaptic membrane.

2.1.2. Acetylcholine receptors

The postsynaptic nicotinic ACh receptor consists of five different subunits forming a cylinder shaped structure that crosses the postsynaptic membrane. In addition to the receptors of the neuromuscular junction, nicotinic receptors are found throughout the CNS and at autonomic ganglia. Binding of ACh, released from the nerve ending following a nerve stimulus, to specific binding sites on the two α-units of the receptor causes the opening of an ion channel situated within the receptor molecule. This allows an influx of Na^+-ions across the membrane causing depolarization of the muscle cell. If the change in current is sufficient and the membrane potential of the muscle cell is

depolarized beyond its intrinsic threshold value an action potential (AP) is initiated and as a result the muscle cell contracts. Most of the ACh in the neuromuscular junction, however, never reaches the postsynaptic membrane, but is degraded shortly after its release by the enzyme acetylcholine esterase. Fortunately, a considerable safety margin exists due to the large amount of postsynaptic receptors available and due to the excess amounts of ACh being released.

ACh receptors have been found also on the presynaptic membrane (Bowman et al. 1990). These are slightly different in configuration as compared to the better known postsynaptic receptors and they are, according to current knowledge, involved in the regulation of availability of transmitter (ACh) at the nerve ending. More precisely, binding of ACh to these presynaptic receptors is believed to initiate a positive feedback mechanism resulting in the mobilization of more ACh in the nerve ending, ready to be released in case of the arrival of any further stimuli (Fox 1993).

2.2. Non-depolarizing neuromuscular blocking agents

In the late sixteenth century, natives in the South American Amazon area killed animals with poison covered arrows that caused their prey to become paralyzed. This poison was prepared from plants by Orinoco Indians and became soon known to the Western world by the name curare. In 1914 a derivative of this poison, d-tubocurarine, was used to determine that ACh was the transmitter at the neuromuscular junction (Dale et al. 1936).

Curare was first used in psychiatric patients receiving electroconvulsive therapy. In the 1940s d-tubocurarine was introduced into anaesthesia practice as a muscle relaxant to facilitate conduction of surgery and received by anaesthetists with great enthusiasm (Griffith 1945). The mechanism of action of the drug was by then recognized as blocking of the synaptic transmission at the neuromuscular junction. In the following decades a number of more refined muscle relaxant drugs have been developed and d-tubocurarine is no longer commonly used in anaesthesia.

2.2.1. Effects of non-depolarizing neuromuscular blocking agents at the neuromuscular junction

Non-depolarizing NMBAs are commonly used as part of a balanced anaesthesia regimen, in order to provide adequate muscle relaxation to facilitate endotracheal intubation and performing of surgery. By using NMBAs sudden coughing or reflex movement, which could lead to serious complications, can be avoided during the surgical procedure. NMBAs are commonly divided into two groups based on their molecular structure, the steroidal compounds and the benzyl-isoquinolones. Of the NMBAs investigated in this study, rocuronium is a steroid muscle relaxant and cisatracurium belongs to the isoquinolone group.

NMBAs are also categorized according to their duration of action into short acting, medium acting and long acting drugs. Most of the commonly used NMBAs, such as rocuronium, vecuronium, atracurium and cisatracurium, present with a medium duration of action. Pipecuronium and pancuronium can be considered long acting NMBAs. The only currently available short acting non-depolarizing NMBA is mivacurium.

Suxamethonium (succinyl choline) is a depolarizing neuromuscular blocking agent originally discovered in the early twentieth century and it has been used as a NMBA since the 1940s. It gained immense popularity over the following decades due to its fast onset and short duration of action. Although rare, the numerous potentially lethal side effects (Claudius et al. 2009a) associated with it have prompted several attempts to find a replacement for suxamethonium.

Both mivacurium and suxamethonium are degraded by a plasma enzyme called pseudo-cholinesterase, and sufficient spontaneous recovery is seen in most individuals without need for pharmacological reversal of the block. Nevertheless, patients with abnormally functioning or subnormal levels of the enzyme may present with dramatically prolonged neuromuscular block (Viby-Mogensen 1980), which in extreme cases may require postoperative sedation and mechanical ventilation.

Non-depolarizing NMBAs bind to the specific binding sites at the α-units of the ACh receptor described above, thus denying the neurotransmitter (ACh) access (Jonsson Fagerlund et al. 2009). Unless two ACh- molecules are able to bind at both sites simultaneously, the ion channel will remain closed. As a consequence, depolarization of the muscle cell and the resulting contraction of the muscle fibre are inhibited.

A competition for the binding sites between NMBA and ACh ensues whenever muscle relaxant is present in the neuromuscular junction. The degree of block is dependent on the amount of NMBA present in the neuromuscular junction in relation to the amount of ACh. Non-depolarizing NMBAs also bind to presynaptic ACh receptors and are thus believed to inhibit the mobilization of ACh as described previously. Consequently, according to recent studies (Bhatt et al. 2007), a shortage of ACh available for excretion following repetitive stimuli develops. This is thought to be the rationale behind the fade phenomenon seen in the muscle response during partial non-depolarizing NMB.

Spontaneous recovery occurs gradually as the amount of NMBA decreases. Speed of recovery depends on the NMBA used, dosage, the anaesthetics used and on numerous individual factors that may affect pharmacokinetics and –dynamics. Recovery from a neuromuscular block can be accelerated by administering a reversal agent such as neostigmine or the novel drug sugammadex. Reversal will be discussed in more detail in chapter 2.4.

2.2.2. Pharmacology of rocuronium and cisatracurium

Both rocuronium and cisatracurium are widely used nondepolarizing NMBAs with an intermediate duration of action (Belmont et al. 1995, Bartkowski et al. 1993, Kisor and Schmith 1999). Rocuronium (Figure 1) is an aminosteroid compound as are, for instance, vecuronium and pancuronium, while cisatracurium (Figure 2) –a stereoisomer of atracurium- belongs to the isoquinolone group of NMBAs. Therefore, the pharmacological properties of these two drugs are quite different.

Rocuronium offers the fastest onset time of all currently available nondepolarizing NMBAs (Bartkowski et al. 1993), while the onset time of cisatracurium is significantly longer. Cisatracurium is approximately four to five times more potent than rocuronium (Naguib et al. 1998), recommended

($2xED_{95}$) doses for endotracheal intubation being 0.15 mg/kg and 0.6 mg/kg, respectively. Rocuronium has a slightly shorter clinical duration and a faster spontaneous recovery rate as compared to the equipotent dose of cisatracurium. However, maximum recovery times are similar (Naguib et al. 1998).

Rocuronium is eliminated through hepatic uptake and biliary excretion and to a lesser part by excretion in the urine. Thus, a significantly prolonged neuromuscular block may be seen in subjects suffering from severe hepatic or renal insufficiency (Atherton and Hunter 1999). Cisatracurium, like atracurium, is eliminated at a constant rate which is independent of liver and kidney function, as it is degraded by non-enzymatic Hofmann elimination in the plasma and tissues (Lien et al. 1996).

Rocuronium and cisatracurium, like all known non-depolarizing NMBAs, bind at the specific ACh binding sites on the nicotinic receptors at the neuromuscular junction, and actually also on nicotinic receptors located elsewhere in the body, for instance in the autonomic ganglia. The latter are involved in the regulation of many functions in the human body. A function of particular importance is the hypoxic ventilatory response, which has been shown to be impaired by partial residual curarization (Eriksson et al. 1992, Eriksson et al. 1993, Eriksson 1996).

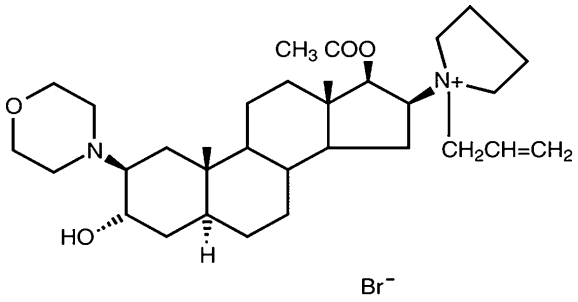

Figure 1. Rocuronium.

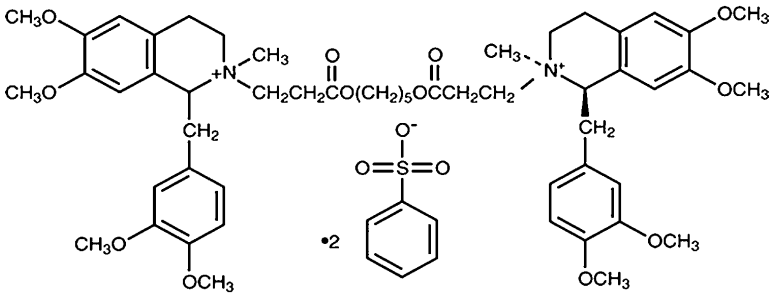

Figure 2. Cisatracurium.

The non-depolarizing NMBAs compete for the binding sites at the postjunctional ACh-receptors with ACh released from the nerve ending. Whenever one or both of the binding sites at the receptor are

occupied by a NMBA molecule the ion channel described above will remain closed and depolarization of the muscle cell and the following contraction of the muscle fibre are prevented. Strength of muscle contraction depends on the number of muscle fibers that contract following a stimulus. With larger amounts of NMBA present more binding sites will be occupied by NMBA and less fibres will contract, which in turn leads to a weaker or completely blocked muscle response.

Spontaneous recovery of the NMB will occur gradually as the NMBA is removed from the neuromuscular junction by redistribution and metabolism. As the amount of NMBA decreases acetylcholine released from the nerve ending becomes more likely to win the competition for the binding sites. When a sufficient amount of NMBA has been removed the clinical block is overcome. Certain amounts of NMBA are still, however, present in the body. The most important pharmacological properties of rocuronium and cisatracurium are presented in Table 1.

Table 1. Pharmacological properties of rocuronium and cisatracurium.

NMBA	ED_{50} (mg/kg)	ED_{95} (mg/kg)	Intubation dose (mg/kg)	Time to maximum block (min)	Clinical duration (min)	Elimination
Rocuronium	0.147	0.305	0.6-1.0	1.7*	36*	liver >70% kidneys <10%
Cisatracurium	0.026	0.04	0.15-0.2	5.2**	45**	Hofmann elimination

*rocuronium intubation dose = 0.6 mg/kg; **cisatracurium intubation dose 0.1 mg/kg
Data from: Naguib and Lien. Pharmacology of muscle relaxants and their antagonists. In: Miller's Anesthesia 2010.

Great variations in recovery times are seen between different individuals after a standardized bolus dose of rocuronium (Debaene et al. 2003) as metabolism occurs through hepatic and renal pathways. There is less variation in recovery times in patients receiving cisatracurium, due to the above mentioned Hofmann elimination (Kisor and Schmith 1999). Hypotermia and impaired blood circulation may however prolong both rocuronium and cisatracurium induced NMB (Cammu et al. 2000, Smeulers et al. 1995, Heier et al. 1990).

2.3. Neuromuscular monitoring

2.3.1. Peripheral nerve stimulation

Assessment of the degree of NMB at the neuromuscular junction can be done through stimulation of a peripheral nerve by applying a 30-70 mA current over the nerve and monitoring the muscle response to that stimulus. The stimulus current and frequency with which the stimuli are applied must be kept unchanged during the entire period of monitoring for the results to be comparable.

Depending on the method used a variety of nerves can be stimulated for this purpose. The ulnar nerve (nervus ulnaris) at the wrist is typically chosen and thus the evoked muscle response is seen at the adductor pollicis muscle of the thumb. Two stimulation electrodes are placed on the skin of the

wrist over the ulnar nerve, the negative electrode is placed distally. Careful positioning of the electrodes is vital as a muscle may become directly stimulated by the current. The resulting twitches could in such a case be misinterpreted as actual responses that are a result of normal synaptic activity in a situation where in reality neurotransmission is still blocked.

A supramaximal stimulus should always be used to ensure that all axons are depolarized. This way the achieved muscle response will always be maximal. Peripheral hypothermia is common during general anaesthesia and may attenuate monitoring results. For optimal results the temperature of the monitored limb should be kept above 32°C (Fuchs-Buder et al. 2007).

There are several stimulation patterns of which the most commonly used are: single twitch, train-of-four (TOF), tetanic stimulation, posttetanic count (PTC) and double burst stimulation (DBS). TOF stimulation is the most widely used stimulation pattern and the method most relevant for this study and is therefore to be presented in more detail.

When a TOF stimulation is applied a series of four equal (0.1-0.2 ms) supramaximal stimuli are given at 0.5 s intervals. This series is repeated every 12-20 seconds (or less frequently) during monitoring of a NMB. When neurotransmission is uninterrupted four equal muscle twitches (T1, T2, T3 and T4) are seen following the TOF stimulation. This is the case whenever no NMBA has been administered or when complete clinical recovery from NMB has been achieved (Ali et al. 1971a, Ali et al. 1971b).

After a bolus dose of NMBA, fade in the TOF response will appear gradually as the block sets on. Fade is a characteristic of a partial non-depolarizing NMB and means that the subsequent responses are weaker than the first response. At deeper level of NMB only part of the responses are detectable (Ali et al. 1971). During deep blockade no TOF responses are detectable. As spontaneous recovery commences the twitch responses reappear gradually (Figure 3). When the first weak TOF response (T1) has reappeared the block is still of 90% depth (Fuchs-Buder et al. 2009). As recovery progresses the number and amplitude of twitches increase until four equal twitches, indicating full recovery, are detected (Ali et al. 1971, Fuchs-Buder et al. 2009; Figure 3).

The train-of-four ratio (TR; T4/T1) is a commonly used measure of depth of blockade and can be calculated from the amplitude of the fourth response (T4) in relation to the first one (T1). This obviously requires that all four twitches are detectable and therefore a TR cannot be measured during deep NMB. The TR should return to at least 0.9 to assure adequate recovery from NMB (Eriksson et al. 1997, Murphy et al. 2006). As compared to the other methods, however, train-of-four measurements yielded by acceleromyography tend to give somewhat higher results. Based on that finding it has been proposed by some authors that the acceleromyographic TR should return to 1.0 to avoid residual curarization (Fuchs-Buder et al. 2007).

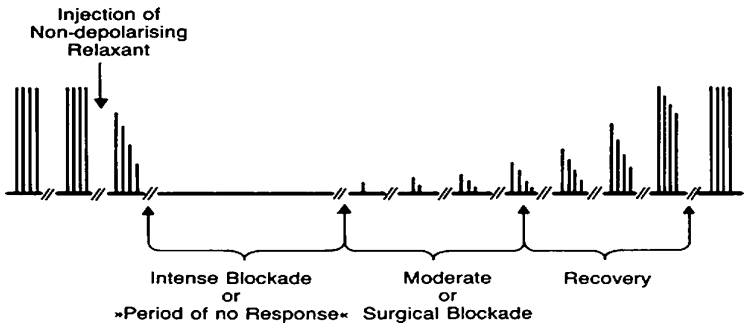

Figure 3. Train-of-four stimulation pattern and muscle responses at various levels of neuromuscular blockade. From: Fuchs-Buder et al. *Good clinical research practice in pharmacodynamic studies of neuromuscular blocking agents II: The Stockholm revision.* Acta Anesth Scand. 2007:51:7 pp 789-808.

2.3.2. Postoperative residual curarization

Traditionally, it was the common opinion of experts that return of a TR ≥ 0.7 would guarantee sufficient recovery from a NMB (Ali et al. 1975, Brand et al. 1977). More recent studies have since shown that a measured TR between 0.7 and 0.9 is associated with various symptoms and findings of postoperative residual curarization (PORC) (Eikermann et al. 2007, Eriksson et al. 1996, Kopman et al. 1997, Sundman et al. 2000). According to the current standard, which is agreed upon by several authorities in the field, return of TR should exceed 0.9 to rule out clinically significant residual paralysis (Eriksson et al. 1997, Murphy et al. 2006).

PORC is a common and potentially life-threatening complication. The incidence of PORC has in many studies been shown to be quite significant (up to 40-62% depending on the source) (Debeane et al. 2003, Baillard et al. 2000, Hayes et al. 2001, Murphy et al. 2005).

Inadequate recovery of neuromuscular function is often a result of inappropriate use, i.e. overdosing, of NMBAs. For instance, administering of additional boluses is often done by routine, but should be done according to actual clinical needs and always be based on objective assessment of the measured degree of block (Eriksson et al. 2003).

As mentioned previously in chapter 2.2., the duration of a block following administration of a NMBA is highly individual and large interindividual variations in the speed of recovery are seen. Debaene and colleagues investigated spontaneous recovery after a single dose of vecuronium, rocuronium or atracurium in 526 patients and found that 45 % of the patients had a TR < 0.9 at arrival in the post anaesthesia care unit (PACU). Even more disturbing was their finding that even 2 h after such a bolus dose residual curarization (TR<0.9) occurred frequently (Debaene et al. 2003). The results of the study by Debaene et al are shown in Figure 4.

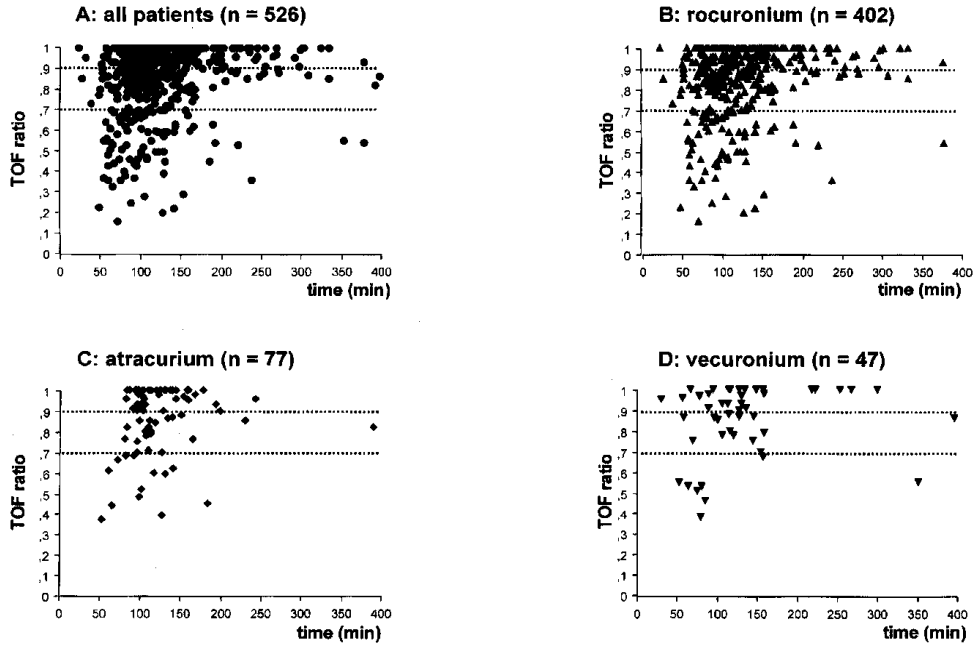

Figure 4. Spontaneous recovery after a single intubation dose of rocuronium, vecoronium or actracurium. From Debaene et al*. Residual paralysis in the PACU after a single intubating dose of nondepolarizing muscle relaxant with an intermediate duration of action.* Anesthesiology 2003: 98:1042-8.

Incomplete reversal of a NMB is often associated with too early administering of a cholinesterase inhibitor, at too deep a level of NMB. Unawareness exists among clinicians regarding the limitations of anticholinesterase reversal agents. Routine reversal of a NMB *per se* is not applied in nearly all hospitals (Fuchs-Buder et al. 2008, Grayling and Sweeney 2007, Naguib et al. 2010, Sorgenfrei et al. 2005). Reversal will be further discussed in chapter 2.4. Failure to use intraoperative neuromuscular monitoring (Baillard et al. 2005) is another major cause for inadequate recovery.

PORC increases the risk for passive regurgitation of gastric contents due to both pharyngeal and laryngeal muscle dysfunction (Eriksson et al. 1997, Sundman et al. 2000). Non-depolarizing NMBAs also interfere with the hypoxic ventilatory response, which is a nicotinic chemoreceptor mediated function (Eriksson et al. 1992, Eriksson et al. 1993, Eriksson 1996). Other important findings related to PORC are upper airway obstruction, atelectasis, hypercapnia and occasional need for re-intubation (Berg et al. 1997, Eikermann et al. 2007). Subjective symptoms related to PORC are fatigue, inability to speak clearly, double vision and shortness of breath (Claudius et al. 2009a), all of which may cause the patient significant discomfort and emotional distress.

The incidence of post-operative pulmonary complications associated with PORC, such as atelectasis and pneumonia, is clearly increased in the elderly population. The use of long acting NMBA pancuronium has also been shown to increase the incidence of pulmonary complications (Berg et al.

1997, Murphy et al. 2004). Since long-term NMB, if at all needed, can be achieved by continuous or repeated dosing of safer medium acting agents, routine pancuronium use should probably be avoided. At least, the potential risks associated with the use of pancuronium, versus possible benefits, should always be assessed.

Simultaneous administration of general anaesthetics -volatile agents in particular- increase the effect of NMBAs and may both sustain and increase depth of the block (Lowry et al. 1998). Intraoperatively administered opioids may continue to suppress the ventilatory drive in the postoperative period. The overall effect of all drugs given on muscle tone and respiratory function is difficult to estimate.

A number of clinical tests for the assessment of muscle recovery have been described during the years. Force of inspiration and achieved tidal volume are parameters that have been widely used as measures of regained ventilatory capacity in patients that are still intubated and connected to the anaesthesia ventilator (Brull and Murphy 2010). Other clinical tests that have been used are grip strength, tongue protrusion, leg elevation and ability to focus on a visible subject (Cammu et al. 2006, Debaene et al. 2003, Kopman et al. 1997). In fact, the mere ability of the patient to open his or her eyes has been considered a sign of adequate muscle recovery. The sustained (5 s) head lift is a popular, but by no means reliable test (Pedersen et al. 1990). In addition, like all the other bedside tests, it requires an awake and co-operative patient. This in turn obviously means exposing the patient to all the risks and discomfort associated with possible existing residual curarization. According to several studies the above described clinical tests cannot be considered reliable (Cammu et al. 2006, Debaene et al. 2003). Clinicians also may perform the tests incorrectly, which further increases the inaccuracy of these methods (Debaene et al. 2003).

In view of the available data on PORC and due to the failing reliability of clinical muscle function tests, intraoperative monitoring of neuromuscular transmission must be seen as mandatory in order to avoid PORC (Kopman et al. 2004, Kopman et al. 2005). However, in actual clinical practice large differences in the use of (any kind of) monitoring of NMB are seen (Fuchs-Buder et al. 2008, Grayling and Sweeney 2007, Sorgenfrei et al. 2005). According to a recently published survey on the management of NMB in Europe and the USA, there is a lack of agreement and awareness among clinicians on the optimal way to monitor and manage a NMB (Naguib and Brull 2009, Naguib et al. 2010). Recently, leading experts (Kopman 2010) within the field have called for consensus-based guidelines in order to increase the quality of management of NMB.

2.3.3. Subjective methods of monitoring

A peripheral nerve stimulator (PNS) is a simple device for applying standardized electrical nerve stimuli. Depending on the device, several stimulus patterns are available and the stimulus current can be set between 0-70 mA. The muscle response is evaluated by the clinician either visually or by using tactile evaluation (Brull and Silverman 1993). These devices are relatively cheap and easy to use and have gained widespread popularity among anaesthetists. Relying on the PNS information may however not be safe, as fade in the TOF response cannot be manually or visually detected at TRs exceeding 0.4 (Drenck et al. 1989, Kopman et al. 2005b). A consequence might be that the clinician

falsely believes full recovery to have been achieved in a situation where in fact a considerable residual NMB exists. For this reason subjective evaluation of the recovery from NMB by using a PNS cannot be considered accurate. It is quite obvious that routine use of objective neuromuscular monitoring is essential to avoid postoperative residual curarization and ensure safe emergence from anaesthesia (Eriksson 2003).

Double burst stimulation (DBS) is a stimulation pattern that was specifically designed to reveal residual curarization. It consists of two subsequent bursts at a 750 ms interval. Each burst consists of three separate impulses. In comparison with TOF, DBS has indeed proved more sensitive in detection of fade during partial blockade, as fade can be detected at TR= 0.6 (Drenck et al. 1989). This level is, however, as previously stated, still not adequate to rule out residual NMB.

2.3.4. Objective methods of monitoring

Objective methods of monitoring NMB are mechanomyography (MMG), electromyography (EMG), acceleromyography (AMG), kinemyography and phonomyography. A common feature of these methods is that the equipment -in addition to applying the stimulus- quantifies the magnitude of the muscle response. The result is displayed as a count of twitches or a TR percentage. This is a major advantage of these devices as compared to the peripheral nerve stimulator. For instance, a TR of >0.4 and <0.9 can be reliably measured, as is not the case when using the PNS. Use of objective neuromuscular monitoring will thus likely reduce the incidence of PORC (Baillard et al.2005, Gatke et al. 2002).

Calibration of the device is performed at induction, after the patient has lost consciousness, but before the initial NMBA bolus is administered. Some devices automatically set the supramaximal level by applying gradually increasing stimuli until the maximal response is determined. Other devices apply a fixed set stimulus (e.g. 60 mA). An initial TOF- stimulus is also applied before the NMBA is administered, to gain a control value to which the subsequent responses will be compared. Regardless of the method chosen, for reliable results, movement of the arm during the period of monitoring must be avoided and the thumb should be able to move freely.

MMG is considered the golden standard method for monitoring NMB (Fuchs-Buder et al. 2007) and the evaluation of any other techniques is done in comparison to this method. MMG measures the actual force of the muscle contraction response to an electrical stimulus. Due to difficult setup of the equipment and the need for a bulky force transducer the method is not very suitable for routine clinical use and has mostly been used for scientific purposes (Trager et al. 2006).

EMG is a method that records the electric activity (i.e. the compound action potential) of the stimulated muscle, which is in proportion to the force of muscle contraction (Fuchs-Buder et al. 2009). The results correlate well with MMG. It can be used, e.g., for the limbs, larynx and diaphragm. Most commonly the ulnar nerve is used. EMG is used both in clinical practice and for scientific purposes.

Being relatively cheap and easy to use, AMG is one of the most frequently used techniques in daily clinical practice. The stimulating electrodes are placed over the ulnar nerve and a piezo-electric element is placed on the distal phalanx of the thumb. Alternatively, other nerves and muscles can be chosen. AMG calculates the force of muscle contraction by measuring the degree of acceleration of the thumb. According to Newton's second law force equals mass times acceleration ($F=m \cdot a$). AMG results correlate fairly well with MMG and EMG, but the method is fairly sensitive to movement and other artifacts (Fuchs-Buder et al. 2009). Fixation of the hand and arm is therefore recommended. A preload may also be applied to the thumb (Claudius et al. 2009b). It has been suggested that TR should return to 1.0, as compared to 0.9 with the other methods (Fuchs-Buder et al. 2007). Normalization of the acceleromyographic TOF-ratios, i.e. all measured TOF data is referred to the initial stable baseline control value, may be performed to diminish the bias of MMG (Claudius et al. 2009b, Fuchs-Buder et al. 2009).

Kinemyography (Datex-Ohmeda NMT MechanoSensor ®) is a well established method that uses a piezoelectric wafer that is positioned in the groove between the thumb and index finger of the monitored hand. The device is integrated in the Datex anaesthesia monitoring system by GE. The method is based on the detection of bending of the piezoelectric sensor wafer strip by movement of the thumb in response to stimulation of the ulnar nerve (Trager et al. 2006). The results are reasonably comparable with those of the other methods described and the method is suitable for clinical use (Trager et al. 2006).

Phonomyography is based on the finding that muscle contraction yields low frequency sounds that can be picked up by special microphones and amplified. Basically any muscle can be monitored. The method is relatively new and seems to correlate well with for instance EMG (Hemmerling et al. 2003, Trager et al. 2006).

2.4. Reversal of a neuromuscular block

As previously discussed, the NMBAs show large variations in pharmacokinetic and -dynamic properties. Spontaneous recovery from NMB is indeed highly individual and dependent on many factors besides the agent administered and dosage regimen. Patient related factors that may affect duration of a NMB are for instance age, sex, BMI, circulatory status, body temperature, renal and hepatic function and concomitant medication (Srivastava and Hunter2009).

As stated earlier, TR must exceed 0.9 (T4/T1=90%) to ensure sufficient neuromuscular recovery (Eriksson et al. 1997, Murphy 2006). A reversal agent should be administered whenever the ratio is below this value, to rule out residual curarization before discontinuing anaesthesia and allowing extubation. In practice, pharmacological reversal of the remaining NMB often becomes necessary, typically after administration of a long acting NMBA or additional boluses of relaxant, in association with very short procedures requiring NMB and for individuals that for any given reason present with a prolonged block. As is the case with monitoring of a NMB, wide variations in the use of routine reversal still exist both regionally and worldwide (Fuchs-Buder et al. 2008, Grayling et al. 2007).

The most commonly used reversal drugs are the cholinesterase inhibitors: neostigmine, pyridostigmine and edrophonium. These drugs inhibit the degradation of neurotransmitter acetylcholine by acting on the enzyme acetylcholine esterase (AChE) in the neuromuscular junction.

In 2008 a novel reversal agent called sugammadex became available in Europe. Sugammadex is a modified γ-cyclodextrin specially designed for rocuronium reversal and it has a completely unique mechanism of action: encapsulation and inactivation of steroidal NMBA molecules (Bom et al. 2002). Sugammadex has made complete reversal possible even at deep levels of NMB (Lee et al. 2009, Pühringer et al. 2008). Thus, sugammadex may not only increase safety of reversal, but also provides new strategies for the use of NMB.

2.4.1. Cholinesterase inhibitors

After ACh is released from the nerve ending into the neuromuscular junction, an enzyme called acetylcholinesterase (AChE) causes its degradation into acetate and choline within a millisecond. By interfering with the actions of this enzyme the breakdown of ACh in the neuromuscular junction can be interrupted. Cholinesterase inhibitors such as neostigmine reverse a NMB indirectly by inactivating AChE in the neuromuscular junction (Barrow and Johnson 1966). As a result the amount of ACh will increase dramatically, while the amount of NMBA molecules remains unchanged. ACh molecules will thus outnumber the relaxant molecules and more probably win the competition for the binding sites at the ACh receptors (Adam et al. 2002). Neostigmine forms a reversible attachment to AChE by forming a covalent bond to the esteratic site of the enzyme.

The recommended neostigmine dose for routine reversal is 50 µg/kg (Abdulatif et al. 1996). Maximal antagonizing effect can be achieved by administering 70 µg/kg. Cholinesterase inhibitors also have a ceiling effect: when all existing enzyme is inhibited the maximum effect is achieved (Bartkowski 1987). Increasing the dose of neostigmine further will thus not increase the amount of ACh, but may bring on more side-effects. Onset of action of neostigmine is seen within 1 minute, but the peak effect does not occur until after approximately 9 minutes (Kirkegaard-Nielsen et al. 1995, Kirkegaard-Nielsen et al. 1996). However, duration of action is only 20-30 minutes (Williams et al. 1978) and therefore there is a risk for recurarization as the effect of neostigmine comes to an end. The NMBA itself is not affected by neostigmine, but is gradually removed from its site of action according to its own pharmacology. Neostigmine is partly metabolized in the liver, but most of the drug is excreted in the urine.

Reversal by neostigmine will be incomplete if reversal is attempted at deep levels of NMB (Bartkowski 1987, Kopman et al. 2005b). Studies have shown that reversal can be effective only after the second train-of-four twitch (T2) has reappeared (Kirkegaard-Nielsen et al. 1995). Effect of reversal by neostigmine is highly dependent on the anaesthetic agent used (Kim et al. 2004).

The cholinesterase inhibitors cause an increase in ACh content not only in the neuromuscular junction, but at all muscarinic sites where ACh acts as a neurotransmitter. Therefore neostigmine presents with many unpleasant muscarinic side effects such as nausea and vomiting, bradycardia, prolongation of the QTc interval (which may provoke potentially lethal cardiac arrhythmias),

bronchoconstriction, hypersalivation, miosis and an increase in intestinal pressure (Van Vlymen and Parlow 1997).

The bradycardic response to neostigmine may progress to asystole and neostigmine must always be administered together with an antimuscarinic drug such as atropine or glycopyrrolate. The net effect of the combination of these drugs on the heart rate is quite varied. Anticholinergic drugs may also cause CNS effects, dryness of the mouth and urinary retention (Mirakhur 1979a and b).

Individuals with Long QT-syndrome (LQTS) are particularly at risk for fatal arrhythmias. One case of cardiac arrest after neostigmine reversal was described by Shields in 2008 (Shields 2008). It is important to point out that many anaesthetic agents, e.g. sevoflurane and opioids, cause a prolongation of the QTc interval and that the overall significance of this remains unknown.

Neostigmine may cause bronchoconstriction although co-administration of an anticholinergic tends to reduce the effect. However, the net effect on airway resistance of this combination of drugs is unpredictable. Patients suffering from asthma or COPD may be particularly susceptible.

2.4.2. Sugammadex

Cyclodextrins are cyclic oligosaccharides that are composed of six to eight α-D-glucopyranoside units in a circular arrangement (Srivastava and Hunter 2009). Largest are the γ-cyclodextrins, which contain eight sugar units. Due to their negatively charge hydroxyl groups these molecules are highly water soluble. The inside cavity of the molecule is lined by carbon atoms and is thus lipophilic. γ-cyclodextrins are used in a variety of applications within the food industry and for various pharmaceutical purposes, e.g. to increase water solubility, stability or bioavailability of lipophilic drugs (Bom et al. 2002, Davis et al. 2004). Ingested γ-cyclodextrins are not absorbed from the gastrointestinal canal into the bloodstream. γ-cyclodextrins are well tolerated (Bom et al. 2002).

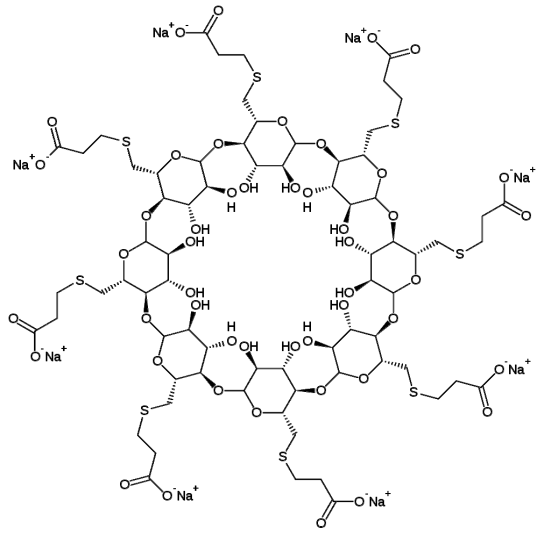

Figure 5. Sugammadex.

Sugammadex (Figure 5) is a modified γ-cyclodextrin, specifically tailored to encapsulate rocuronium and remove it from its site of action and thereby reverse NMB. The affinity of sugammadex for rocuronium is extremely strong, with an estimated 25 million complexes formed for each one that dissociates (Bom et al. 2002). Sugammadex also binds the other steroidal NMBAs, although with a somewhat lower affinity. It is registered for reversal of rocuronium and vecuronium NMB. Sugammadex has no intrinsic pharmacological activity apart from the encapsulation of steroid relaxant. It is not capable of encapsulating isoquinolone NMBAs nor does it bind suxamethonium.

The binding of rocuronium to the cavity of sugammadex through non-covalent thermodynamic forces, van der Waals and hydrophobic interactions is, although very stable, not completely irreversible (Bom et al. 2002). The specific hydroxyl groups that are linked to the sugar ring are essential for the stability of the complex of the two drugs. The distribution volume is 10-15 l, thus sugammadex remains mainly in the plasma and extracellular space. One molecule of sugammadex is required for each molecule of rocuronium to be bound. The complex formed remains water soluble and is excreted unchanged by the kidneys (Gijsenbergh et al. 2005). Plasma elimination half-life ($T_{1/2}$) of sugammadex is 2.2 hours and most of the drug is excreted within 24 hours of administration. Renal failure reduces excretion of sugammadex markedly (Staals et al. 2010).

Encapsulation and inactivation of unbound rocuronium in the plasma leads to a decrease in the plasma concentration of rocuronium, thus creating a concentration gradient. This gradient in turn causes rocuronium to be removed from the tissues (the neuromuscular junction) to the plasma, only to be further encapsulated by sugammadex (Bom et al. 2002). Successively more rocuronium is removed resulting in complete and rapid reversal of the NMB (Gijsenbergh et al. 2005). The speed and effect of reversal has been convincingly demonstrated in a number of studies (Adam et al. 2002, Groudine et al. 2007, Ploeger et al. 2009, Sorgenfrei et al. 2006).

Due to its intense affinity to rocuronium, sugammadex is not likely to bind other drugs or steroidal hormones. Extensive in vitro testing also shows that significant drug interactions are unlikely to occur, with the possible exception of oral contraceptives containing progesterone. A decrease in plasma progesterone levels due to encapsulation by sugammadex may impair the effect of oral contraceptives (European Medicines Agency 2010).

Recommended sugammadex dosage is 2 mg/kg (actual body weight) for reversal of an intermediate (T2 has reappeared at time of reversal) rocuronium or vecuronium NMB (Sorgenfrei et al. 2006). Full reversal has been shown to occur in approximately 1.7 minutes in patients who have received rocuronium 0.6 mg/kg, while a vecuronium 0.1 mg/kg dose is fully reversed in 2.3 minutes (Suy et al. 2007), Similarly, full recovery occurs 17.9 min after neostigmine reversal (Khuenl-Brady et al. 2010).

Reversal of a deep block requires a larger dose of rocuronium. If 1-2 PTC twitches (but no TOF twitches) are detectable at the time of reversal the recommended sugammadex dose is 4 mg/kg (Groudine et al. 2007, Jones et al. 2008). On the average, reversal can be expected in 2.7 min (*versus* 49 min after neostigmine 70 µg/kg) (Jones et al. 2008). Speed of reversal by sugammadex is independent of the drug (sevoflurane versus propofol) used for maintenance of anaesthesia (Rex et al. 2009).

The ability of sugammadex to achieve immediate reversal of a very deep NMB makes this drug unique. By administration of 16 mg/kg sugammadex a very deep block induced by a large dose of rocuronium (1.2 mg/kg), intended for so called crush induction, is reversed in about 3.2 minutes (Lee et al. 2009, Mirakhur 2009, Sparr et al. 2007). Thus complete neuromuscular recovery from a 1.2 mg/kg rocuronium NMB after reversal by a large dose of sugammadex actually occurs faster than spontaneous recovery of a single 1 mg/kg dose of suxamethonium (Lee et al. 2009).

At the time of writing, sugammadex has been used in an estimated more than 400 000 patients. It appears well tolerated and safe for both adult and paediatric patients (de Boer et al. 2007, Plaud et al. 2009). As it, unlike the cholinesterase inhibitors, has no direct effect on cholinergic transmission, the muscarinic side-effects related to neostigmine reversal are also avoided. To date, sugammadex has not, however, been licensed in the United States and Canada, as the US Food and Drug Administration (FDA) has decided to await further information on possible allergic reactions. This decision is based on findings in preclinical studies performed on volunteers.

Currently, administration of sugammadex is not recommended to paediatric patients younger than 2 years, as more information is needed regarding the safety of sugammadex in the paediatric population. The only recommended dose for children thus far is 2 mg/kg (European Medicines Agency 2010). Due to limited information on possible fetal effects of sugammadex, caution is recommended when using sugammadex in pregnant patients, although according to rat studies the drug does not pass through the placenta (European Medicines Agency 2010).

Until further information is available regarding the safety of sugammadex in patients with severe renal or hepatic dysfunction, sugammadex should probably be avoided in these patients. Mild renal insufficiency, however, is not considered a contraindication (European Medicines Agency).

Sugammadex opens up new possibilities regarding the use of NMB, as proper intubation conditions can be achieved equally fast by using a large dose of rocuronium as by using suxamethonium (Andrews et al. 1999). Deep NMB can if necessary be sustained until the end of surgery and normal intubation doses of rocuronium be used for very short procedures requiring NMB, without postsurgery delays caused by inability of the reversal agent (neostigmine) to reverse the block. Although fortunately rare, a ‘cannot intubate, cannot ventilate’ –situation (CICV) may in a worst case scenario lead to hypoxic brain damage and death. Lives may be saved in the future as immediate reversal is for the first time possible in a CICV situation.

2.5. Anaesthetics and neuromuscular blockade

2.5.1. General anaesthetics and ligand-gated ion channels

General anaesthetics are a group of pharmacological substances with quite heterogeneous chemical structures. Most general anaesthetics are believed to exert their effect through the GABAergic system. Gamma-aminobutyric acid (GABA) is the most important inhibitory neurotransmitter in the

CNS and about one third of all synapses in the CNS are believed to be GABAergic (Krasowski et al. 1998).

Inhibition through the GABAergic system is of major importance as it controls brain excitation and thus regulates basic functions like anxiety, muscle tension and vigilance. Glycine is another major inhibitory neurotransmitter in the CNS, but unlike GABA it is mostly present in the brainstem and the spinal cord.

GABA is synthesized from the amino acid L-glutamate and is stored in vesicles in the nerve ending. When GABA is released into the synaptic cleft it binds to specific carrier proteins that transport the transmitter into postsynaptic neurons. GABA binds to GABAergic receptors of which three different subtypes are defined: the $GABA_A$-, $GABA_B$- and $GABA_C$- receptors. Of these receptors the $GABA_A$-receptor is the most important one. It is the target of many sedative drugs, such as benzodiazepines, barbiturates and general anaesthetics and binding of such an agonist to the receptor mediates inhibitory nerve transmission in the brain.

Like the nicotinic receptors (e.g. the ACh receptor discussed previously) the $GABA_A$- receptor consists of five subunits which form an integral ion-channel (Barnard et al. 1998). Binding of an agonist causes a change in the conformation leading to opening of the ion-channel. Mainly chloride-ions pass through and cause hyperpolarization of the post-synaptic membrane.

There are several subtypes of the $GABA_A$- receptor. $GABA_B$-receptors bind for instance baclofen, which is used for the treatment of spasticity, while $GABA_C$- receptors are less well known.

Some anaesthetics, like ketamine, xenon and nitrous oxide, exert their effect through another system, the NMDA-system (Yamakura and Harris 2000). The possible mechanism of action of these drugs is inhibition of the excitatory transmitter glutamate at the NMDA- receptor. These receptors are, like the above described GABA- receptors, ion-channels. They consist of seven subunits and the ligands, either agonists or antagonists, can bind at several sites.

General anaesthetics decrease muscle tone by several mechanisms. Intravenous anaesthetics, such as propofol and thiopentone, probably exert their effect through central mechanisms, while volatile anaesthetics act at the neuromuscular junction.

2.5.2. Nitrous oxide

Nitrous oxide (N_2O), in room temperature, is a colourless, non-flammable gas with both analgesic and anaesthetic properties. It was discovered in 1700s and first used as an anaesthetic during a dental extraction by an American dentist named Horace Wells in 1844 (Goerig et al. 2002).

Figure 6. Nitrous oxide.

The pharmacological mechanism of action of N_2O (Figure 6) is not completely known. However, it acts mainly through blocking of the NMDA-receptor and by weak potentiation of the inhibitory GABAergic system and also by acting on a number of other ligand-gated ion channels (Krasowski et al. 1998, Yamakura et al. 2000). The anti-nociceptic effect of N_2O is believed to be the result of inhibition of glutamate receptors (Yamakura and Harris 2000).

The use of inhaled N_2O during surgical procedures requiring general anaesthesia is common worldwide. N_2O is only a weak anaesthetic and is therefore usually combined with other agents, most frequently with more powerful inhalational anaesthetics such as sevoflurane, isoflurane or desflurane, to achieve a sufficient level of general anaesthesia.

Besides its rapid onset of action, the motive for including N_2O in a general anaesthesia regimen is to a large part one of reducing costs. N_2O is a cheap drug and it significantly reduces the demand of the more expensive volatile agents or propofol. It is also easy to administer and causes little adverse circulatory effects, such as hypotension. This is a definite benefit especially in the elderly population.

N_2O is usually administered as a 2:1 mixture with oxygen and delivered through the anaesthesia ventilation system along with possible volatile anaesthetics. Inhalation of nitrous oxide (in a 1:1 ratio with oxygen) is also frequently used to relieve pain associated with childbirth.

Nitrous oxide reacts with ozone and is thus a major greenhouse gas and air pollutant, which remains in the athmosphere for up to centuries. An increase in the use of rebreathing gas flow systems and in the utilization of low fresh gas flows is seen in Finnish anaesthetic practice from 1995 until 2002. In practice the patient re-breathes the same anaesthesia gases with constant addition of oxygen and removal of carbon dioxide. As a result the consumption of inhalational anaesthetics is decreased and this has lead to significantly reduced costs as well as reduced air pollution. The routine use of N_2O has as a result of this technique been decreasing in Finland and elsewhere, but is nevertheless still common worldwide.

NMBAs have until recently usually been studied during nitrous oxide supplemented with intravenous anaesthetics, as it has been well established that volatile anaesthetics affect the pharmacodynamics of muscle relaxants (Hemmerling et al. 2001, Olkkola and Tammisto 1994). Unlike volatile agents, N_2O has been believed to have no effect on NMB. However, some recent studies indicate that N_2O actually may potentiate the action of mivacurium and rocuronium (Kopman et al. 2005a, Plaud et al. 2002). In these studies bolus administration of NMBA was used. In the study by Plaud maximum mivacurium NMB was increased in patients receiving nitrous oxide and propofol 15 min prior to administration of the NMBA as compared to patients receiving only propofol. Kopman's group found that the ED_{50} –dose of rocuronium was significantly lower in patients receiving TIVA and a mixture of N_2O and oxygen than in patients receiving only TIVA with a mixture of air and oxygen.

2.5.3. Volatile anaesthetics

Despite extensive research, the exact mechanism of action of halogenated ether anaesthetics, such as sevoflurane in the central nervous system (CNS), remains unknown. Ligand-gated ion channels in

the CNS, the GABAergic system in particular, are nevertheless the main target of these agents. Inhalational agents such as ether, halothane and enflurane were in routine use during several decades. They have since been successively replaced in many countries, first by isoflurane and later by sevoflurane and desflurane, which are currently in routine use in Finland and elsewhere. The molecular structure of sevoflurane is seen in Figure 7.

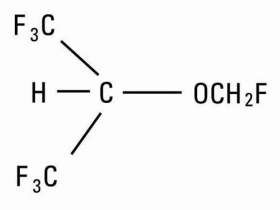

Figure 7. Sevoflurane.

Sevoflurane (1,1,1,3,3,3- hexafluoro- 2- propylether) is the most widely used volatile anaesthetic in Finland. It is primarily used for maintenance of general anaesthesia after induction by propofol or some other intravenous anaesthetic. Mask induction by sevoflurane is also feasible and fairly often used in small children. Certain short surgical procedures, e.g. tympanostomies, can be carried out during mask inhalation of sevoflurane, with or without the addition of an intravenous opioid. Spontaneous breathing is usually maintained although sevoflurane does depress respiratory function in larger concentrations.

Sevoflurane is generally well tolerated and well suited for both anaesthetic procedures of a longer duration as for day surgery procedures. It causes vasodilation, which may lead to hypotension especially in the eldery and in hypovolemic patients. Sevoflurane also dilates the airways and is therefore well suited for asthmatic patients. Allergic reactions to sevoflurane are very rare. Degradation of sevoflurane yields a product called Compound A, which in large concentrations has been associated with renal toxicity (Goldberg et al. 1999). The clinical significance of this finding is unclear. Epileptiform EEG activity has been shown to occur in healthy volunteers during 1.5 and 2.0 MAC sevoflurane anaesthesia (Jääskeläinen et al. 2003). Due to this finding sevoflurane should probably be avoided in individuals with a history of epilepsy.

Volatile agents significantly reduce the dose requirements of NMBAs (Hemmerling et al. 2001). Previous studies have quantified the interactions between volatile anaesthetics and NMBAs using constant end-tidal concentrations of volatile anaesthetics (Kansanaho and Olkkola 1995, Kansanaho and Olkkola 1996b, Kansanaho et al. 1997). Volatile anaesthetics increase NMB by acting both on pre- and postsynaptic ACh receptors in the neuromuscular junction. The additive effect on NMB is dependent on length of exposure to the anaesthetic and a marked increase in effect can be seen after exposure exceeding 4 hours. The effect is also enhanced at higher end-tidal concentrations (Kansanaho and Olkkola 1995). The effect of reversal of a NMB by neostigmine has been shown to be significantly impaired in patients receiving sevoflurane for maintenance of anaesthesia as compared to propofol (Kim et al. 2004, Reid et al. 2001).

2.5.4. Propofol

Propofol (2.6 diisopropylphenol) has been used as an intravenous anaesthetic since 1986. The drug is used for induction and maintenance of general anaesthesia and for sedation of mechanically ventilated adult patients in intensive care units.

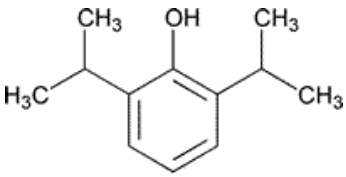

Figure 8. Propofol.

Propofol (2.6 diisopropylphenol) (Figure 8) binds to the $GABA_A$- receptor as previously discussed and activates it (Krasowski et al. 1998). It may also bind to the receptor in the presence of bound GABA transmitter and in this case propofol intensifies the effect of GABA (Korpi at al. 2002). The exact binding site of propofol at the receptor is unknown.

Propofol is short acting with a rapid onset of action. As compared with the previously more common intravenous barbiturate thiopentone, recovery is significantly faster and propofol also causes less emesis. Propofol causes amnesia, but has no analgetic properties.

Fat soluble propofol is administered as a mixture containing soy oil, which may provoke allergic reactions in some patients. Propofol is rapidly redistributed in the body and it is metabolized by conjugation in the liver. Prolonged infusion of propofol at a constant rate leads to accumulation and prolonged anaesthetic effect. Adverse effects (AEs) of propofol are hypotension, transient apnea and pain on injection. The most severe AE is the potentially lethal so called propofol infusion syndrome. It has been reported in paediatric intensive care patients occasionally.

Propofol is often used for maintenance of anaesthesia as an alternative to volatile anaesthetics. Administration of propofol as a continuous intravenous infusion supplemented with opioids and a NMBA is referred to as total intravenous anaesthesia (TIVA). Several advantages, such as rapid recovery and a lower incidence of post operative nausea and vomiting (PONV) are associated with TIVA (Smith et al. 1999). By using intravenous agents the potential adverse effects of volatile anaesthetics on theatre staff and the environment are also avoided.

Propofol reduces muscle tone significantly mainly through central mechanisms. Tracheal intubation can indeed be performed without administration of a NMBA, by combining sufficient doses of propofol and an opioid. The same is true for sevoflurane. Subanaesthetic doses of propofol -in the absence of NMB- have been shown to increase the incidence of pharyngeal dysfunction with a decrease in upper esophageal sphincter tone (Sundman et al. 2001). The overall effect of propofol on NMB is according to several studies, however, significantly lower than that of volatile anaesthetics (Hemmerling et al. 2001, Wulf et al. 1998).

2.5.5. Target controlled infusion of propofol and remifentanil

Target controlled infusion (TCI) is a computerized system for delivering intravenous drugs, in particular anaesthetic drugs, such as propofol and opioids. The rationale behind this system is to deliver a particular drug according to the desired (target-) concentration either in the plasma or at the "effect site", i.e. the brain. The TCI system is still unfamiliar to many anaesthesists worldwide and will therefore be presented shortly in this chapter.

TCI systems have inbuilt pharmacokinetic models for several drugs. These models are derived from previous pharmacokinetic studies. The 3- compartment model concept (Glass et al. 2010) is often used to mathematically describe the behavior of intravenously administered anaesthetics. The central compartment (V1) is the compartment into which the drug is injected, i.e. the plasma. From the plasma the drug is quickly redistributed into compartment V2 and more slowly to V3. The fast compartment V2 represents the vessel rich tissues, while V3 -the slow compartment- consists of tissues with lesser blood flow. The pharmacokinetic models take into account several patient factors that affect kinetics, such as age, sex, length, weight and lean body mass (Schnider et al. 1998). For the delivery of propofol several models are available, the most frequently used being the Marsh model (Marsh et al. 1991) and the Schnider model (Schnider et al. 1998).

TCI has been used for intravenous opioid delivery with sufentanil, alfentanil and the ultra-short acting opioid remifentanil. The Minto model (Minto et al. 1997) is used for infusing remifentanil. Remifentanil is degraded by unspecific esterases in the tissues (Egan et al. 1996) and unlike other opioids does not accumulate even during longtime infusions. Remifentanil TCI and propofol TCI are often used in combination. Remifentanil TCI can also be successfully used in combination with a volatile anaesthetic. The TCI technique allows a more precise titration of the drug as compared to manually adjusted infusions. However, the ratio of the actual blood concentration to the predicted blood concentration varies between individuals.

2.6. Monitoring depth of anaesthesia

Anaesthesia induces significant changes in the electroencephalogram (EEG). In the view of the clinical anaesthetist it is of great importance to be able to assess the level of hypnosis during anaesthesia in order to optimize patient safety and minimize the incidence of intraoperative awareness and recall (Ekman et al. 2004, Myles et al. 2004). Another motive for measuring the level of hypnosis intra-operatively is the possibility of reducing the consumption of anaesthetics, with the possible benefits of reduced costs and faster recovery (Yli-Hankala et al. 1999, Liu 2004, Ekman et al. 2004).

Analyzing real-time raw EEG signals during anaesthesia is difficult. In recent years several tools for the measurement of the level of hypnosis have been developed. Among the best evaluated ones are the EEG-derived indices bispectral index (Rampil 1998 and Rampil et al. 1998) and spectral entropy (Viertiö-Oja et al. 2004). These monitors analyze and convert EEG signals into a continuous index between 0 and 100. Measurement of auditory-evoked potentials (AEP) derived from the EEG

response to auditory stimuli is another much used method for assessing depth of anaesthesia. Changes in amplitude and latency of Pa and Nb waves from the primary auditory cortex are seen with increasing levels of anaesthesia (Thornton 1991).

2.6.1. The bispectral index

The bispectral index (BIS) is one of the most studied measures of anaesthetic depth. It is derived from the bispectrum of the EEG, which reflects the effects of anaesthetics on the brain. The BIS integrates several disparate descriptors of the EEG and is a composition of time domain, frequency domain and high-order spectral sub-parameters (Rampil et al. 1998). The EEG information is processed and then presented as an index as described above. Zero represents an iso-electric EEG and indicates a very deep level of anaesthesia, while 100 indicates full awakeness. An index between 40 and 60 is recommended as the most optimal anaesthetic level during surgical procedures.

The BIS formula is synthesized empirically from EEG and behavioral scales derived from a large volume of clinical data from a prospectively collected database, but the algorithm has not been published in detail. The BIS monitor uses different algorithms to calculate the bispectral index during different states of anaesthesia (Bruhn et al. 2000b).

The index is calculated from each consecutive 60 s of sampled EEG that is initially filtered to exclude high- and low-frequency artifacts and divided into 2 s epochs. Computation of the bispectral parameter requires averaging several epochs and there exists a 75% overlap between adjacent epochs. The BIS value reported thus represents an average value derived from the previous 60 s of useable data (Rampil et al. 1998).

Since the index is calculated from events in the EEG signal that have already passed, there is a considerable time lag between changes in the EEG and the changes being reflected in the index. The monitor cannot predict any future changes in anaesthesia depth. The BIS is insensitive to the specific anaesthetic or sedative agents used. The BIS monitor has been validated with several intravenous and inhalational anaesthetics (Vakkuri et al. 2004). However, the effect on the EEG of nitrous oxide and the NMDA antagonist ketamine differ from that of GABAergic anaesthetics, resulting in increased beta activity and the anaesthetic effect of these drugs cannot reliably be measured by any of the EEG-derived indices (Maksimow et al. 2006, Park et al. 2006).

2.6.2. Spectral entropy

Spectral entropy measures the regularity (or rather *irregularity*) of the frequency distribution. The human EEG is chaotic during the awake state and becomes slower and more regular with increased levels of anaesthesia. The Entropy Module™ of the S/5 Anesthesia Monitor (GE Healthcare Finland, Helsinki, Finland) measures depth of anaesthesia with a single algorithm. It calculates two different indicators: the state entropy (SE), reflecting the EEG-dominant part of the spectrum and the response entropy (RE), which includes both EEG- and EMG-dominant parts (Viertiö-Oja et al. 2004). In the analysis of SE electrical activity between 0.8 and 32.0 Hz is interpreted, while in the analysis of RE entropy content between 0.8 to 47.0 Hz is studied. Thus, unlike BIS, spectral entropy distinguishes

between pure EEG activity and EEG-activity influenced by EMG-derived signals. A different scaling is used for the SE (0-91) and RE values (0-100) and the RE value is always higher or equal to SE. The purpose of this is to make EMG activity detectable at any level of anaesthesia. Anaesthesia can be considered optimal when both values are equal and ranging between 40 and 60.

Spectral entropy seems to correlate equally well with different stages of sedation and hypnosis as does the BIS. According to one study the SE index correlates even better with sedation levels compared to BIS (Schmidt et al. 2004). Some other advantages of the Entropy Module™ in comparison with BIS have also been documented. Vakkuri et al. showed that RE indicates emergence from anaesthesia significantly faster than both SE and BIS (Vakkuri et al.2004). Spectral entropy has been validated for use with propofol, thiopentone, sevoflurane, isoflurane and desflurane, but not for use with ketamine (Bruhn et al. 2000, Schmidt et al. 2004).

2.7. The afferentation theory

It has been proposed that the reliability of BIS measurements may be adversely affected by frontal EMG activity from the muscles of the forehead (Dahaba 2005, Bruhn et al. 2000a). Whether the changes seen in the index values of these monitors following frontal EMG activity are merely artefacts or the result of actual arousal activity is still under debate (Bruhn et al. 2000a, Ekman et al. 2007a, Messner et al. 2003). Increasing frontal EMG activity itself is mostly associated with light anaesthesia. On the other hand, it has been suggested that agents or maneuvers that stimulate muscle stretch receptors (i.e., muscle afferents) may produce cerebral stimulation. This theory is known as the afferentation theory (Lanier et al. 1994). According to this theory increases in stretch receptor activity, regardless of the source (e.g., drug effect, active muscle movement), should have a similar stimulatory effect on the brain.

Neuromuscular blockade eliminates such EMG activity. Nevertheless, the data on the effect of NMB on BIS and AEP is conflicting (Greif et al. 2002, Inoue et al. 2006, Vasella et al. 2005). BIS response to noxious stimuli has been shown to depend on the degree of NMB during sevoflurane anaesthesia in one study (Ekman et al. 2007a). The same group performed another study to further elucidate the influence of neuromuscular block on the EEG response to a noxious stimulus during anaesthesia and the result of that study indicated that NMB actually may exert an effect on the level of hypnosis (Ekman et al. 2007b). In other studies no effect of NMB on BIS or AEP indices could be detected (Greif et al. 2002, Vasella et al. 2005).

3. OBJECTIVES OF THE STUDY

This study is essentially twofold and investigates the interaction between nitrous oxide and NMBAs (Study I-II) and factors related to the reversal of a rocuronium induced neuromuscular block by the novel reversal agent sugammadex (Study III-IV).

The specific objectives of this study were:

1. To study the effect of nitrous oxide on the infusion requirements of rocuronium during propofol-remifentanil anaesthesia.
2. To study the effect of nitrous oxide on the infusion requirements of cisatracurium during propofol-remifentanil anaesthesia.
3. To study the time gap, after the administration of sugammadex or neostigmine, between loss of visual fade by using a PNS until objective TOF ratio has returned to >0.90.
4. To study the effect of reversal of a rocuronium induced NMB by sugammadex on depth of anaesthesia as quantified by the bispectral index, spectral entropy and response entropy.

4. PATIENTS AND METHODS

4.1. Patients

A total of 220 patients were investigated in the four studies of this thesis. The main patient characteristics and methods are presented in Table 2. All patients provided informed written consent prior to enrollment. The patients were scheduled for elective oto-rhino-laryngeal surgery requiring general anaesthesia. Study III was in part (n=20) conducted at Oulu University Hospital and these patients were scheduled for elective general surgery.

Table 2. Main characteristics of patients and methods in Studies I-IV.

Study Nr	Group	Nr of patients (F/M)	Age (years)	ASA (1/2/3)	BMI (kg/m^2)	NMBA	Anaesthetic regimen	Reversal
I	Air-TIVA	35 (12/23)	45 ± 15	24/5/6	25 ± 3	Rocuronium by closed-loop infusion	Propofol/Remi-fentanil by TCI	Neostig-mine
	N_2O-TIVA	35 (14/21)	49 ± 13	17/14/4	25 ± 3		Propofol/Remi-fentanil by TCI + N_2O	
II	Air-TIVA	35 (15/20)	47 ± 13	23/12/0	25 ± 3	Cis-atracurium by closed-loop infusion	Propofol/Remi-fentanil by TCI	Neostig-mine
	N_2O-TIVA	35 (13/22)	49 ± 12	18/16/1	25 ± 3		Propofol/Remi-fentanil by TCI + N_2O	
III	Neostig-mine	23 (13/10)	39 ± 16	15/8/0	26 ± 4	Rocuronium by repetitive boli	Sevo- or desflurane combined with fentanyl, alfentanil or remifentanil	Neostig-mine
	Sugam-madex	24 (15/9)	43 ± 15	15/7/2	25 ± 4			Sugam-madex
IV	NA	29 (19/10)	49 ± 12	13/14/2	27 ± 5	Rocuronium by repetitive boli	Propofol/Remi-fentanil by TCI	Sugam-madex

Values are mean ± SD or number of patients. F = female; M = male; ASA = American Society of Anesthesiologists' physical status classification; BMI = body mass index; NMBA = neuromuscular blocking agent and its mode of administration; TIVA = total intravenous anaesthesia; TCI = target controlled infusion; NA = not applicable

Patients with significant cardiac, renal or hepatic insufficiency, elevated intracranial pressure, a body mass index (BMI) exceeding 32.5 and patients suffering from marked ventilatory impairment due to underlying respiratory disease, were excluded. Other criteria for exclusion were certain diseases, such as muscular dystrophy, myopathy or cerebral palsy and concomitant medication known to affect neuromuscular transmission (I-IV). In addition, pregnant and lactating females were excluded and a pregnancy test was performed in premenopausal female patients when indicated (III). Randomization was used in studies I-III. All patients in Study IV received the same treatment. Thus, no randomization or blinding was used in Study IV.

4.2. Anaesthesia and ventilation

In Studies I-II and IV patients were anaesthetized by target controlled infusion (TCI) of propofol and remifentanil. Premedication consisting of oral midazolam 3.75-7.5 mg (I, II) or oral paracetamol 1.5-2 g and oral diazepam 5-10 mg (IV) was administered along with any continuous medication (I-II, IV). At induction, the propofol target was set at 4 µg/ml intitially and increased to 6 µg/ml, whenever clinically indicated (I-II). In Study IV the initial propofol target was 6 µg/ml. The remifentanil target was set at 2 ng/ml (I-II, IV). Effect site targeting was used for both propofol and remifentanil (I-II, IV).

In Studies I-II the patients were randomly assigned to receive a gas mixture of either nitrous oxide with oxygen (N=35) or air with oxygen (N=35) throughout the procedure. Initially, these gas mixtures were delivered by mask ventilation until the patients were intubated and connected to the anaesthesia ventilator. Inspiratory oxygen was set at 30% and fresh gas flow kept at 10 l/min during mask ventilation and at 5 l/min during mechanical ventilation in order to keep gas (nitrous oxide) concentrations as constant as possible. In Study IV the patients were ventilated by mask at induction using 100 % oxygen. After intubation the patients were mechanically ventilated by a mixture of oxygen and air

After intubation the target of propofol was kept at 4 µg/ml and the remifentanil target was adjusted according to clinical need between 1.5-6 ng/ml (I-II, IV). With clinical signs of nociception as judged by excessive salivation, lacrimation and changes in cardiovascular parameters (over +30% increase in systolic blood pressure or systolic blood pressure higher than 180 mmHg) remifentanil target was increased as clinically indicated (I-II, IV).

In Study III all patients were anaesthetized according to the normal clinical routine of the respective hospitals. In addition to their normal continuous medication in the morning of the day of surgery, the patients were given the standard premedication of the study centre. The patients received propofol and an opioid (fentanyl, alfentanil or remifentanil) at induction and after tracheal intubation anaesthesia was maintained by either sevoflurane or desflurane in a mixture of oxygen and air supplemented with an opioid. Regional anaesthesia was applied whenever clinically indicated, mainly for the management of postoperative pain.

4.3. Neuromuscular blockade and reversal

Rocuronium was used in Studies I, III and IV, while in Study II cisatracurium was used.

In Studies I-II a standardized initial bolus dose of NMBA (0.6 mg/kg rocuronium in Study I; 0.1 mg/kg cisatracurium in Study II) was administered after calibration of the neuromuscular monitoring device. The dose was calculated per ideal body weight as defined by Devine's equation (Devine 1974). A closed-loop feedback controlled computerized infusion (Olkkola and Schwilden 1990) of respective NMBA was used to maintain a stable 90% (T1 = 10% of the control value) NMB for at least 90 minutes. After the 90 minute study period had been completed delivery of NMB was discontinued and spontaneous recovery from NMB was allowed whenever a continued NMB was not regarded necessary. Neostigmine was administered for reversal whenever a spontaneous recovery of TR ≥ 0.9

had not been achieved. Neuromuscular monitoring was thus continued in each patient until an objectively measured TR of at least 0.9 had been obtained.

In Studies III-IV bolus dosage of rocuronium was used throughout the procedure. In Study III, an initial bolus dose of rocuronium 0.6-1.0 mg/kg was administered to facilitate endotracheal intubation. Incremental doses of rocuronium 5-10 mg were administered whenever two visible twitch responses would reappear using a PNS. At the end of anaesthesia the patients in Study III were randomly assigned to receive either neostigmine or sugammadex for reversal of NMB. The clinical anaesthetist responsible for the patient was blinded to the reversal agent used and also to the objective measurements of NMB.

In Study IV, an initial 0.6 mg/kg bolus dose of rocuronium was administered before tracheal intubation. The dose was calculated per ideal body weight as defined by Devine's equation (Devine 1974), similarly as in Studies I-II. Neuromuscular blockade was sustained at a level where T1 (-T2) remained detectable, using additional bolus administration of rocuronium, when necessary. Sugammadex was administered to all patients participating in this study.

4.3.1. Closed-loop feedback controlled infusion

In Studies I-II maintenance of NMB was achieved by intravenous infusion of the NMBA through an indwelling cannula in a forearm vein. The infusion was controlled by a model-driven closed loop feedback system as described previously (Olkkola and Schwilden 1990). The Relaxograph®(Datex, Helsinki, Finland) monitor and an infusion pump (Fresenius Infusomat CP-IS®, Fresenius AG, Bad Homburg, Germany) were connected to a Compaq® Portable 386 Computer (Compaq Computer Corporation, Houston, Texas, USA) by means of a serial RS232C interface. The infusion was programmed according to the desired level of neuromuscular blockade (i.e. the set-point), which in Studies I-II of this thesis was set to 90% (T1 = 10% from control). The infusion started at the point when T1 first increased above 10% from control as a sign of beginning recovery from the initial NMBA bolus. Unlike the commonly used open-loop infusion systems, in which drug dosage is determined by the clinician, the closed-loop system determines the infusion rate (input) at a particular time by adjusting it to the latest measurement of drug effect (output), which in this case was the degree of NMB measured every 20 seconds by the Relaxograph®.

Controller performance was measured by calculating the mean offset from set-point and the mean SD from set-point during feedback infusion. The measured values for effect and rate of the infusion were saved on the computer. The possible effect of nitrous oxide on rocuronium (in Study I) and cisatracurium (in Study II) was then quantified by comparing the asymptotic steady state rates of infusion for 90% block between the groups in the respective studies.

Cumulative dose of NMBA = $D \cdot (1 - e^{-kt}) + I_{ss}t$

where D is the amount of muscle relaxant in its apparent distribution volume, k is the relative rate of distribution of muscle relaxant, I_{ss} is the asymptotic steady state infusion rate of either rocuronium or cisatracurium and t is the duration of infusion of the NMBA.

Figure 9 shows the data for one representative patient in the rocuronium study (Study I) and demonstrates the calculation of I_{ss}.

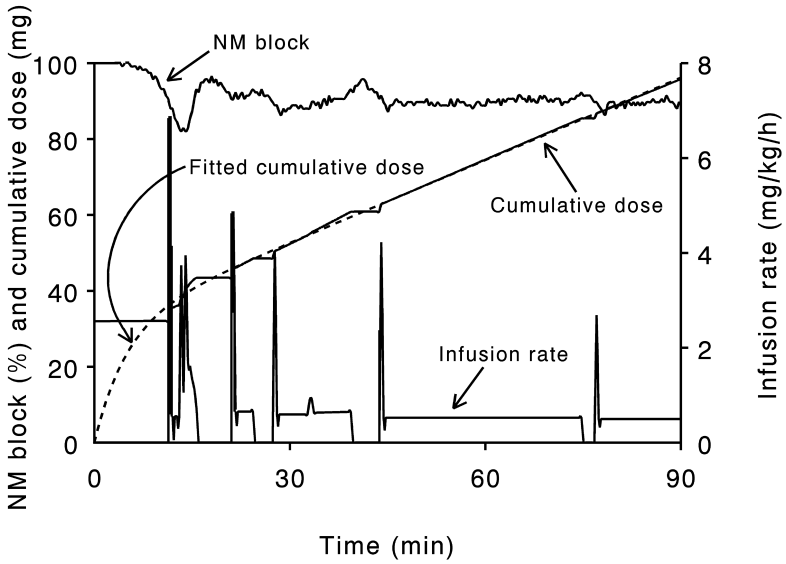

Figure 9. Data for one representative patient in the Nitrous Oxide- TIVA group showing the rate of infusion (I_{ss}) necessary to produce a constant 90% NMB by closed-loop infusion of rocuronium, the corresponding cumulative dose requirements of rocuronium, the fitted cumulative dose requirements and the measured NMB (Study I).

4.3.2. Level of block during the study period and at reversal

In Studies I-II the above described closed-loop feedback system was used to maintain a 90% NMB (T1 = 10% from control) until the 90 minute study period had been completed. After this, the infusion was either discontinued or, when clinically indicated, continued as long as regarded necessary. Thus, the degree of NMB was 90% or less at the time of reversal. Reversal was achieved by neostigmine administered with glycopyrrate. A TR ≥ 0.9 was awaited before ending monitoring.

In Study III the most relevant issue in terms of NMB was to achieve uniform levels of blockade in all patients at the moment of reversal. Thus, the initial rocuronium bolus (0.6-1 mg/kg) was determined by the anaesthetist responsible for the patient according to estimated duration of anaesthesia and other relevant factors. As will be further described in the chapter on neuromuscular monitoring, the clinician relied on visual evaluation of the TOF responses only. NMB was sustained during the entire procedure and incremental doses of rocuronium 5-10 mg were administered by the clinician at the (visual) reappearance of the second TOF twitch (T2). The reversal agent (either sugammadex or neostigmine according to the randomization plan) was administered by a nurse or the assisting investigator at the point when the clinical anaesthetist reported two visible twitch responses in three consecutive series of TOF-stimulation applied at 15 s intervals with a PNS.

The patients in Study IV received a 0.6 mg/kg bolus dose of rocuronium at induction as previously described. NMB was sustained during the entire procedure, until administration of the reversal drug sugammadex, at a level where electromyographic TOF responses T1 (-T2) were detectable. Thus, additional bolus administration of rocuronium was used whenever more than two responses were measured. After induction of anesthesia, a Datex ElectroSensor®-neurotransmission monitor was used to obtain control electromyographic values. The train-of-four sequence (100 ms pulse width, 2 Hz frequency) was then assessed every 20 s until anaesthesia had been completed and complete reversal, as defined of a train-of-four ratio of >0.9, had been achieved.

4.4. Clinical monitoring

4.4.1. Cardioventilatory monitoring

In all studies (I-IV) cardiorespiratory parameters were measured according to the normal routine of the hospital(s). This includes noninvasive measurement of blood pressure, heart rate, continuous ECG, minute ventilation, respiration rate, inspiratory and end-tidal O_2 and CO_2, airway pressure and O_2-saturation. During maintenance of anaesthesia, end-tidal pCO_2 was maintained between 34-40 mmHg (4.5-5.3%). Routine monitoring also included inspiratory and end-tidal concentrations of N_2O.

In Study III volatile anaesthetics were administered and thus, in addition to the above mentioned parameters, inspiratory and expiratory concentrations of either sevoflurane or desflurane were monitored but not reported in this thesis. All physiological data including the cardiorespiratory parameters, muscle relaxation and the indices for the depth of anaesthesia were collected at 10 or 30 s intervals directly from the patient monitors using the GE Collect by Windows® software (GE Healthcare).

4.4.2. Neuromuscular monitoring

Studies I-II: After induction of anaesthesia, but before administering either rocuronium (Study I) or cisatracurium (Study II) for NMB, a Relaxograph® neuromuscular transmission monitor (Datex, Helsinki, Finland) was used to obtain control electromyographic values. The TOF sequence was assessed (frequency of stimuli, 2 Hz; pulse width, 100 μs) by means of stimulating surface electrodes placed adjacent to the ulnar nerve at the wrist. Recording electrodes were placed on the first dorsal interosseus muscle and index finger (Kalli 1990). The stimulus output was a rectangular wave with a current range of 0-70 mA and the machine calibrated automatically by searching for the optimum signal levels before setting the supramaximal level. A second calibration of the neuromuscular monitoring device was carried out 10 min after induction of anaesthesia. A stable baseline calibration signal was awaited before administration of NMBA. The degree of NMB was assessed every 20 s with the Relaxograph® and is defined as the ratio of the measurement of T1 to the corresponding control value.

Study III: The degree of NMB was measured by one single monitor, but using two separate methods as previously described. Specifically, objective monitoring of the evoked muscle responses was

applied over the ulnar nerve by use of the TOF-Watch® (TOF-Watch®, Organon Inc.), that applies the acceleromyographic technique for the measurement of NMB. Calibration of the device was carried out in accordance with the instructions provided by the manufacturer by applying an initial tetanic stimulus.The train-of-four-mode of stimulation (2 Hz; 0.2 ms) was applied at 15 s intervals throughout the procedure. The anaesthetist in charge of the patient was blinded to the objective measurements and quantified the degree of neuromuscular block only by visual evaluation of the muscle contraction response.

Study IV: After induction of anaesthesia a Datex ElectroSensor neuromuscular transmission monitor (Datex, Helsinki, Finland) was used to obtain control electromyographic values. The train-of-four sequence (200 μs pulse width, 2Hz frequency) was then assessed every 20 s until anaesthesia has been completed.

4.4.3. Additional monitoring relevant to the study

The Bispectral index (BIS) was used for the assessment of anaesthetic depth in Studies I-II. Skin temperature of the hand was measured and kept above 33°C (Studies I-III). If necessary, forced air warming blanket was used.

In Study IV the main focus was on detecting a possible change in the level of anaesthesia after reversal by sugammadex and therefore depth of anaesthesia was monitored by two different methods in all subjects, the Bispectral Index and State Entropy. After termination of surgery, in order to gain stable baseline levels of BIS- and entropy values, without any presence of noxious stimuli, the patients were kept anesthetized for an additional 5 minutes before reversal of neuromuscular blockade by sugammadex. Sugammadex 2 mg/kg was then administered and administration of propofol and remifentanil, as well as recording of BIS and state entropy values, was continued for another 10 minutes. After this, anaesthesia was discontinued and the patients were extubated and transferred to the post anaesthesia care unit (PACU).

4.5. Statistical analysis

The number of patients recruited in each of the four studies was calculated prior to the commencement of each study. Based on previous studies (Olkkola and Tammisto 1994; Hemmerling et al. 2001), studies I and II were powered to demonstrate a 15% difference in the NMBA infusion requirements at a level of significance of $P = 0.05$ and power of 80%. The same power and level of significance was used also in study IV where the sample size was based on the anticipated difference of 5 BIS-units before and after the administration of sugammadex (Laitio et al. 2008). Study III was powered to demonstrate a 7-minute difference in the time from the administration of neostigmine or sugammadex to the achievement of TR of 0.90 at a level of significance of $P = 0.05$ and power of 90% (Flockton et al. 2008; Naguib and Lien 2010).

Statistical analysis of the data was performed by using Student's t-test, Mann- Whitney U-test and Chi-square test as appropriate (I-III). In Study IV, the data were compared using analysis of variance

for repeated measures. Results are given as mean ± SD and $P < 0.05$ indicates a difference of statistical significance. All data of the four studies were analyzed using the statistical program Systat for Windows®, version 10.2 (Systat Software, Richmond, CA, USA).

5. RESULTS

Patient characteristics and controller performance were similar in both groups in Studies I and II. The same applies to peripheral skin temperature, end-tidal carbon dioxide and average values for bispectral index and remifentanil consumption. Slightly more patients appeared to receive ephedrine in the groups receiving nitrous oxide as compared to the groups receiving air-oxygen mixture, but the difference between the groups was not statistically significant in either of the two studies. The main results of Studies I and II are presented in Figure 10 and Table 3.

5.1. Effect of nitrous oxide on rocuronium infusion requirements

In Study I the mean steady-state infusion rates of rocuronium were 33.0 ± 9.8 and 36.9 ± 13.2 mg/h in the Nitrous oxide-TIVA and Air-TIVA groups, respectively. When taking in account the ideal body weights (IBW) the mean infusion rates were 0.50 ± 0.14 and 0.55 ± 0.17 mg/kg/h, respectively. The mean values for the cumulative dose of rocuronium, I_{ss} and I_{ss}/ IBW were thus 6-10 % higher in the Air-TIVA group. This difference was not, however, statistically significant.

5.2. Effect of nitrous oxide on cisatracurium infusion requirements

The mean steady-state rates of cisatracurium infusion in Study II were 0.072 ± 0.018 and 0.066 ± 0.017 mg/kg/h in the Air-TIVA and Nitrous oxide-TIVA groups, respectively (Figure 10). This difference in infusion rates between the groups was not statistically significant.

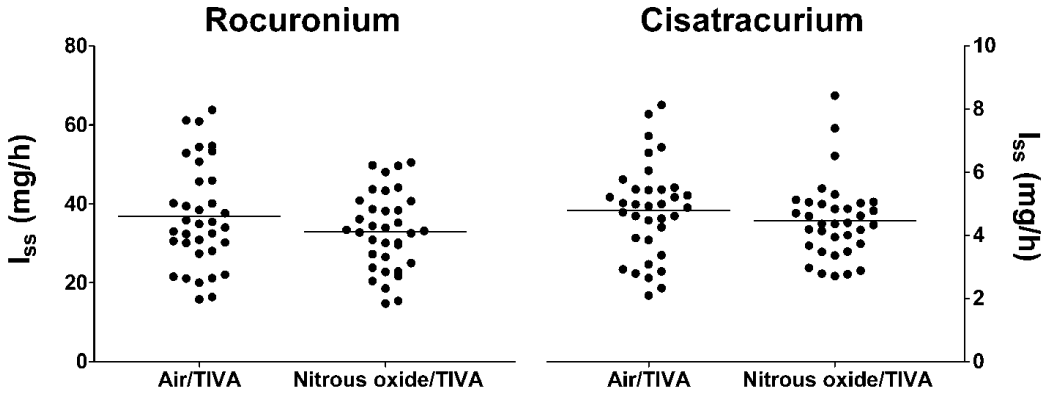

Figure 10. Scattergram of the individual values of the rate of infusion (I_{ss}) necessary to produce a constant 90% NMB during TIVA with air (Air-TIVA) and nitrous oxide (Nitrous oxide-TIVA). The mean value in each group is represented by a horizontal line (Study I, II).

Table 3. Steady-state rate of infusion of rocuronium (Study I) and cisatracurium (Study II) controlled by closed-loop feedback system to maintain neuromuscular blockade constant at 90% during total intravenous anesthesia (TIVA) with air (Air/TIVA) or with nitrous oxide (N_2O/TIVA).

Study Nr	Group	Controller performance		Time to 10% recovery of T1 following the initial bolus (min)	Cumulative dose of rocuronium (I) or cis-atracurium (II) /IBW (mg/kg)	Steady-state rate of infusion of rocuronium (I) or cisatracurium (II)	
		Offset from set-point (%)	SD from set-point (%)			I_{ss} (mg/h)	I_{ss}/IBW ($mg \cdot kg^{-1} \cdot h^{-1}$)
I	Air/TIVA	0.3 ± 1.3	2.5 ± 1.3	25 ± 8	1.3 ± 0.3	36.9 ± 13.2	0.55 ± 0.17
	N_2O/TIVA	0.2 ± 0.9	2.8 ± 1.0	27 ± 8	1.2 ± 0.2	33.0 ± 9.8	0.50 ± 0.14
	Mean difference (95% CI)	-0.1 (-0.7, 0.4)	0.2 (-0.3, 0.8)	2.4 (-1.5, 6.3)	-0.1 (-0.2, 0.1)	-3.9 (-9.4, 1.7)	-0.05 (-0.12, 0.03)
II	Air/TIVA	1.0 ± 1.5	3.0 ± 1.3	31.5 ± 6.1	12.4 ± 2.6	4.8 ± 1.5	0.07 ± 0.02
	N_2O/TIVA	1.1 ± 1.3	3.0 ± 1.2	34.4 ± 7.6	12.1 ± 2.2	4.5 ± 1.2	0.07 ± 0.02
	Mean difference (95% CI)	0.1 (-0.5, 0.7)	0.0 (-0.7, 0.5)	2.9 (-0.4, 6.2)	0.3 (-1.5, 0.8)	0.3 (-1.0, 0.3)	0.00 (-0.01, 0.00)

Values are mean ± SD. There were no statistically significant differences between the groups. ASA = American Society of Anesthesiologists' physical status classification; CI = confidence interval of the difference in mean values; IBW = ideal body weight; I_{ss} = asymptotic steady-state rate of infusion; I_{ss}/IBW = asymptotic steady-state rate of infusion per kg ideal body weight; NMB = neuromuscular blockade; T1 = first twitch in the train-of-four sequence.

5.3. Safety and accuracy of reversal and monitoring of a NMB

Patient characteristics in the two groups of Study III were similar and there were no significant differences in the conduct of anaesthesia between the groups. Times from reversal until return of TR 0.90 were 13.3 ± 5.7 min in the neostigmine and 1.7 ± 0.7 min in the sugammadex groups, respectively (P < 0.001). The potentially unsafe periods of recovery, i.e the times from loss of visual fade to the return of TR 0.90, were 10.3 ± 5.5 and 0.3 ± 0.3 min, respectively (P < 0.001). The main results of Study III are displayed as scattergrams in Figure 11.

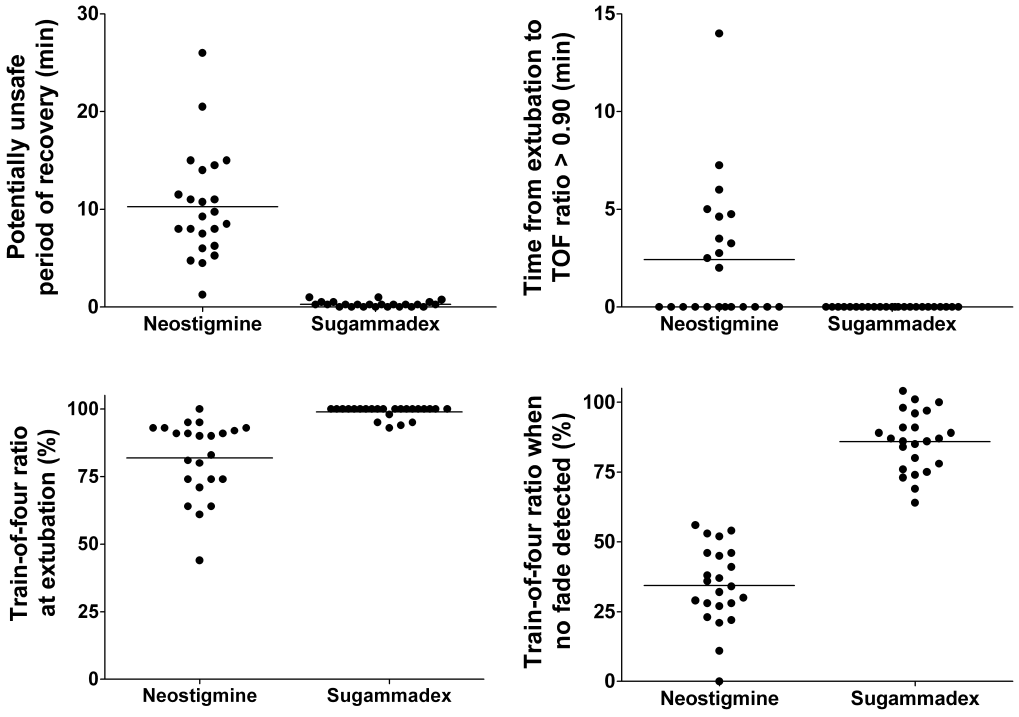

Figure 11. Scattergrams of the potentially unsafe period of recovery, the TR at the time of extubation and at the time no visual fade could be detected and the time elapsed from extubation until return of a TR > 0.90 of each individual in the two groups. The mean values are represented by a horizontal line. (Study III).

TRs at the time of loss of visual fade were 0.34 ± 0.14 and 0.86 ± 0.11 and at the time of extubation 0.82 ± 0.14 and 0.99 ± 0.02 in the neostigmine and the sugammadex groups, respectively (P < 0.001). The main results of Study III are presented in Table 4.

Table 4. Variables describing the use of rocuronium and recovery of neuromuscular block in the two groups.

Variable	**Group**		***P*-value***
	Neostigmine	**Sugammadex**	
Initial dose of rocuronium (mg)	48 ± 8 (35-65)	48 ± 8 (35-62)	0.903
Total dose of rocuronium (mg)	63 ± 22 (40-125)	61 ± 16 (38-95)	0.932
TOF ratio at time of 'no fade'	0.34 ± 0.14 (0.00-0.56)	0.86 ± 0.11 (0.64-1.04)	<0.001
Time from 'no fade' to TOF ratio 0.70 (min)	4.6 ± 2.7 (0.3-10.3)	0.0 ± 0.1 (0.0-0.3)	<0.001
Time from 'no fade' to TOF ratio 0.80 (min)	7.1 ± 4.3 (1.0-20.5)	0.1 ± 0.1 (0.0-0.5)	<0.001
Time from 'no fade' to TOF ratio 0.90 (min) = *unsafe period of recovery*	10.3 ± 5.5 (1.3-26.0)	0.3 ± 0.3 (0.0-1.0)	<0.001
Time from reversal to TOF ratio 0.70 (min)	7.6 ± 3.4 (2.5-15.8)	1.3 ± 0.6 (0.5-3.0)	<0.001
Time from reversal to TOF ratio 0.80 (min)	10.1 ± 4.7 (3.3-23.4)	1.4 ± 0.6 (0.6-3.0)	<0.001
Time from reversal to TOF ratio 0.90 (min)	13.3 ± 5.7 (3.5-28.9)	1.7 ± 0.7 (0.7-3.5)	<0.001
TOF ratio at extubation	0.82 ± 0.14 (0.44-1.00)	0.99 ± 0.02 (0.93-1.04)	<0.001
Time from extubation to TOF ratio 0.90 (min)	2.4 ± 3.4 (0.0-14.0)	0.0 (0.0-0.0)	<0.001

*Determined by Mann-Whitney U-test.

Values are mean ± SD (range); TOF ratio = Train-of-four ratio; 'No fade' = no fade in the train-of-four response can any longer be detected by the anaesthetist, who is blinded to the objective measurements.

5.4. Effect of sugammadex reversal on the bispectral index and entropy

All patients in Study IV received the same treatment, thus no randomization or blinding was used. The mean averaged BIS, state entropy and response entropy values were 31.7 ± 9.9, 35.3 ± 12.9 and 36.8 ± 13.3 before and 32.0 ± 11.9, 36.3 ± 15.9 and 38.4 ± 18.0 after sugammadex administration, respectively. There were no statistically significant differences between the measured *pre* and *post* sugammadex values. Mean results are shown in Figure 12.

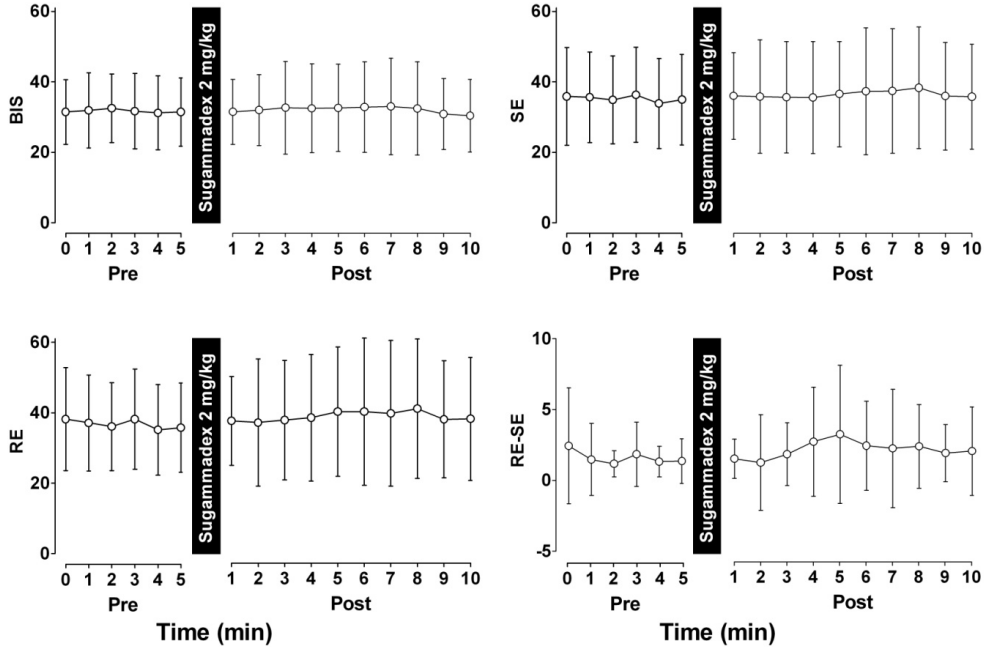

Figure 12. The values (mean and SD) of bispectral index (BIS), state entropy (SE), response entropy (RE) and difference between response entropy and state entropy (RE-SE) before and after the administration of sugammadex 2 mg/kg for the reversal of rocuronium-induced NMB. (Study IV).

6. DISCUSSION

6.1. General methodological considerations

All studies were approved by Ethics Committee of the Hospital District of Southwest Finland and by the Finnish Medicines agency and they were reported to the European EudraCT clinical trials register. The studies were performed according to the revised Declaration of Helsinki and also in accordance with the good clinical research practice guidelines regarding studies of neuromuscular blocking agents (Fuchs-Buder et al. 2007). All studies were adequately powered to be able to demonstrate a clinically significant difference between the treatment groups. Studies I, II and IV used an open design whereas in study III a double-blind design was employed. Studies I-III were randomized studies whereas the nature of study IV did not necessitate randomization. The studies can therefore be expected to produce unbiased results.

In order to be able to quantify the NMBA infusion requirements during maintenance of anaesthesia, a minimum study period of 90 minutes was used in studies I-II. All patients, except 20/50 of the study patients in study III were anaesthetized at the Department of Oto-rhino-laryngology at Turku University Hospital. The patients at the Department of Oto-rhino-laryngology underwent different surgical procedures, typically various forms of sinus surgery, middle and inner ear surgery, parotidectomies and neck dissections. Patients undergoing extensive head- and neck surgical procedures, such as major tumor resections and reconstructive surgery were excluded. To avoid possible bias associated with marked obesity, only patients with a body mass index (BMI) of less than 32.5 were included. Other criteria for exclusion were clinically significant renal, hepatic or ventilatory dysfunction, raised intracranial pressure, pregnancy or lactation. Patients with muscular dystrophies, myopathy or cerebral palsy and patients with a previous history of intolerance to any of the study drugs were also excluded. Medication known to interfere with neuromuscular transmission and simultaneous participation in other studies were also causes for exclusion. As excessive perioperative bleeding might have affected infusion requirements through altered hemodynamics, blood loss exceeding 15 ml/kg was a cause for exclusion from the studies. As a conclusion it seems fair to assume that the type of surgery affected neither the conduct nor the results of the studies performed entirely at the Department of Oto-rhino-laryngology. 40% of the patients in study III underwent scheduled general surgery at Oulu University Hospital. Because the same exclusion criteria were used for these patients, too, it is unlikely that the validity of the study would have been jeopardized by accrual of patients from two centres.

The measurement of NMB is sensitive to body temperature (Heier et al. 1990). For reliable quantifying of NMB, it is of vital importance to avoid hypothermia. In the present study the peripheral temperature of the monitored hand was measured continuously and kept above 33°C by the use of warm blankets to avoid any systematic errors in neuromuscular monitoring.

Objective neuromuscular monitoring was used in all four studies. Studies I, II and IV used electromyography (EMG), which records the compound muscle action potential after stimulation of the corresponding nerve (Fuchs-Buder et al. 2007). In Study III acceleromyography (AMG) was used for the objective measurements and the results obtained were compared to visual assessment of the muscle twitch response as previously described. AMG measures acceleration of the thumb after stimulation of the ulnar nerve (Viby-Mogensen et al. 1988). According to Newton's second law force equals mass times acceleration. As mass (the thumb) remains constant, the degree of acceleration is directly proportional to the force of the muscle response. Mechanomyography (MMG), as previously discussed, is considered the golden standard method of monitoring NMB for clinical research purposes. MMG is, however, a rather bulky and difficult method to use in the everyday clinical setting. EMG measurements correlate well with results obtained by MMG, while AMG cannot be used interchangeably with either EMG or MMG (Fuchs-Buder et al. 2007). Results obtained by AMG tend to be slightly higher in comparison with those obtained by the other methods. It has been recommended by some experts that the AMG measurements be normalized to the control TR value in order to improve accuracy of the data, although this issue remains somewhat controversial (Claudius et al. 2009b). In Studies I and II it was of critical importance to maintain of a stable level of NMB throughout the entire 90-minute study period in order to obtain reliable data on the NMBA infusion requirements. As EMG can be considered reasonably accurate and superior in reliability to any of the other monitoring methods available this method was chosen for the studies. AMG is widely used in clinical anaesthesia management and was regarded appropriate for Study III as the same device could easily be used for subjective (visual) and objective assessment at the same time. Using the same device, instead of two separate ones, for both monitoring methods was regarded necessary in order to improve comparability (and thus reliability). Although it is inferior to EMG in regard of accuracy, AMG can still be considered reasonably reliable for the comparison of train-of-four ratios. As described earlier, the results of Study III were also normalized.

6.2. Effect of nitrous oxide on rocuronium and cisatracurium requirements

Nitrous oxide had no statistically significant effect on rocuronium or cisatracurium infusion requirements. While looking at the scattergrams of the individual I_{ss} values in the current studies (Figure 10), it is fair to conclude that the effect of N_2O on rocuronium and cisatracurium requirements is negligible and has no clinical significance. The 95% confidence intervals of the differences in mean I_{ss} and Iss/IBW values add to this conclusion. Studies I and II were adequately powered to observe as small as a 15% difference at a level of significance of P = 0.05 and a power of 80%. Thus the results of Study I and II differ from the results of previous studies using bolus administration of mivacurium, rocuronium and vecuronium (Plaud et al. 2002; Kopman et al. 2005a; Fiset et al. 1991), in which N_2O was shown to potentiate the effect of the NMBA.

The two studies included in this thesis differ in terms of methodology from previous studies on the interaction between N_2O and muscle relaxants in several ways. Unlike the Plaud, Kopman and Fiset studies, which all used bolus techniques, Studies I and II used a closed-loop feedback control

method of administering NMBA. The computerized closed-loop feedback infusion of rocuronium (in Study I) and cisatracurium (in Study II) kept the level of NMB at a fairly constant level of 90% during the entire 90 minute study period. This made it possible to quantitate the interaction of N_2O with NMBAs by assessing relaxant infusion requirements. The Kopman study (Kopman et al. 2005a) used the single-dose technique for the quantitation of the N_2O-rocuronium interaction. They observed a 20% decrease of the mean ED50. Because at least a 90% NMB is required for adequate surgical relaxation, it seems more relevant to study the interaction of N_2O and muscle relaxants using constant infusion of the muscle relaxant under investigation as was done in Studies I and II. This study setup allows longer exposure to N_2O which in turn will increase the validity and reliability of the study. The longer the exposure, the better is the possibility to quantitate the true effect of N_2O on muscle relaxants. In previous studies the exposure to N_2O has been significantly shorter than in the present study - only 15 min in rocuronium and mivacurium studies and 5 min in vecuronium study (Plaud et al. 2002, Kopman et al. 2005a, Fiset et al. 1991), compared to the 90 minute study period of Studies I and II.

Due to the above described differences in methods of administration of the study drugs, the results of Studies I and II cannot be directly compared to the previous studies. The reason for the disagreement between the studies of this thesis and the previous ones is, however, still not clear. Ideally, the effect of anaesthesia on the pharmacodynamics on both bolus dosage and continuous infusion should have been investigated consecutively in all patients. Plaud and his group suggest that N_2O affects the neuromuscular junction directly and independently on its rate of accumulation in the muscle (Plaud et al. 2002). Saturation of muscle tissue with N_2O is less than 30% after 15 min administration of N_2O, supporting the idea of an accumulation-independent effect of N_2O on the neuromuscular junction. Others have proposed that N_2O exerts its effect by changing the transfer of muscle relaxants to the site of action (Kopman et al. 2005a). It has generally been assumed that N_2O has no effect on the pharmacokinetics of muscle relaxants (Stanski et al. 1979, Stanski et al. 1980, Cannon et al. 1987, Shanks et al. 1987, van den Broek 1994). Although obviously not providing any further information concerning the possible mechanism of action, the results of Studies I and II strongly suggest that N_2O has only a negligible, if any, effect on the pharmacological response of muscle relaxants.

There is some evidence that propofol affects the potency of NMBAs. In one study a 20-min infusion of propofol resulted in a 50% rise in the potency of mivacurium as compared to a 5-min propofol infusion (Hemmerling et al. 2008). However, studies I-II used a target controlled infusion of propofol and the target was kept unchanged at 4 μg/ml in all patients during the maintenance of anaesthesia for the entire study period. The use of propofol is therefore unlikely to have affected our conclusions on the effect of N_2O on muscle relaxants. Because remifentanil is not known to affect the level of NMB (Naguib and Lien 2010) and because its cumulative dose during the 90-min study period and BIS-levels were similar in the groups, it can be assumed that the results of these studies reflect the true effect of N_2O on rocuronium and cisatracurium requirements.

6.3. Safe reversal of the NMB: impact of the reversal agent and monitoring method chosen

During recent years it has become widely agreed upon by experts that objective methods of monitoring NMB are superior to subjective methods both in regard of accuracy and reliability (Viby-Mogensen et al. 1985). Nevertheless, subjective methods such as visual or tactile assessment of the muscle response after stimulation by a PNS - or even plain evaluation of various clinical signs of neuromuscular recovery - remain widely relied upon among clinical anaesthetists (Murphy et al. 2008, Kopman et al. 2004). As lack of a fade in the muscle twitch response following a train-of-four stimulus has traditionally been considered as a sign of full recovery, it is also frequently assumed among clinicians that reversal of the NMB is unnecessary whenever visual or tactile fade has disappeared. Previous studies, however, have shown that visual and tactile fade cannot be detected at TR > 0.4 (Viby-Mogensen et al. 1985, Murphy et al. 2008b). It is evident that current clinical management of NMB often conflicts with the current recommendations made by experts in this field (Kopman 2010, Brull and Murphy 2010). Clearly, this may compromise patient safety.

Numerous studies have shown that sugammadex reverses a rocuronium induced NMB significantly faster when compared to neostigmine (de Boer et al. 2007, Flockton et al. 2008, Naguib and Lien 2010, Jones et al. 2008). Study III of this thesis investigated the speed of reversal of an intermediate level rocuronium induced NMB by either sugammadex or neostigmine. At the same time the study compared two methods of monitoring the muscle response to TOF stimulation during the recovery phase. Visual assessment of the muscle twitch response was compared with objectively measured data. One objective of Study III was indeed to investigate at which TR visual fade disappears and the muscle twitches are sensed as equal. The time elapsing from this moment until the return of TR > 0.90 is referred to in this thesis as *the potentially unsafe period of recovery*. As expected, the results on speed of reversal were comparable with results of the previous studies. Most importantly, however, Study III specifically demonstrates in a randomized controlled trial that the use of neostigmine in combination with visual assessment of the muscular response to PNS may increase the risk of postoperative residual curarization and exposes patients to an unnecessary risk of aspiration and hypoxia (Eriksson 1996, Eriksson et al. 1997, Murphy et al. 2008a).

Visual evaluation of the muscle response was used in all patients simultaneously with acceleromyography, an objective index of neuromuscular block. Visual, rather than tactile, evaluation was chosen in order to avoid any disturbance of the objective measurements. Any movement of the monitored hand during TOF stimulation could have affected the objectively measured TR results thus making them unreliable. Visual and tactile evaluations of NMB produce comparable results at low to moderate levels of fade (Viby-Mogensen et al. 1985). Blinding was used in respect of the objectively measured results and the reversal agent chosen. The patients were also randomized to receive either neostigmine or sugammadex. Blinding and randomization are essential as these measures increase the reliability of the results of this study.

Well-trained anaesthesia specialists could not detect a fade in the TOF response visually at levels beyond an average TR of 0.34 in the neostigmine group. This finding is in good agreement with previous results (Viby-Mogensen et al. 1985, Murphy et al. 2008b). The corresponding TR values in the sugammadex group were significantly higher. This can be explained by the significantly faster onset of action of sugammadex. *The potentially unsafe period of recovery,* as defined earlier, was reduced from an average time of 10 min with neostigmine to less than 20 seconds with sugammadex. There was also a significant interindividual variation of recovery times within the neostigmine group since the longest potentially unsafe period of recovery in this group was 26.0 minutes, whereas in the sugammadex group the longest potentially unsafe period was just 1.0 minutes. In the sugammadex group the lowest TR value when fade could not be detected was 0.64, whereas in the neostigmine group values less than 0.10 were observed. At extubation the values for TR were also significantly lower in the neostigmine group.

All acceleromyographic data was subsequently normalized which made the differences between the groups even greater. As all previous studies with sugammadex have used non-normalized TR values, the primary intent was, however, to present only the non-normalized results. According to the study protocol, objective monitoring was thus discontinued when a non-normalized TR > 0.9 had been reached and therefore it was impossible to recalculate the duration of *the potentially unsafe period of recovery* using normalized data. It is easy to conclude that normalization of this value would have added a few minutes to the results. It is fair to assume that the results of this study would have been even more obvious if either EMG or MMG had been used instead of AMG, as AMG tends to yield slightly higher TRs. This was exemplified in a study by Kopman (Kopman et al. 2004). A rocuronium NMB was reversed at T2 with neostigmine 50 µg/kg, similarly as in the study of this thesis, but electromyography rather than acceleromyography was used for monitoring of NMB. The reversal times were somewhat longer when NMB was monitored electromyographically.

Maintenance of anaesthesia was conducted by the use of volatile agents (sevoflurane or desflurane) and opioids (fentanyl, alfentanil or remifentanil) according to the clinical routine at the two study centres. Another potential option would have been to use a standardized anaesthesia regimen similar to that of Studies I-II and IV, as it can be argued that such standardization would have increased the reliability of the results. However, because the use of volatile anesthetics and opioids did not differ between the two groups, conclusions on the neuromuscular recovery can be assumed valid. The initial dose of rocuronium varied between 0.6 and 1.0 mg/kg and subsequent doses were either 5 or 10 mg (at two visible twitch responses) according to the judgement of the clinical anaesthetist. As the main focus of the study was to compare reversal by sugammadex with neostigmine, standardization of the level of NMB at the moment of reversal - and not previous dosage regimen - was critical for the reliability of the results. Nevertheless, the cumulative dose of rocuronium was similar in both groups and did not jeopardize the validity of the results.

6.4. Effect of sugammadex reversal on depth of anaesthesia

Previous findings suggest that arousal may be induced by the reversal of NMB by neostigmine (Vasella et al. 2005) and sugammadex (Pühringer et al. 2008). Vasella offered the previously described afferentation theory as an explanation, as the arousal effect appeared to correspond to a sudden increase in afferent signals from muscle stretch receptors (Lanier et al. 1994, Vasella et al. 2005). Pühringer et al discovered abnormally elevated blood pressure levels in two subjects after sugammadex reversal in a phase II trial consisting of a total of 176 patients (Pühringer et al. 2008). They proposed reversal-agent-induced arousal, like that previously associated with neostigmine, as a possible explanation. Study IV of this thesis, although focusing only on sugammadex, was designed with the specific aim to further evaluate this matter. The results showed no influence by sugammadex-induced reversal of an intermediate level rocuronium NMB on the depth of anesthesia. Even a complete neuromuscular recovery did not affect the depth of anaesthesia as measured by BIS or entropy. Thus, the results of this study do not support the afferentation theory or the theory of sugammadex-induced arousal.

All patients in Study IV received a standardized (2 mg/kg) dose of sugammadex for reversal of the NMB and the level of NMB at the time of reversal was standardized. The present study can be criticized for not including a group where NMB would have been antagonized with neostigmine. Indeed, a randomized controlled trial using either neostigmine or sugammadex would have added valuable information in distinguishing whether arousal is associated with reversal of a neuromuscular block in general or if it is indeed related to the use of neostigmine, as indicated by the results of the Vasella study (Vasella et al. 2005).

In this open study all patients received the same treatment and thus no randomization, blinding or allocation into separate groups was used. Sample size (n= 30) was determined on the basis of previous studies (Laitio et al. 2008). It was calculated that 28 patients would be required to demonstrate a difference of 5 BIS-units before and after the administration of sugammadex at a level of significance of $P = 0.05$ and power of 80%. Accordingly, the study was adequately powered to show a clinically significant effect of sugammadex on depth of anaesthesia. Although the speed of sugammadex reversal is not affected by the type of anaesthesia (Rex et al. 2009), also the anaesthetic regimen (propofol-remifentanil TCI) was standardized. Nevertheless, it was important to maintain delivery of the anaesthetics unchanged throughout the study, as the main focus was to determine the possible effect of rapid reversal of a moderate rocuronium NMB by sugammadex on the previously described EEG-derived measures of anaesthetic depth, i.e the bispectral index and entropy levels.

All patients served as their own controls and the targets of the TCI devices were kept unchanged at the time of administration of the reversal agent and thereafter for the entire remaining time of the study. Patients were not manipulated during the initial 5-min stabilization period and the 10-min period following sugammadex. Thereby any possible effects of noxious or other surgery related stimuli on the results were eliminated. Obviously, blood pressure was measured using

noninvasive methods every 5 minutes throughout the course of anaesthesia but this occurrence is not likely to invalidate the measurement of the depth of anaesthesia.

The interaction of muscle relaxation and noxious stimulation on the depth of anaesthesia has been studied previously. Ekman and his co-workers measured the levels of BIS and auditory evoked potentials (AEP) under light sevoflurane anaesthesia during neuromuscular blockade and after neostigmine reversal (Ekman et al. 2007a and b). Deeper levels of NMB significantly attenuated the effect of the noxious stimulus as compared to the effect during a less profound block or after neostigmine reversal. The degree of neuromuscular blockade influenced BIS and AEP levels only in the presence of noxious stimuli. The results of Study IV cannot be directly compared to the results of the Ekman studies as study methodology differs in several respects. Most importantly, in Study IV noxious stimuli were eliminated.

Compared to the study of Vasella and his co-workers, Study IV used a similar anaesthetic regimen. Anaesthesia was maintained with a target-controlled infusion of propofol and remifentanil. However, there was a difference in depth of anaesthesia between the two studies. The mean BIS values before and after sugammadex administration in Study IV ranged from 30 to 33, while in the Vasella study the mean BIS values prior to neostigmine varied from 54 to 56. The Ekman studies (Ekman et al. 2007a and b) were conducted under light sevoflurane anaesthesia as compared to a deeper level of propofol-remifentanil TIVA in Study IV. As previously discussed, there is a possibility that prevailing depth of anaesthesia does affect the relationship between level of NMB and its possible effect on anaesthetic depth. If afferentation has only a minor central effect, it is possible that the phenomenon could not be observed at deeper levels of anaesthesia, but it would be measurable during lighter anaesthesia. The entire concept of the afferentation theory is still, however, not established.

The validity of the methods used in Study IV to assess depth of anaesthesia may be debated. Simultaneous registration of auditory evoked potentials (Thornton 1991) would have added valuable information. However, because the patients of this study consisted of oto-rhino-laryngological patients, some of which suffered from impaired hearing and/or had surgery of the ear region, auditory evoked potentials were not measured. In view of the available data on the EEG-derived measures of anaesthetic depth (Schmidt et al. 2004; Vakkuri et al. 2004; Viertiö-Oja et al. 2004) it seems fair to assume that the chosen methods provide a fairly reliable estimate of actual depth of anaesthesia and therefore it can be concluded that sugammadex does not affect the depth of anaesthesia.

7. SUMMARY AND CONCLUSIONS

7.1. Effect of nitrous oxide on rocuronium and cisatracurium requirements

The mean steady-state rates of infusion of rocuronium per kg ideal body weight to maintain the degree of neuromuscular block at 90% were 0.55 ± 0.17 $mg \cdot kg^{-1} \cdot h^{-1}$ for patients anaesthetized with air and total intravenous anaesthesia and 0.50 ± 0.14 $mg \cdot kg^{-1} \cdot h^{-1}$ for patients anaesthetized with nitrous oxide and total intravenous anaesthesia. For cisatracurium the corresponding figures were 0.07 ± 0.02 and 0.07 ± 0.02 $mg \cdot kg^{-1} \cdot h^{-1}$, respectively. Nitrous oxide does not affect rocuronium or cisatracurium infusion requirements at steady state in a clinically significant degree.

7.2. Safe reversal of the NMB: impact of the reversal agent and monitoring method chosen

The potentially unsafe period of recovery, i.e the time from loss of visual fade to the return of TR 0.90, was 10.3 ± 5.5 min after the reversal of a moderate rocuronium-induced neuromuscular block by neostigmine and 0.3 ± 0.3 min after sugammadex. The use of sugammadex for reversal of neuromuscular block is associated with both a statistically and clinically significant shorter period of potentially unsafe recovery.

7.3. Effect of sugammadex reversal on depth of anaesthesia

The mean averaged BIS, state entropy and response entropy values were 31.7 ± 9.9, 35.3 ± 12.9 and 36.8 ± 13.3 before and 32.0 ± 11.9, 36.3 ± 15.9 and 38.4 ± 18.0 after sugammadex administration, respectively. Reversal of an intermediate rocuronium block at deeper levels of propofol-remifentanil anaesthesia by 2 mg/kg sugammadex does not affect the depth of anaesthesia as determined by BIS or entropy levels. Accordingly, reversal of neuromuscular block by sugammadex does not increase the risk for premature emergence in patients anaesthetized with remifentanil and propofol.

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