- CHAPTER II -

GENERAL INTRODUCTION

CELLULAR TARGETS INVOLVED IN THE ANTI-INFLAMMATORY EFFECTS OF KETAMINE – A LITERATURE REVIEW -

1. KETAMINE

1.1. HISTORY

Ketamine is a dissociative anaesthetic drug which was synthesised in 1962 by Stevens and first used in humans in 1965 by Corssen and Domino.[1] In 1970 ketamine was introduced in veterinary medicine for anaesthesia in the cat[2] and many species followed soon after (for review see 3 and 4). In 1977 the combination xylazine and ketamine was evaluated for anaesthesia in horses.[5] Currently, ketamine is used in a variety of clinical settings regarding equine anaesthesia and analgesia. Besides the application for induction of anaesthesia, ketamine has also been used as an adjunct to inhalant anaesthesia,[6-8] in different total intravenous anaesthesia (TIVA) protocols[9-11] and for epidural analgesia[12] and peripheral nerve blocks.[13] More recently, studies focus on the antinociceptive effects of a subanaesthetic continuous rate infusion (CRI) of ketamine in conscious horses.[14,15]

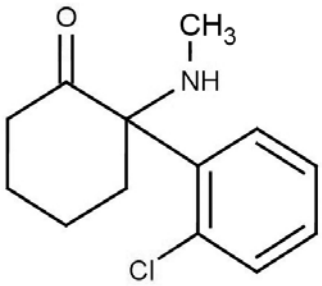


Figure 1. Structural formula of ketamine

1.2. PHYSICOCHEMICAL PROPERTIES

Ketamine is a congener of phencyclidine and is chemically designated as (±) 2-(ortho-chlorophenyl)-2-(methylamino)-cyclohexanone (Fig. 1). Ketamine has a molecular weight of 238 Da, forms a white crystalline salt with a pKa of 7.5 and has relatively high lipid solubility.[16] For veterinary use, commercially available ketamine solution is a racemic mixture consisting of two optical enantiomers, R(-) and S(+)ketamine, in equal amounts and the preservative benzethonium chloride.[17] For human use, also solutions of S(+)-ketamine only, the most active form, are available. Ketamine is prepared in a slightly acidic solution with a pH of 3.5 to 5.5.[16]

1.3. PHARMACODYNAMICS

1.3.1. MECHANISM OF ACTION

Ketamine interacts with multiple binding sites, including N-methyl-D-aspartate (NMDA) and non-NMDA glutamate receptors, nicotinic and muscarinic cholinergic, adrenergic, γ-aminobutyric acid type A ($GABA_A$), dopamine, serotonin and opioid receptors.[18-20] In addition, interactions with voltage-dependent ion channels such as Na^+, K^+ and L-type Ca^{2+} channels have been described.[19] All of these interactions may play a role in the pharmacological and clinical effects of ketamine. However, non-competitive NMDA receptor antagonism accounts for most of the amnestic, analgesic, psychotomimetic and neuroprotective effects of ketamine.[18,19] By binding to the phencyclidine site of the NMDA receptor, ketamine blocks the open Ca^{2+} channel pore coupled to the NMDA receptor and inhibits glutamate activation of the receptor, thereby inhibiting the excitatory effect of glutamate on neurons in the central nervous system.[18,19]

1.3.2. EFFECTS ON THE CENTRAL NERVOUS SYSTEM

Ketamine produces dose-related unconsciousness and analgesia**.** The primary site of central nervous system action of ketamine appears to be the thalamoneocortical projection system. Electroencephalographic (EEG) studies showed that ketamine selectively depresses neuronal function in especially the association areas of the cortex[21] and the central nucleus of the thalamus.[22] Moreover, depressant effects of ketamine have been noted in nociceptive cells in the medial medullary reticular formation[23] and laminae I and V of the dorsal horn.[24] In conjunction with its depressant effects, ketamine also stimulates parts of the limbic system, including the hippocampus.[21,25] This functional dissociation between the thalamoneocortical and limbic systems produces a characteristic anaesthetic state often referred to as dissociative anaesthesia since ketamine interrupts flow of information from the unconscious to conscious parts of the brain, rather than producing a generalised depression of all brain centres.[3]

Induction of ketamine anaesthesia is often accompanied with increased skeletal muscle tonus generally described as catalepsia. Moreover, limb movements and tonic-clonic spasms of limb muscles occur in absence of external stimuli and are not dose-related. The mechanism of ketamine-induced catalepsia is not clearly understood, but studies indicate that it may be due to a deficiency of dopamine function or an imbalance in cholinergic-dopaminergic function.[26] Patients anaesthetised with ketamine may keep their eyes open, maintain corneal, laryngeal

and pharyngeal reflexes to some extent and show variable degrees of lateral nystagmus.[27,28]

In humans, ketamine produces undesirable psychological reactions like vivid dreaming, extracorporeal experiences and illusions often associated with excitement, confusion, euphoria and fear. Ketamine-induced depression of auditory and visual relay nuclei, leading to misperception of auditory and visual stimuli may be responsible for this reaction.[17] Hallucinatory behaviour, like nystagmus, neighing and slow head and limb movements can also be observed in horses following ketamine anaesthesia. Concomitant administration of benzodiazepines generally attenuates or treats this behaviour.

As a sympathomimetic agent, ketamine significantly increases cerebral blood flow, intracranial pressure and cerebrospinal fluid pressure as a result of cerebral vasodilation and elevated systemic blood pressure.[29,30] Therefore, ketamine should be used with caution in patients with cerebral trauma or intracranial masses.[31] On the other hand, as a non-competitive NMDA receptor antagonist ketamine might have neuroprotective effects, since excessive activation of NMDA receptors has been implicated in the pathogenesis of cerebral ischemia and other neurodegenerative disorders.[19,32] Moreover, during ketamine anaesthesia cerebrovascular autoregulation seems to be preserved.[33]

It has been known since the late 1970s that the ketamine enantiomers exhibit pharmacological and clinical differences.[34] Receptor studies in rodents show that S(+)-ketamine has a 4-fold higher affinity for NMDA receptors than its stereoisomer R(-)-ketamine. In rodents and humans, S(+)-ketamine exhibits 1.5 to 3-fold greater anaesthetic potency than the R(-) compound.[19,34] Moreover, S(+)-ketamine induces less spontaneous movements and psychotomimetic side effects than R(-)-ketamine.[19,33,34]

1.3.3. ANALGESIC EFFECTS

At anaesthetic concentrations ketamine produces profound analgesia. In addition, continuous rate infusions of subanaesthetic ketamine concentrations have proven to be effective as an adjunct to inhalation anaesthesia and in the management of acute postoperative pain. When low dose ketamine infusions are combined with systemic opioid administration, an opioid-sparing effect up to 50% is observed.[20,35] Moreover, by using low dose infusion rates the occurrence of ketamine-related side effects is reduced to a minimum.[20,33,36]

The analgesic potency of ketamine is mainly mediated through binding to NMDA receptors. NMDA receptors are highly involved in the pathogenesis of

hyperalgesia (i.e., a heightened sense of pain), allodynia (i.e., pain caused by a stimulus that does not normally cause pain) and the wind-up phenomenon (i.e., temporal summation of painful stimuli in the spinal cord). As a non-competitive NMDA receptor antagonist ketamine has proven to be useful in reducing these pathological pain states.[36-38] The influence of ketamine on pre-emptive analgesia (blocking pain mediators before pain is initiated) and chronic pain treatment is still controversial.[36,39,40]

It was presumed that ketamine mainly attenuated acute somatic nociception, but recent data, obtained from rodent and human experimental studies, demonstrate that ketamine also significantly attenuates acute visceral nociception.[41-43]

Epidural administration of ketamine produces dose-dependent analgesia in man,[44] dogs[45] and rats.[46,47] In horses, doses of 0.5 - 2.0 mg/kg produced analgesia of the tail, perineum and upper hind limb for 30 to 75 minutes, respectively.[12]

In rodents and humans, S(+)-ketamine exhibits 3-fold greater analgesic potency than the R(-) compound.[19,34] The analgesic potency of (S+)-ketamine is approximately twice that of the racemic mixture.[19,34]

1.3.4. EFFECTS ON THE CARDIOVASCULAR SYSTEM

As a sympathomimetic drug, ketamine stimulates the cardiovascular system, which is characterised by increases in heart rate, mean aortic pressure, pulmonary arterial pressure, central venous pressure and cardiac output.[26] The increase in cardiac output is generally attributed to increases in heart rate and cardiac contractility.[48] In vivo, ketamine-induced increases in blood pressure and cardiac contractility are usually considered to be indirect and mediated through sympathomimetic effects mediated in the central nervous system[49] and inhibition of neuronal reuptake of catecholamines by sympathetic nerve endings.[50] In contrast, in isolated heart muscle preparations of rats and dogs or in sympathoadreno-depleted patients, it is stated that ketamine acts as a direct myocardial depressant.[51-53] The centrally mediated sympathetic responses to ketamine usually override the direct depressant effects of ketamine.[28]

Increase in both blood pressure and heart rate seems paradoxical since increases in blood pressure normally elicit baroreflex-induced decreases in heart rate. Irnaten et al.[54] found that the ketamine-induced increase in heart rate is mediated through decreased activity of parasympathetic cardiac neurons in the brainstem.

The increase in haemodynamic variables is associated with increased cardiac work and myocardial oxygen consumption. The normal heart is able to increase myocardial oxygen supply by increasing cardiac output and decreasing coronary

vascular resistance, so that coronary blood flow is appropriate for the increased oxygen consumption.[55]

Since ketamine preserves cardiovascular function, its use is recommended for anaesthesia of critically ill patients in which there is a risk of cardiac depression and hypotension.[56-58] However, stimulation of the cardiovascular system might be detrimental to patients with little cardiac functional reserve.[3] Some investigators have reported that in patients with shock, the induction of anaesthesia with ketamine can cause marked cardiovascular depression.[59] This likely results from the prior depletion of catecholamine stores in combination with a direct myocardial depressant effect of ketamine now coming to the foreground.[28]

Horses are known to maintain relatively normal haemodynamics when receiving ketamine in combination with xylazine[5] or guaiphenesin for short-term anaesthesia.[60] In halothane anaesthetised horses, incremental ketamine infusions have been demonstrated to improve haemodynamics, especially cardiac output.[6] Cardiac arrhythmias are uncommon in patients under ketamine anaesthesia.[61]

Following epidural administration, ketamine produced a slight but insignificant increase in heart rate, whereas other cardiovascular parameters remained within physiological limits.[12]

1.3.5. EFFECTS ON THE RESPIRATORY SYSTEM

Ketamine has minimal effects on the respiratory system. Following administration, respiration rate is usually maintained or mildly increased, while alveolar ventilation can be transiently decreased, depending on the dose administered.[26] Arterial blood gases are generally preserved when ketamine is used alone. However, by using adjuvant sedatives or anaesthetic drugs, respiratory depression and a subsequent slight increase in arterial carbon dioxide tension ($PaCO_2$) can occur. Ketamine does not affect the central respiratory drive as reflected by an unaltered response to carbon dioxide.[62] At higher dosages, ketamine may produce an apneustic and irregular pattern of breathing.[28]

Ketamine decreases airway resistance, which is probably a result of the sympathomimetic response to ketamine as well as a direct relaxant effect on bronchial smooth muscles.[63] In contrast, upper airway obstruction may occur following ketamine administration, since ketamine can increase salivation and secretion of respiratory tract mucus. Moreover, although pharyngeal and laryngeal reflexes remain relatively intact after ketamine administration, silent aspiration can occur during ketamine anaesthesia thus consequently increasing the risk of aspiration pneumonia.

In horses, the combination of xylazine and ketamine did not affect respiratory rate.[5,11] Only mild respiratory acidosis was observed which can probably be more attributed to ventilation-perfusion mismatch caused by lateral recumbency rather than by the depressant effect of ketamine on the respiratory system per se.[5] Similar results were observed in a study done by Taylor and Luna,[10] where continuous infusion of a detomidine-ketamine-guaiphenesin combination only slightly increased $PaCO_2$ without significantly changing respiratory rate and arterial oxygen tension (PaO_2).
Epidural administration of ketamine at 0.5 – 2.0 mg/kg did not affect respiratory rate and arterial blood gases in horses.[12]
The observed decrease in airway resistance in humans following ketamine administration is believed to occur in horses as well.[64]

1.4. PHARMACOKINETICS

Ketamine can be administered intravenously, intramuscularly, subcutaneously, orally or as in form of an epidural or spinal injection. In horses, the vast majority of clinical use involves intravenous and intramuscular routes, although intramuscular injection of ketamine can induce some tissue irritation since the pH of an aqueous solution of ketamine is 3.5 to 5.5.[16]
Following intravenous bolus injection, ketamine is rapidly distributed. Because of its small molecular weight, a pK_a near the physiological pH, rather low protein binding and high lipid solubility, ketamine rapidly crosses the blood-brain barrier and has an onset of action within 30 seconds following intravenous injection, with a maximal effect occurring in approximately one minute.[28] Termination of effect after a single bolus injection of ketamine is caused by rapid redistribution of the drug from highly vascularised organs (brain and blood) to less perfused tissues in the body.[65]
Ketamine is metabolised extensively by the hepatic cytochrome P_{450} system in all species including the horse. The major pathway involves N-demethylation to form norketamine, which is then hydroxylated and oxidated to form hydroxynorketamine and 5,6-dehydronorketamine, respectively. Subsequently, these products are conjugated to more water-soluble glucuronide derivates and excreted by the kidneys.[17]
The activity of the metabolites of ketamine has not been studied well, but norketamine has been shown to have analgesic properties, although significantly less than the parent compound.[66] Metabolites may also have some slight additive effect to the action of the parent drug.[19]

Ketamine has a wide therapeutic index, although repetitive administration of ketamine has been demonstrated to result in ketamine tolerance in several laboratory animals by inducing multiple forms of hepatic P_{450} enzymes.[67]
Hijazi et al.[68] found that pharmacokinetic variables of ketamine were greater in intensive care patients than in healthy volunteers and in surgical patients. The increase in the volume of distribution was greater than the increase in clearance, resulting in a longer estimated half-life of ketamine in the intensive care group.
The enantiomers of ketamine also differ in their pharmacokinetic profile. In animal laboratory studies, the therapeutic index (i.e. ratio of LD_{50} to ED_{50}) was found to be greater for S(+)-ketamine than for either R(-)-ketamine or the racemic mixture.[66] In humans, following ketamine infusion both higher plasma concentrations of R(-)-ketamine and R(-)-norketamine were found when compared to S(+)-ketamine and S(+)-norketamine, respectively.[66] Overall, plasma clearance of S(+)-ketamine was 16-35% greater than that of R(-)-ketamine.[66,69] This difference is thought to be due to the fact that R(-)-ketamine inhibits the elimination of S(+)-ketamine.[70] S(+)-ketamine is not inverted into R(-)-ketamine.[71] Edwards et al.[66] also found that uptake into most tissues and metabolism in some tissues is enantioselective, whereas Henthorn et al.[69] found no stereoselective influence on peripheral tissue distribution. Moreover, work with human liver microsomes indicated that the rate of N-demethylation was greater with S(+)-ketamine than with R(-)-ketamine or the racemic mixture.[66]
In equines, the pharmacokinetics of ketamine have been investigated after single bolus injection of anaesthetic dosages and following continuous rate infusions of subanaesthetic dose rates in conscious and anaesthetised horses. As in all other species studied, plasma ketamine concentrations in the horse follow a biexponential decline with a rapid initial distribution phase being followed by a slower elimination phase.[72,73] In xylazine premedicated horses, distribution and elimination half-lives measured 2.9 and 42 minutes, respectively, following a single bolus injection of 2.2 mg/kg ketamine.[72] In halothane anaesthetised horses, a similar distribution half-life was found following 2.2 mg/kg ketamine IV, whereas elimination half-life was considerably slower (65.8 ± 3.5 min).[73] Extending ketamine induction by simultaneous infusions of propofol and ketamine increased elimination half-life to 81.1 min.[74] In contrast, subanaesthetic continuous rate infusions in conscious horses revealed a longer distribution half-life and a shorter elimination half-life when compared to the previous described studies.[15] In horses, no differences in plasma concentrations of S(+)- and R(-)-ketamine have been found, whereas plasma concentrations of S(+)-norketamine were continuously

higher than R(-)-norketamine during target-controlled ketamine infusions.[8] Similar to the results found by Henthorn et al.,[69] no differences in tissue distribution of the individual enantiomers could be demonstrated in that study. Also, no stereoselective biotransformation of ketamine to norketamine could be demonstrated in horses.[75]

1.5. CLINICAL USE OF KETAMINE IN HORSES

Ketamine should not be used as a monoanaesthetic in horses, since its anaesthetic effect is often associated with poor muscle relaxation and hallucinatory behaviour.[4] Hence, preanaesthetic sedation is mandatory before ketamine is administered. Acepromazine premedication is inadequate prior to ketamine induction alone since it is not able to attenuate the ketamine-induced muscle rigidity.[4] (Fielding 2006). Most commonly, α_2-agonists, such as xylazine (1.0-1.1 mg/kg IV), detomidine (20 µg/kg IV) and romifidine (80-100 µg/kg IV) are used for this purpose. Administration of an α_2-agonist, followed in 5 to 10 minutes by a bolus injection of ketamine (2.2 - 3.0 mg/kg IV), induces a short period of safe and effective anaesthesia. Lateral recumbency is assumed in 1 to 3 minutes after ketamine injection and when no other anaesthetic is given, duration is about 10-15 minutes and recovery to standing requires 25-30 minutes.[5,76] Anaesthesia can be prolonged by redosing with one third to one half of the original dose of each drug.[4] Alternatively, following premedication, guaiphenesin (55 mg/kg IV) or benzodiazepines (diazepam 0.22 mg/kg IV; midazolam 0.1 mg/kg IV) can be given prior to the bolus injection of ketamine (2.2 mg/kg IV). This regimen improves muscle relaxation and shows less cardiovascular depression when compared to the combination of an α_2-agonist and ketamine only.[4,17,77] The use of benzodiazepines might be preferred to guaiphenesin, since benzodiazepines appear to be more effective in reducing hallucinatory behaviour.[17,77]

For total intravenous anaesthesia following ketamine induction, continuous infusion of the anaesthetic combination detomidine (0.02 mg/mL)-ketamine (2 mg/mL)-guaiphenesin (100 mg/mL) (Triple-drip infusion) at 1 mL/kg/h has been demonstrated to be safe and effective for use in horses.[9,10] Moreover, Mama et al.[11] showed that various infusions of xylazine and ketamine can be used for total intravenous anaesthesia in healthy adult horses.

Beside its use for induction and total intravenous anaesthesia in horses, ketamine is increasingly used during inhalation anaesthesia to reduce the concentration of inhalation anaesthetic required[6] and/or to provide additional analgesia.[8] Muir and

Sams[6] found that plasma ketamine concentrations over 1.0 μg/mL significantly reduced the minimal alveolar concentration (MAC) of halothane by a maximum of 37% in halothane anaesthetised healthy experimental horses. Knobloch et al.[8] showed a significant inhibition of the nociceptive withdrawal reflex under ketamine infusion during isoflurane anaesthesia.
Recently, Fielding et al.[15] studied the clinical and analgesic effects of subanaesthetic continuous rate infusions of ketamine in healthy conscious horses. Infusion rates up to 0.8 mg/kg/h did not cause any behavioural or clinical changes. However, for the infusion rates tested no analgesic effect could be demonstrated.
Ketamine has also been used for local analgesia in horses. Following epidural administration, ketamine dosages of 0.5 - 2.0 mg/kg were effective in producing analgesia of the tail, perineum and upper hind limb for 30 to 75 minutes, respectively.[12] In another study, an abaxial sesamoid block with ketamine ensured adequate analgesia for 15 minutes.[13]

2. POTENTIAL CELLULAR TARGETS FOR KETAMINE

Beside its anaesthetic and analgesic effects ketamine has demonstrated to possess anti-inflammatory properties. In several studies in rodents, ketamine suppressed the lipopolysaccharide (LPS)-induced tumour necrosis factor-alpha (TNF-α), interleukin-1-beta (IL-1β) and interleukin-6 (IL-6) response both in vitro and in vivo.[78-86] In a human whole blood assay, the LPS-stimulated production of TNF-α, IL-6 and IL-8 significantly decreased in the presence of ketamine.[87-91] In clinical studies in humans, ketamine given before surgical procedures, such as hysterectomy and coronary artery bypass grafting, significantly reduced IL-6 serum concentrations.[92-94] In addition, in a model of Gram-negative bacterial sepsis in rats, ketamine significantly increased survival compared to rats treated with saline.[82]
In rats and humans, ketamine also attenuated LPS-stimulated neutrophil adhesion both in vitro and in vivo by reducing expression of adhesion molecules on neutrophils and endothelial cells.[89,95-99]
Ketamine also significantly inhibited hypotension and metabolic acidosis and improved survival in rats and dogs injected with LPS.[80,82,100]
Finally, ketamine may also affect inflammatory mediators like nitric oxide (NO), prostaglandin E_2 (PGE_2) and reactive oxygen species (ROS) (see respective subchapters).

2.1. LPS AND ITS SIGNALLING PATHWAYS

Bacterial LPS is a major component of the outer membrane of Gram-negative bacteria. LPS is comprised of three interconnected structures: an inner hydrophobic lipid A component, a core oligosaccharide structure and an outer O-specific polysaccharide chain.[101-103] The O-polysaccharide portion of the complex is composed of repeating polysaccharide units that extend into the environment of the bacterium and acts as an antigen for the production of antibodies.[103] This 'O-antigen' is highly variable among bacterial species, accounting for the serospecificity of Gram-negative bacterial species.[101,103] The core region is composed of a specific arrangement of monosaccharides and 3-deoxy-D-manno-octulosonic acid (KDO) residues`, which remains constant across groups of bacteria.[101-103] Lipid A consists of β(1-6)-linked disaccharides to which amide or ester-linked medium chain fatty acids are attached.[102,103] The lipid A component is very similar among bacterial species and is responsible for the endotoxic properties of the LPS molecule.[101-103]

2.2. LPS AND ITS RESPONSE IN EQUINES

LPS is released from Gram-negative bacteria during periods of bacterial multiplication or death. In healthy horses, large numbers of Gram-negative bacteria, and consequently large amounts of LPS, reside in the intestinal tract.[104] It has been estimated that at least 2.25 gram of free LPS exists in the caecum and ventral colon of healthy, adult horses.[105] Normally, movement of LPS into the systemic circulation is minimised through a highly efficient intestinal mucosal barrier, which is composed of tight junctions between individual epithelial cells, secretions from epithelial cells and resident bacterial populations. Small amounts of LPS may traverse the mucosal barrier, but most is rapidly cleared by Kupffer cells in the liver or neutralised by circulating LPS antibodies.[106] However, when intestinal blood flow is reduced, as may occur in horses suffering from gastrointestinal disorders, the integrity of the mucosal barrier may become impaired. Under these circumstances, LPS readily crosses the damaged mucosal barrier and enters the mesenteric venous system, lymphatic system or peritoneal cavity. When, in addition, the clearance capacity of hepatic Kupffer cells is exceeded, large amounts of LPS will enter the systemic circulation and trigger various cells to produce a plethora of inflammatory mediators, which subsequently induce a myriad of responses, a complex phenomenon generally known as endotoxaemia. The clinical effects associated with equine endotoxaemia include fever, tachycardia, hypotension, hyperpnoea, leukopenia followed by leukocytosis,

metabolic acidosis, hyperglycaemia followed by hypoglycaemia, abdominal discomfort, haemoconcentration, increased vascular permeability, disseminated intravascular coagulation (DIC) and, ultimately, multiple organ dysfunction and death.[106,107]

Besides gastrointestinal disorders, equine endotoxaemia may develop from other Gram-negative infections, such as pleuritis, pneumonia, peritonitis or endometritis.[108]

2.3. MOLECULAR MECHANISMS OF LPS-INDUCED SIGNAL TRANSDUCTION PATHWAYS

2.3.1. LPS-BINDING PROTEIN AND CD14

Once in circulation, LPS forms a high affinity complex with LPS binding protein (LBP).[109-111] This glycoprotein is normally present in plasma at trace concentrations, but its concentration rapidly increases during the acute phase response.[112] The role of LBP is to transfer LPS to the cell surface of monocytes, macrophages and neutrophils where it binds to cluster of differentiation 14 (CD14).[109-112] This receptor exists as a membrane bound form (mCD14) on myeloid cells and a soluble form (sCD14) in plasma. Soluble CD14 functions to enhance LPS responses in cells that do not ordinarily express CD14 such as endothelial and epithelial cells.[110-111] Although CD14 was initially identified as a LPS receptor,[109] it lacks transmembrane and intracellular domains and thus cannot initiate signal transduction by itself.[110-113] However, CD14 plays a pivotal role in LPS-induced signal transduction since blockade of CD14 with monoclonal antibodies prevented cytokine synthesis in whole blood following LPS exposure. Moreover, formation of the CD14/LPS complex significantly reduces the concentration of LPS required for activation of macrophages when compared to unbound LPS alone.[110,111] So far, the role of CD14 in LPS-induced signal transduction appears to be that of binding LPS and presenting it to the LPS receptor complex.[111-113]

2.3.2. LPS RECEPTOR COMPLEX

The LPS receptor complex is composed of Toll-like receptor 4 (TLR4) and myeloid differentiation protein-2 (MD-2).[111,112,114,115] Toll-like receptors (TLRs) are the mammalian homologues of a plasma membrane receptor in Drosophila named Toll.[116,117] TLRs are predominantly expressed by immune cells including monocytes, macrophages and neutrophils.[116,118] TLRs are categorised as pattern recognition receptors (PRRs) as they recognise pathogen associated molecular patterns (PAMPs).[111,119] In mammalian species, at least 11 members of TLRs (TLR1-

TLR11) have been identified. All TLR subtypes contain an extracellular leucine-rich repeat (LRR) motif, a single transmembrane region and an intracellular Toll/interleukin-1 (TIR) domain.[111,112,117] The LRR motifs form a horseshoe structure believed to be directly involved in the recognition of PAMPs.[117] The cytoplasmatic TIR domain is essential for the assembly of downstream signal transduction pathways.[117,120] Different microbial pathogens activate different TLR subtypes. TLR4 is generally considered to be the predominant signal-transducing receptor for LPS, although TLR4 has been demonstrated to be activated by other mediators such as fibronectin and heat shock proteins.[111,116,117]

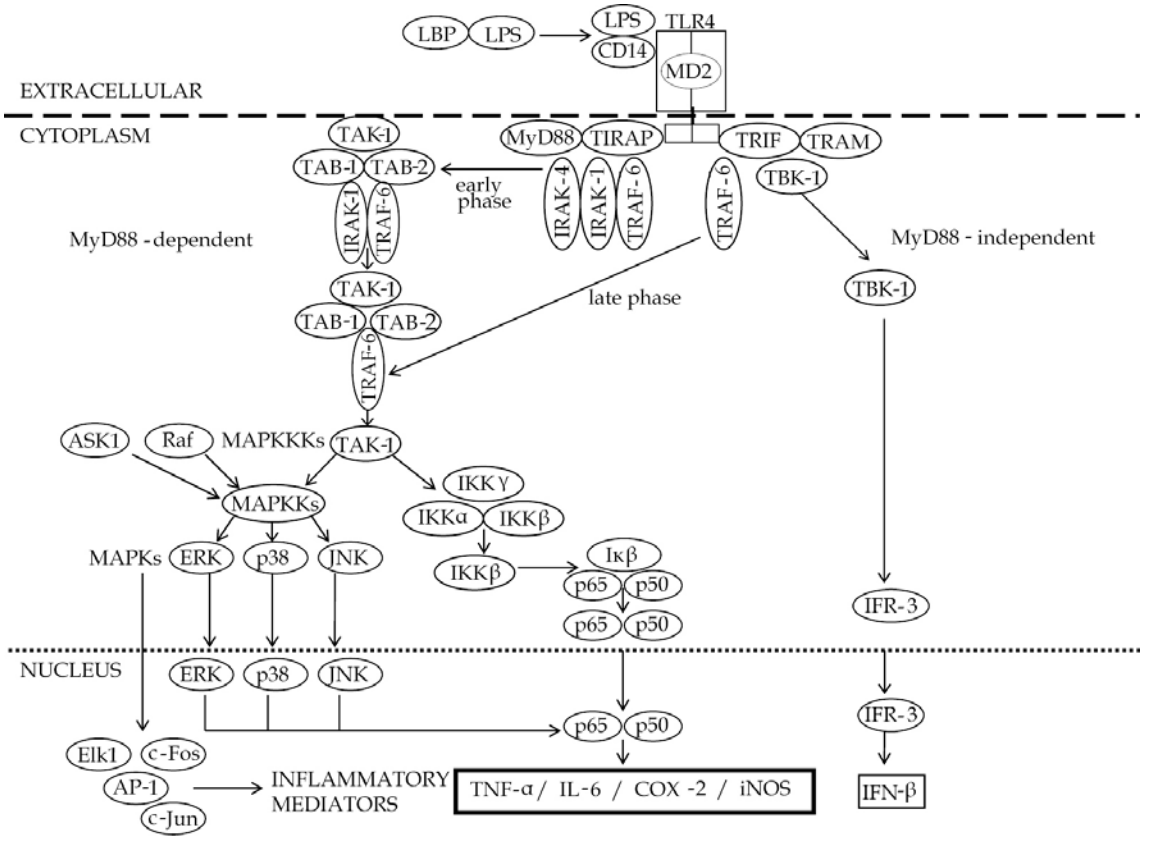
However, TLR4 alone is not able to recognise LPS and induce signalling, but is highly dependent on the presence of the extracellular glycoprotein MD-2.[121,122] MD-2 binds to the lipid A moiety of LPS and the extracellular LRR-domain of TLR4 and thus enables LPS ligand recognition by TLR4.[123] For an efficient LPS response, a physical association between MD-2 and TLR4 is crucial.[124] After ligand binding, TLR4 dimerise and undergo the conformational change required for the recruitment of downstream signalling molecules.[117]

To date, there are no reports on the influence of ketamine on the LPS-activated CD14 protein or LPS receptor complex.

In agreement with previously published reports in rodents, all three components of the LPS receptor complex (i.e., CD14, MD-2 and TLR4) have been identified in horses.[125-127]

2.3.4. DOWNSTREAM SIGNAL TRANSDUCTION PATHWAYS OF TLR4

The TLR4-mediated signal transduction cascade in response to LPS can be divided into two synergistically acting pathways: a myeloid differentiation factor 88 (MyD88)-dependent pathway that leads to an early activation of nuclear factor-kappa B (NF-κB) and mitogen-activated protein kinase (MAPK), and a MyD88-independent pathway associated with a delayed activation of NF-κB and MAPK (Fig. 2).[111,112,116,117,128]


LBP
LPS
LPS
CD14
TLR4
MD2
EXTRACELLULAR
CYTOPLASM
TAK-1
TAB-1
TAB-2
IRAK-1
TRAF-6
MyD88 - dependent
MyD88
TIRAP
TRIF
TRAM
TBK-1
early phase
IRAK-4
IRAK-1
TRAF- 6
TRAF-6
MyD88 - independent
late phase
TBK-1
TAK-1
TAB-1
TAB-2
TRAF-6
ASK1
Raf
MAPKKKs
TAK-1
IKKγ
MAPKKs
IKKα
IKKβ
MAPKs
ERK
p38
JNK
IKKβ
Iκβ
p65
p50
p65
p50
IFR-3
NUCLEUS
ERK
p38
JNK
p65
p50
IFR-3
Elk1
c-Fos
AP-1
c-Jun
INFLAMMATORY MEDIATORS
TNF-α / IL-6 / COX -2 / iNOS
IFN-β

In the MyD88-dependent pathway, the adaptor molecules MyD88 and TIR domain-containing adaptor protein (TIRAP) are recruited to the activated receptor complex and associated with interleukin-1 receptor-associated protein kinase 1 (IRAK-1), IRAK-4 and tumour necrosis factor receptor-associated factor 6 (TRAF-6). Following activation, IRAK-1 and TRAF-6 dissociate from this complex and associate with another complex at the plasma membrane composed of transforming growth factor-β-activated kinase 1 (TAK-1) and two TAK-1 binding proteins, TAB-1 and TAB-2. Then IRAK-1 is degraded and the remaining complex, consisting of TRAF-6, TAK-1, TAB-1 and TAB-2, translocates to the cytosol, where ubiquitination of TRAF-6 induces the activation of TAK-1. TAK-1, in turn, phosphorylates mitogen-activated kinase kinases (MAPKKs) and the inhibitors of κB (IκB) kinase (IKK) complex. Stimulated MAPKK activates the transcription factor activating protein 1 (AP-1) through activation of the mitogen activated kinases (MAPKs), c-Jun N-terminal kinase (JNK) and mitogen-activated protein kinase p38 (p38). Activation of IKK leads to phosphorylation of inhibitor of kappa B (IκB) and subsequent activation of the transcription factor NF-κB (described below).[111,112,116,117,128]

In the MyD88-independent signal transduction pathway, the adaptor molecules TIR-domain-containing adaptor protein inducing IFN-β (TRIF) and TRIF-related adaptor molecule (TRAM) are recruited to the stimulated LPS receptor complex and associated with TRAF-6 and TRAF-family-member-associated NF-κB activator-binding kinase 1 (TBK-1). Activation of TRAF-6 mediates NF-κB activation, whereas stimulation of TBK-1 leads to phosphorylation of the transcription factor interferon-regulatory factor 3 (IRF-3) and the subsequent expression of interferon (IFN)-inducible genes through the Janus activated kinase (JAK) – signal transducer and activator of transcription (STAT) signalling pathway.[111,112,116,117,128]

2.4. MAPKs

MAPKs are a highly conserved family of serine/threonine/tyrosine kinases which comprise three major subfamilies: extracellular signal-regulated kinase (ERK), mitogen-activated protein kinase p38 (p38) and c-Jun N-terminal kinase (JNK).[129] The basic MAPK signalling cascade consists of three sequentially acting protein kinases, i.e. MAPK kinase kinase (MAPKKK, also called MKKK or MAP3K), MAPK kinase (MAPKK, also called MKK or MAP2K) and the specific MAPK. Activation of MAPK pathways is spatially regulated, i.e. activation of one MAPK

does not normally lead to the activation of other MAPKs.[129] However, since each individual MAPK cascade shares a number of upstream and downstream kinases, complex cross-talk and signal convergence occur among MAPK family members.[130] The vast majority of defined substrates for MAPK are transcription factors, among which are AP-1, Elk1, c-Fos, c-Jun and NF-κB.[129,131] However, MAPKs have the ability to activate many other substrates including other protein kinases, phospholipases and cytoskeletal-associated proteins.[129] The fact that MAPKs are able to phosphorylate many different substrates indicates that MAPKs affect many different biological functions.

2.4.1. ERK

The ERK subfamily is made up of 5 isoforms (ERK1-5), of which ERK1 (44 kDa) and ERK2 (42 kDa), commonly referred to as ERK1/2, are most extensively studied. ERK 1/2 are widely expressed in mammalian cells and primarily involved in proliferation, transformation and differentiation.[130,132] Hence, ERK activity is predominantly induced by growth-promoting mitogenic stimuli. However, there is increasing evidence that ERK activation plays a crucial role in LPS- and cytokine-induced inflammatory responses, since ERK has been reported to act as an important regulator of NF-κB activation and NF-κB-dependent gene expression in response to LPS and IL-1.[131,133] Moreover, ERK has demonstrated to be involved in nuclear export of LPS-induced TNF-α mRNA.[134]

In general, ERK activation is initiated by phosphorylation and activation of Raf (MAPKKK) and MEK1/2 (MAPKK),[129,130,135] even though involvement of other members of the MAPKKK family, such as ASK1, Tpl2 and MEKK3 has been described (Fig. 2).[129,134,136]

PD098059 and U0126 have demonstrated to be effective inhibitors of ERK activation. Both compounds do not directly inhibit ERK activation, but interfere with upstream protein kinases.[134]

2.4.2. MAPK P38

The mammalian MAPK p38 subfamily consists of at least four different isoforms, p38α, p38β, p38γ and p38δ.[129] Two isoforms, p38α and p38β, are ubiquitously expressed. In most inflammatory cells, p38α (38 kDa) is the major isoform of p38 MAPK. Expression of p38γ is largely restricted to skeletal muscle, whereas p38δ gene expression is found in the lung, kidney, testes, pancreas and small intestine.[137,138]

MAPK p38 is activated by many stimuli including LPS, pro-inflammatory cytokines (e.g. TNF-α, IL-1), hormones and stresses such as osmotic shock and heat shock.[130,139] Upon stimulation, TAK-1, MAPKKK4 and apoptosis signal-regulating kinase 1 (ASK1) are described as the proximal upstream MAPKKKs that initiate the p38 pathway, whereas MAPKK3, MAPKK4 and MAPKK6 are the immediate upstream MAPKKs to activate p38 (Fig. 2).[136-138]
The main biological response of p38 activation involves the production and activation of inflammatory mediators, such as TNF-α, IL-1, IL-6, IL-8 and COX-2, to initiate leukocyte recruitment and activation. MAPK p38 can regulate expression of pro-inflammatory cytokines at both transcriptional and post-transcriptional level.[134,137,138]
SB203580 is widely used as the specific signal transduction inhibitor of both p38α and p38β. SB203580 potently inhibits the production of pro-inflammatory cytokines such as TNF-α and IL-1 in vitro and in vivo.[137-139] Moreover, administration of SB203580 has beneficial effects in animal disease models such as collagen-induced arthritis and endotoxin-induced septic shock.[138]

2.4.3. JNK

JNK consists of three isoforms (JNK1-3), of which JNK1 (55 kDa) and JNK2 (46 kDa) are widely distributed, whereas JNK3 is mainly located in neuronal tissue. JNK is activated in response to an array of stimuli including LPS, pro-inflammatory cytokines (e.g., TNF-α, IL-1), osmotic stress and ultraviolet irradiation.[130] Upon exposure, JNKs are phosphorylated and activated by the MAPKKs MAPKK4 and MAPKK7, which are activated by 13 distinct upstream MAPKKKs including TAK1, Tpl2, and ASK1 (Fig. 2).[129,136,139] This diversity of MAPKKKs allows a wide range of stimuli to activate the JNK pathway.[132]
Gene disruption experiments in mice have demonstrated that JNK is essential for c-Jun phosphorylation and the subsequent activation of the transcription factor AP-1. JNKs may also enhance the activity of other transcription factors including Elk-1 and NF-κB.[131,139] Moreover, JNKs are required for some forms of stress-induced apoptosis and the regulation of inflammatory genes including cytokines, growth factors, cell surface receptors and adhesion molecules.[139]
The selective inhibitor, SP600125, has been a useful tool in assessing the role of JNK in various disease models in rodents and humans.[139]

2.4.4. MAPKs: THE ROLE OF KETAMINE

Only few reports describe the influence of ketamine on MAPK phosphorylations. Tian et al.[140] and Miao et al.[141] found that pretreatment with ketamine did not significantly influence JNK1/2 and JNK3 activation following global ischemia in the hippocampus of the rat. Boulom et al.[142] demonstrated that ketamine per se induced MAPK activation and expression of ERK2 in human aortic smooth muscle cells.

2.4.5. MAPKs IN EQUINES

Both ERK1 and ERK2 have been identified in different maturation states of equine oocytes,[143,144] while existence of JNK and p38 isoforms is only described in an equine macrophage cell line.[145]

2.5. NF-κB

NF-κB was first described in 1986 as a nuclear factor necessary for immunoglobulin κ light chain transcription in B cells,[146,147] but is now known to exist in virtually all cell types. NF-κB is the key regulatory transcription factor which mediates the transcription of an exceptionally large number of genes including those involved in immune and inflammatory responses.[131,148,149]

Five distinct NF-κB subunits have been identified and cloned in mammalian cells. These include NF-κB1 (p50/p105), NF-κB2 (p52/p100), RelA (p65), RelB and c-Rel.[131] NF-κB1 and NF-κB2 undergo proteolytic processing to liberate p50 and p52, respectively. NF-κB/Rel proteins can exist as distinct homo- or heterodimers, although the most predominant form of NF-κB is the p50/p65 heterodimer. NF-κB/Rel proteins share a highly conserved 300 amino acid long Rel homology domain (RHD), responsible for dimerisation, DNA binding and association with their inhibitory cytoplasmic proteins, termed inhibitor of κB (IκB). Moreover, RHD contains a nuclear localisation sequence (NLS), which promotes NF-κB translocation to the nucleus.[119,131,150,151]

LPS-induced NF-κB activity is biphasic: an early phase occurring at 0.5-2 hours, and a late phase occurring at 8-12 hours post stimulation. LPS and other early released inflammatory mediators like platelet-activating factor (PAF) cause the early phase NF-κB activation, whereas TNF-α and IL-1β mediate the late phase activation.[152,153]

2.5.1. THE INHIBITORY PROTEIN, IκB

In resting cells, NF-κB/Rel dimers are mainly sequestered in the cytoplasm in an inactive form by association with IκB.[119,131,151] The IκB family contains nine known mammalian members, IκBα, IκBβ, IκBγ, IκBε, IκBζ, Bcl-3, p105, p105 and molecule possessing ankyrin-repeats induced by lipopolysaccharide (MAIL), among which IκBα is the most abundant.[119,131,154] A common structure for all IκB proteins is the ankyrin repeat domain (ARD), which mediates IκB binding to the NF-κB dimers, masking the NLS on NF-κB proteins.[119,131,151] IκB proteins are different in their structure, preference for binding of NF-κB dimers, biological functions and modes of activation. There are also differences in the mechanism of regulating IκB gene expression. IκBα, IκBβ and IκBε are constitutively expressed, but IκBζ and MAIL are induced by LPS and pro-inflammatory cytokines.[131] Contrasting sharply with the function of other IκBs, MAIL serves as a transcriptional enhancer, enhancing LPS-induced IL-6 expression by over 20-fold. IκBα, IκBζ and MAIL are NF-κB regulated genes. NF-κB activation increases these IκBs, which in turn inhibits NF-κB activation. IκBs inhibit NF-κB activation through three mechanisms: by sequestrating NF-κB dimers in the cytoplasm, facilitating dissociation of DNA-bound NF-κB dimers from their DNA binding sites and exporting NF-κB dimers from the nucleus.[131]

2.5.2. CYTOPLASMIC NF-κB ACTIVATION: CANONICAL AND NON-CANONICAL PATHWAYS

NF-κB is known to be activated by a plethora of activators, including bacteria and their products, pro-inflammatory cytokines and different forms of stress.[131,149] The biological events underlying NF-κB activation can be divided into two phases: a cytoplasmic and a nuclear phase.[131,151,154,155]

The signal transduction pathways leading to cytoplasmic activation of NF-κB are multiple and complex, but have in common that they all lead to the generation of DNA-binding dimers. To date, three major pathways mediating NF-κB activation have been identified: canonical (classical), non-canonical (alternative) and atypical pathways.[119,131,151,154-156] Canonical pathways are stimulated by inflammatory mediators (e.g. LPS, TNF-α, IL-1), which rapidly induce NF-κB activation. In contrast, non-canonical pathways are preferentially induced by B cell activation signals (e.g., lymphokine-β, B-cell activating factor (BAFF)) and mediate a delayed but sustained activation of primarily RelB-containing NF-κB dimers (Fig. 2). The atypical pathway is triggered by DNA damage such as UV irradiation.[119,131,151,154-156]

However, both the non-canonical and atypical pathways will not be further described as they are, based on their inducers, beyond the scope of this thesis.
Activation of NF-κB via the canonical pathway is largely dependent on the β subunit of IKK.[119,131,155,156] IKK is a large protein complex composed of three subunits: IKKα, IKKβ and IKKγ (also called NF-κB essential modulator (NEMO) or IKK associated protein (IKKAP)). IKKα and IKKβ are catalytic subunits, with IKKβ being far more potent in mediating NF-κB than IKKα. Non-catalytic IKKγ functions as a regulatory subunit by associating IKKα/IKKβ dimers to upstream signalling molecules. Upon complex assembly, activated IKKβ rapidly mediates phosphorylation, ubiquitination and degradation of IκB proteins, especially IκBα. Degraded IκBα releases NF-κB and exposes NLS on NF-κB dimers, consequently allowing translocation of NF-κB to the nucleus.[119,131,155,156]

2.5.3. NUCLEAR NF-κB ACTIVATION: ADDITIONAL REGULATORY MECHANISMS

In addition to nuclear translocation, NF-κB activity is controlled by additional regulatory mechanisms.[151,154,155,157,158] These include regulation of nuclear import and export of NF-κB dimers, regulation of the recruitment of NF-κB dimers to the promotor or enhancer sites of NF-κB target genes, regulation of NF-κB transcriptional activity after recruitment and positive or negative feedback mechanisms. Recruitment and transcriptional activity are mainly regulated by posttranslational modifications of NF-κB proteins. Particularly p65 is subjected to a variety of these modifications, including phosphorylation, acetylation, S-nitrosylation and S-glutathionylation.[151,154,155,157,158] It appears that these nuclear events are mainly of relevance to determine strength and duration of the NF-κB transcriptional response.[151]
Upon nuclear activation, NF-κB can mediate transcription of numerous inflammatory genes, which play important roles in the development of endotoxaemia. These genes include cytokines (e.g. TNF-α, IL-1β, IL-6), chemokines, immunoreceptors, inducible enzymes (e.g., inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2)), adhesion molecules and acute-phase proteins.[131,149]

2.5.4. NF-κB: THE ROLE OF KETAMINE

There are a few reports on the influence of ketamine on IκB and NF-κB activity. In LPS-treated rat mononuclear cells, Yu et al.[159] showed a reduced expression of IκBα, which markedly increased following exposure to LPS and ketamine at

different concentrations (10-5000 μM). In addition, Shen et al.[160] demonstrated that pretreatment with ketamine (50 mg/kg) significantly blocked ischemia-induced decrement of IκB-α in the hippocampus of the rat. Regarding NF-κB, ketamine reduced LPS-induced NF-κB activation in human glioma cells,[161] in rat mononuclear cells[159] and in rat monocytes[162] in vitro. In vivo, ketamine suppressed NF-κB activity in the intestine, liver and lung of endotoxin-challenged rats,[163] in mouse brain cells[161] and in the hippocampus of the rat following global ischemia.[160]

2.5.5. NF-κB IN EQUINES

In only one study in horses, NF-κB activity has been described. Janicke et al.[164] identified low levels of constitutive NF-κB activity in the nucleus of equine vascular smooth muscle cells and reported on translocation of an additional NF-κB protein 2 hours after stimulation with LPS and interferon-gamma (IFN-γ). Simultaneously, increased expression of COX-2 and limited expression of iNOS was found.

2.6. CYTOKINES

Cytokines are largely inducible proteins with a molecular mass > 5 kDa, which can be secreted by any cell in the body, with the possible exception of erythrocytes, and which can bind to and activate a range of cells. Cytokines highly participate in both homeostatic regulation and pathogenesis of numerous diseases. LPS is able to induce most cells to synthesise a range of cytokines.[102]

Cytokines generally have no enzymatic activity and act at local level. They induce their biological effects only when they bind to their specific receptors on the surface of target cells, inducing specific intracellular signal transduction pathways, and eventually resulting in the switching on of particular sets of genes, including adhesion receptors, acute-phase proteins, lipid-metabolising enzymes, nitric oxide synthase (NOS) and cytokines.[102] The affinity of cytokine receptors for their ligands is extremely high and most cells have only very small numbers of receptors. Furthermore, only a few receptors need to be occupied to produce a maximal response.[102] Thus, very low levels of cytokines can induce biological effects in vivo. However, exaggerated production of cytokines in vivo may cause profound systemic alterations with potentially life-threatening consequences.[102,165]

Generally, cytokines are divided into pro-inflammatory (e.g., TNF-α, IL-1, IL-2, IL-3, IL-5, IL-6, IL-9, IL-11, chemokines, IFNs, platelet-derived growth factor (PDGF)) and anti-inflammatory cytokines (e.g., IL-1 receptor antagonist (IL-1ra), IL-4, IL-10,

IL-13, transforming growth factor β (TGF-β)).[102,166] In the scope of this thesis only TNF-α and IL-6 will be described.

2.6.1. TNF-α

TNF-α, also known as cachexin, consists of two forms: a soluble form (17 kDa) and its membrane bound precursor (26 kDa). The trimeric form of soluble TNF-α is mainly responsible for bioactivity.[165,167]

TNF-α is predominantly produced in mononuclear phagocytes, although it is also produced in numerous other cell types.[167] Upon stimulation by a variety of stimuli (e.g., LPS, synthetic lipid A, IL-1, TNF, leukotrienes, nitric oxide (NO), reactive oxygen species (ROS), parasites, viruses), transcription and translation of the TNF-α gene is rapidly upregulated, enabling the release of large quantities of soluble TNF-α.[167] Expression of TNF-α is mainly regulated by MAPKs and the transcription factors NF-κB and AP-1,[131,134,137] although many other factors at distinct levels have also been demonstrated to be involved in TNF-α expression.[167]

The activities of TNF-α are mediated through at least two types of membrane-associated receptors called type I (TNFR1) and type II (TNFR2) receptors. Each of the TNF receptors is present on virtually all cells, except erythrocytes.[167] TNFR1 is responsible for the major TNF-α bioactivity, including cytotoxicity and activation of neutrophils and endothelial cells, whereas TNFR2 alone is not sufficient to stimulate these functions. However, in combination with TNFR1, TNR2 has been demonstrated to potentiate the effects of TNFR1.[167] Both types of receptors can be released from the membrane and appear in the circulation. These soluble receptors (sTNFRs) bind to TNF-α and function as TNF-binding proteins that neutralise TNF-α activity. However, low amounts of sTNFRs may augment the long-term effects of TNF-α by providing a reservoir of bioactive TNF-α that is slowly released.[167]

Three lines of evidence indicate that TNF-α is the primary mediator of endotoxaemia. First, increased concentrations of TNF-α have been detected in the circulation of endotoxic animals and humans. The kinetic profile of TNF-α is extremely rapid. Following induction of endotoxaemia, plasma concentrations of TNF-α rapidly increase, peak between 90 to 120 minutes and become undetectable within 4 to 6 hours.[165,167,168] Clearance from the circulation is mainly caused by binding to TNF-α receptors and soluble TNF-α binding proteins, following which the whole complex is excreted by the kidneys.[167] Second, systemic administration of purified recombinant TNF-α fully mimics the pathophysiological changes of

LPS-induced endotoxaemia like neutrophil activation, adherence of activated neutrophils to vascular endothelium, upregulation of pro-coagulant factors, downregulation of anti-coagulant factors and phospholipase A_2 activation.[168] Apart from direct effects, the biological effects of TNF-α are largely mediated indirectly by initiating the release of other pro-inflammatory mediators, such as IL-1, IL-6, IFN-γ, prostanoids, leukotrienes, PAF, NO and ROS. Ultimately, this plethora of mediator-induced effects will lead to endotoxaemia which is clinically characterized by fever, hypotension, haemoconcentration, metabolic acidosis, hyperglycaemia followed by hypoglycaemia, diffuse capillary leakage, DIC, hemorrhagic necrosis of tissues, multiple organ failure and death.[167,168] The third and most convincing evidence for the role of TNF-α in endotoxaemia is derived from studies in which monoclonal antibodies directed against TNF-α prevented most of the injurious effects of endotoxaemia.[165,167]

2.6.1.1. TNF-α: ROLE OF KETAMINE

In many experiments with LPS-challenged rodents[78,80-83,86] and humans,[87-91] ketamine significantly reduced TNF-α production in a dose-dependent manner both in vitro and in vivo. No conflicting reports have been found.

2.6.1.2. TNF-α IN EQUINES

In vitro, TNF-α concentration significantly increased in LPS-treated equine peritoneal macrophages when compared to non-stimulated cells. Among the LPS doses tested (0.5 ng/mL, 5 ng/mL and 5 μg/mL), no differences in TNF-α concentrations were found.[169] In another in vitro study, Barton et al.[170] found that LPS-treated peritoneal macrophages obtained from healthy horses and horses suffering from acute gastrointestinal disease both showed a significant increase in TNF-α activity compared to non-treated macrophages, although LPS-induced TNF-α activity was significantly less for macrophages from horses with acute gastrointestinal disease. These results suggest that these macrophages may exhibit early endotoxin tolerance.[170]

In experiments with LPS-challenged horses, plasma TNF-α concentrations significantly increased following bolus injection of 5 μg/kg LPS and low dose infusions of 0.03 μg/kg LPS for 1-4 hours.[171,172] The kinetic profile of equine TNF-α was extremely consistent with those found in other species and independent of the route of LPS administration (i.e., intraperitoneally or intravenously) and the way of LPS administration (i.e., bolus injection or slow infusion).[165,171,172] In response to

LPS, horses seemed depressed, had signs of mild to moderate abdominal pain, developed tachycardia and fever and had leukopenia followed by leukocytosis. Moreover, these symptoms were positively correlated with TNF-α activity.[171,172]
In horses suffering from gastrointestinal disorders, both serum and peritoneal fluid TNF-α activity were significantly higher compared to healthy horses.[173-175] In horses with gastrointestinal disorders, peritoneal fluid TNF-α activity was significantly greater than serum TNF-α activity.[175] Mortality and marked increase in serum TNF-α activity were greater in horses with intestinal inflammatory disorders or strangulating intestinal obstruction than in horses with non-strangulating intestinal obstruction. Serum TNF-α activity and mortality were positively correlated.[173]
Similar results were found in foals, in which serum TNF-α activity was highly correlated with clinical criteria of sepsis. Moreover, an association was apparent between disease severity and serum TNF-α activity.[176]
MacKay[177] found that TNF-α associated cytotoxicity was significantly higher in sera of equine patients suffering from any naturally acquired disease than in sera of healthy horses. Sera from horses with either peritoneal leakage of gastrointestinal tract contents or any bacterial infection were significantly more cytotoxic than sera from horses without these clinical factors. Horses that showed the highest serum TNF-α cytotoxicity were all suffering from gastrointestinal disorders and had a clinical profile suggestive of endotoxaemia.
In vitro, TNF-α antibodies significantly inhibited TNF-α activity in equine peritoneal macrophages.[178] In experiments with LPS-challenged horses, administration of TNF-α antibodies significantly reduced serum TNF-α activity,[179] TNF-α-induced cytotoxicity[172] and the haematological and clinical responses associated with LPS.[179]

2.6.2. IL-6

Interleukin-6 (IL-6), formerly known as interferon-β2, B-cell stimulatory factor-2, hybridoma plasmacytoma growth factor, 26 kDa protein or hepatocyte-stimulatory factor, is a multifunctional cytokine which consists of two N-glycosylation sites that are variably glycosylated. As a result, IL-6 is secreted as a heterogeneous set of proteins with molecular masses ranging from 19-30 kDa.[167,180] Only unglycosylated IL-6 is biologically active.[180]
Under physiological conditions, IL-6 mRNA is constitutively expressed at low levels in several tissues and cell types, including leukocytes, spleen, liver, kidney

and intestines.[167] However, during inflammation, trauma or immunologic challenge, IL-6 is synthesised in nearly all tissues and cell types, with endothelial cells, fibroblasts and mononuclear phagocytes being its major producers.[167,180] Upon stimulation, expression of IL-6 mRNA is mainly regulated by the JAK-STAT pathway and the nuclear factor for IL-6 expression (NF-IL-6).[167,181,182]

The biological effects of IL-6 are mediated through interaction with the IL-6 receptor complex on target cells. IL-6 first binds to a low-affinity ligand-binding subunit which is stabilised and activated when bound by an intracellular signal transducing subunit.[181,183] The kinetic clearance of synthesised IL-6 is biphasic, consisting of a rapid initial elimination ($t_{½}$ ~3 min) and a slower second elimination ($t_{½}$ ~55 min).[180]

IL-6 exerts numerous biological effects. As the major stimulator and regulator of the acute phase response and following tissue trauma and infection, IL-6 induces the biosynthesis of the full spectrum of acute phase proteins in hepatocytes. In contrast, TNF-α and IL-1 stimulate the generation of only a limited subset of acute phase proteins.[167,180] IL-6 also plays an essential role in the functioning of the immune system by stimulating humoral as well as cellular defence mechanisms. Moreover, IL-6 regulates growth and differentiation of various cell types, stimulates haematopoiesis and induces fever through a PGE_2-dependent mechanism.[167,180] Ultimately, the biologic role of IL-6 depends on the level in serum and tissue compartments. Relatively low levels may be beneficial as they stimulate repair mechanisms, including the production of anti-inflammatory cytokines, whereas very high levels accelerate inflammatory disorders and organ dysfunction.[167,180]

Evidence indicates that IL-6 is highly involved in the pathogenesis of endotoxaemia and other inflammatory diseases.[183,184] In experimental studies in humans and rodents, intravenous injection of LPS or TNF-α resulted in a sharp increase in serum IL-6 concentrations,[167] whereas the administration of anti-TNF-α antibodies considerably reduced LPS-induced IL-6 production.[185]

Although generally known as a pro-inflammatory cytokine, IL-6 also exerts anti-inflammatory activities by suppressing LPS-induced synthesis of TNF-α and counteracting the effects of TNF-α and IL-1.[167,186] According to Tilg et al.,[187] these anti-inflammatory properties might be due to the induction of IL-1ra and the release of sTNFR. In contrast to these anti-inflammatory properties, high concentrations of IL-6 have been associated with fatal outcome in humans, making it a useful marker of the pro-inflammatory response.[188,189]

2.6.2.1. IL-6: ROLE OF KETAMINE

In LPS-treated rodents, ketamine significantly inhibited LPS-induced IL-6 production in a dose-dependent manner.[80-83,86] Similar results were found in human whole blood assays, in which LPS-induced IL-6 production decreased in the presence of ketamine.[88,91] Moreover, ketamine attenuated the IL-6 response after various surgical interventions in humans.[92,93]

2.6.2.2. IL-6 IN EQUINES

In vitro, IL-6 activity significantly increased in LPS-treated equine peritoneal macrophages when compared to non-stimulated cells. IL-6 activity peaked at 6 or 12 hours after exposure to 5 or 500 ng/mL and 0.5 ng/mL endotoxin, respectively, and remained high through 24 hours of incubation.[190]

In experiments with LPS-challenged horses, plasma IL-6 concentrations significantly increased following low dose infusions of 0.03-1 μg/kg LPS over 1 hour.[191,192] Depending on the endotoxin concentration infused, serum IL-6 activity was significantly increased above baseline from 2 to 12 hours following the start of infusion. Peak plasma IL-6 activity was observed between 3 and 4 hours after LPS stimulation. The IL-6 response of endotoxin-treated horses started 1 hour after TNF-α appeared in the circulation. In response to LPS, horses became lethargic, had signs of mild to moderate abdominal pain, developed tachycardia and fever and had leukopenia followed by leukocytosis. Significant positive association and linear correlation was only apparent between serum IL-6 activity and rectal temperature.[191]

In horses suffering from gastrointestinal disorders, both serum and peritoneal fluid IL-6 activity were significantly higher when compared to healthy horses.[174,175] IL-6 concentrations were significantly positively correlated with LPS concentrations.[174] In horses with gastrointestinal disorders, peritoneal fluid IL-6 activity was significantly greater than serum IL-6 activity.[175] Blood and peritoneal fluid IL-6 activity was significantly higher in horses with inflammatory or strangulating lesions than in horses with non-inflammatory or non-strangulating lesions. Diagnostic accuracy for non-survival was greatest (80%) when blood IL-6 activity exceeded 60 U/mL.[175]

In experiments with LPS-challenged horses, administration of TNF-α antibody significantly reduced serum IL-6 activity as well as the haematological and clinical responses associated with circulating LPS.[179]

2.7. iNOS

In mammals, three distinct isoforms of nitric oxide synthase (NOS) exist: endothelial NOS (eNOS, NOS I; 135 kDa), neuronal NOS (nNOS, NOS III; 160 kDa) and inducible NOS (iNOS, NOS II; 130 kDa).[193] In general, nNOS and iNOS are soluble, whereas eNOS is membrane bound,[193] although all three can have either cytosolic or membrane bound locations.[194] Recently, the existence of a fourth NOS enzyme, mitochondrial NOS (mtNOS) was reported in rat and mouse.[195] However, it has yet to be determined that this NOS protein is specifically associated with mitochondria, since mtNOS seems more likely to be a membrane-associated iNOS.[194]

The three main isoforms share structural similarities and have nearly identical catalytic mechanisms.[193,194,196,197] Beside the cosubstrates nicotinamide adenine dinucleotide phosphate (NADPH) and O_2, they all require a number of cofactors for activity including haem, tetrahydrobiopterin (H_4B), calmodulin, flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN).

NOS catalyse the biosynthesis of nitric oxide (NO) by a two step oxidative conversion of L-arginine to NO and L-citrulline via the more labile intermediate N-hydroxy-L-arginine, utilising NADPH as an electron donor.[194,197-199] The functional NOS protein is a homodimer, with each subunit comprising of a reductase domain at the C-terminus, an oxygenase domain at the N-terminus and a calmodulin-binding domain in their midregion.[194] The reductase domain contains the FAD and FMN moieties and share extensive homology with cytochrome P_{450} reductase. This domain transfers electrons from NADPH to the oxygenase domain. The oxygenase domain actually catalyses the conversion of arginine into citrulline and NO and contains the binding site for haem, H_4B and arginine. The calmodulin binding domain plays a pivotal role in activation of the different isoforms.[194,197]

Commonly, two isoforms, nNOS and eNOS are constitutively expressed, whereas iNOS is expressed upon cell activation.[193,200,201] However, recent experimental studies demonstrated that all three can be constitutively expressed in some cells or tissues, and all three can be induced, albeit by different stimuli.[194,202] Activation of nNOS and eNOS is calcium-dependent. In response to increases in intracellular calcium concentrations, calcium binds to calmodulin after which the calcium-calmodulin complex binds to the calmodulin binding domain and activates NOS. In contrast, activation of iNOS is independent of intracellular calcium concentrations, as calmodulin is prebound to the molecule even at very low calcium concentrations.[193,194,201,203] As nNOS and eNOS activity is triggered by calcium influx, the activity is transient and results in a low production of NO (low-

output), while iNOS can be active for hours to days and generate 1000-fold higher amounts of NO (high-output).[200,203-205]

nNOS is expressed in the central and peripheral nervous system and in skeletal muscles.[193,203] So far, four splice variants (nNOSβ, nNOSγ, nNOSμ and nNOS-2) have been detected.[194] Cells that express eNOS include vascular endothelial cells and cardiomyocytes. No splice variants of eNOS have been reported.[194] iNOS is inducible in a wide range of cells and tissues[193,194,201] by a variety of stimuli, including bacterial products (e.g., LPS), cytokines (e.g., TNF-α, IL-1β, IFN-γ), viral products, cyclic AMP (cAMP)-elevating agents, protein kinase stimulating agents and growth factors.[193,200,206] Stimuli that induce iNOS expression differ between species and between cell types within the same species.[193] For example, rodent and bovine cells can be more easily triggered than human and pig cells.[201] The majority of human cells require a complex cytokine combination (e.g. TNF-α, IL-1β and IFN-γ) for iNOS induction.[207] In addition, LPS and INF-γ are strong inducers of iNOS in monocytes, whereas TNF-α and IL-1β have only marginal effects in monocytes.[200] In contrast, most tissue-derived cells, such as hepatocytes, mesangial cells and vascular smooth muscle cells are more sensitive to iNOS expression when treated with TNF-α or IL-1β.[193,200,201] iNOS requires a delay of 6-8 hours before the onset of NO production, reflecting the time taken for mRNA and protein synthesis.[200]

Expression of iNOS is regulated by a large array of mechanisms at both transcriptional, posttranscriptional, translational and posttranslational level.[206-208] Once iNOS is expressed, its enzymatic activity can no longer be regulated.[193,207] Overall, the transcription factor NF-κB seems to play a central role in regulating LPS-induced iNOS expression.[209] Besides NF-κB, involvement of transcription factors like interferon regulatory factor 1 (IRF-1), signal transducer and activator of transcription 1 (STAT-1), AP-1 and cAMP-induced transcription factors (e.g., cAMP-responsive element binding protein (CREB), CCAAT-enhancer box binding protein (C/EBP)) have been demonstrated.[201,206,207] Moreover, several studies have now identified the involvement of all three MAPK (i.e., JNK, p38 and ERK) pathways in regulating iNOS expression.[206,207,210,211] iNOS is even able to trigger transcription of his own biosynthetic machinery since iNOS produced NO can activate all three MAPK cascades and the transcription factors NF-κB and AP-1.[200] Yet, a negative feedback on its own expression has also been demonstrated.[204]

2.7.1. NO

Nitric oxide (NO) is a simple diatomic molecule whose physico-chemical and biological properties are determined by its small size (30 kDa), the absence of charge and its single unpaired electron. As an uncharged molecule, it freely crosses cell membranes and it is readily diffusible in body fluids and tissues. Its lone outer electron renders it a radical and therefore chemically reactive. However, for a radical it is relatively stable as it does not react with itself and has a physiological half-life of seconds to minutes. In aqueous solutions NO undergoes oxidation into the relatively unreactive ions, nitrate (NO_3^-) and nitrite (NO_2^-).[203,212-214]

NO acts as a molecular messenger in various physiological and pathological processes in many organ systems.[203,206,215] It has been considered that 'low-output' NO, derived from constitutively active nNOS and eNOS, is an important regulator of physiological homeostasis, whereas 'high-output' NO, produced by iNOS, is correlated with the pathogenesis of inflammatory diseases such as asthma, arthritis, colitis, tumour development, neurodegenerative diseases and septic shock.[205-207]

Under physiological conditions, NO has been reported to be an important free radical scavenger and thus limiting the toxic effects of free radials such as superoxide anion (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radical (·OH). NO may also have the ability to prevent superoxide production by directly inhibiting NADPH oxidase.[216] In the brain, NO acts a neuromodulator that influences functions like memory, behaviour, nociception, vision and olfaction.[203,204] In the peripheral nervous system, NO acts as a neurotransmitter participating in functions such as smooth muscle control, gastrointestinal motility, and neuroendocriene function.[203,204,214] In skeletal muscles NO functions as a signal transducer regulating both metabolism and muscle contractility. In blood vessels, NO functions as a vasodilator thereby regulating blood flow and pressure.[204,214] Within cardiomyocytes, NO affects calcium currents and, consequently, myocardial contractility.[203,214] Furthermore, it inhibits platelet aggregation, leukocyte adhesion to endothelial cells and stabilises cell membranes resulting in reduced ischaemia-reperfusion injury.[203,204,214,216] In activated macrophages, NO synthesis leads to non-specific cytotoxicity against bacteria, protozoa and tumour cells.[204,205,207]

In addition, large amounts of NO have been demonstrated to inhibit cytokine production in stimulated macrophages by directly inhibiting NF-κB expression,[216] while smaller amounts induced this production.[205]

Under pathophysiological conditions, exaggerated NO production may lead to an array of detrimental effects, among which are excessive vasodilation and loss of systemic resistance, increased vascular permeability and airway and tissue oedema.[217-219] Moreover, increased NO production may induce hepatocyte dysfunction,[220] reduced gastrointestinal motility,[203] neurodegenerative disorders[219] and myocardial depression resulting in negative inotropy.[218]
When NO reacts with free radicals, more potent and highly reactive oxidants like peroxynitrite ($ONOO^-$) and nitrogen dioxide (NO_2) will be formed.[221] Subsequently, these free radicals induce platelet aggregation, disseminated intravascular coagulation, stimulation of lipid peroxidation and inhibition of mitochondrial respiration, finally leading to cellular damage.[203,217] Ultimately, lysis of cells and its organelles might lead to multiple organ dysfunction and death.[221]

2.7.2. iNOS EXPRESSION AND NO PRODUCTION: ROLE OF KETAMINE

In rodents, controversial effects regarding the influence of ketamine on LPS-induced iNOS expression and NO production have been found. In vitro, ketamine inhibited LPS-induced iNOS expression and NO production in a murine macrophage-like cell line[222] and in rat alveolar macrophages.[223] Ex vivo, ketamine blunted iNOS expression in several abdominal organs of endotoxin-challenged rats.[224,225] In contrast, Shibakawa et al.[226] found no significant inhibition of nitrite release in LPS-treated primary glial cell cultures following ketamine exposure.

2.7.3. iNOS EXPRESSION AND NO PRODUCTION IN EQUINES

In vitro, iNOS expression significantly increased in LPS-treated equine alveolar macrophages,[227] articular chondrocytes[228] and e-CAS cells[145] when compared to non-treated cells. In vascular smooth muscle cells only very limited expression of iNOS was found following simultaneous stimulation with LPS and IFN-γ. Basal NO synthesis was demonstrated in non-stimulated equine chondrocytes,[228] synoviocytes,[228] alveolar macrophages[227] and e-CAS cells.[145] Upon stimulation with LPS, NO synthesis significantly increased in equine chondrocytes in a dose-dependent manner.[228] In equine alveolar macrophages, significant increase in NO production was only detected in cells stimulated with LPS concentrations below 1 μg/mL,[227] whereas NO production in equine synoviocytes was neither significantly increased nor decreased by exposure to LPS.[228] In addition, NO was not detected in any supernate from peritoneal macrophage monolayers.[229]
Ex vivo, no substantial difference was detected in iNOS activity between synovial membranes obtained from healthy horses or from horses whose metacarpo-

phalangeal joints were exposed to IL-1β.[230] In vivo, Mirza et al.[231] showed a significant increase in iNOS staining in mucosal leukocytes and vasculature in horses suffering from a strangulating large colon volvulus versus clinically healthy horses. Moreover, higher nitrite concentrations were measured in articular cartilage, subchondral bone and the underlying trabecular bone of the proximal articular surface of the first phalanx of horses suffering from osteoarthritis than in healthy horses.[232] In addition, mares suffering from breeding-induced endometritis showed greater iNOS expression in their uterine biopsies and higher NO concentrations were measured in their uterine secretions compared with resistant mares.[233]

2.8. COX-2

Cyclooxygenase (COX) enzymes, also known as prostaglandin endoperoxide synthases, are fatty-acid oxygenases of the myeloperoxidase superfamily that are responsible for producing prostaglandins.[234] COX enzymes catalyse the sequential oxygenation and reduction of membrane-derived arachidonic acid to form the endoperoxides prostaglandin G_2 (PGG_2) and prostaglandin H_2 (PGH_2), respectively.

COX enzymes contain 3 major domains: an N-terminal epidermal growth factor (EGF) domain, a helical membrane binding domain and a large catalytic domain at the C-terminus.[235] The EGF domain functions as a dimerisation domain since it contains conserved disulfide bonds.[234] Via the membrane binding domain, COX enzymes are largely anchored to the luminal side of the endoplasmic reticulum membrane and the nuclear envelope, although they have also been detected in lipid bodies, mitochondria, vesicles and nucleus.[234,236] The membrane binding domain contains 4 helices, which surround the opening where fatty acids enter the enzyme.[235] The catalytic domain has two distinct cyclooxygenase and peroxidase active sites which sequentially catalyse the oxidation and reduction of arachidonic acid, respectively.[234,235] COX enzymes have short catalytic life times, since the enzymes are autoinactivated.[234]

COX enzymes (72 kDa) consist of two isoforms: COX-1 and COX-2, sharing a 61% homology at the amino acid sequence.[234,235] In 2002, a splice variant of COX-1 has been identified in canine and human cerebral cortex and the human heart. This enzyme, designated as COX-3, shows a similar structure but slower enzymatic activity when compared to the parental isoform.[235,237] Splice variants of COX-2 have also been reported, but have failed to show enzymatic activity.[235]

COX-1 is ubiquitously and constitutively expressed in mammalian tissues and cells and is mainly involved in 'housekeeping' functions such as gastric mucosal integrity, crypt cell regeneration after injury, platelet aggregation, renal blood flow and glomerular filtration.[234,237] It is mainly utilised in the immediate prostaglandin biosynthesis.[236]

COX-2 is expressed by cells that mediate inflammation (e.g., macrophages, monocytes, synoviocytes) and has been recognised as the isoform that is primarily responsible for the synthesis of prostaglandins involved in pathological processes, particularly those related to acute and chronic inflammatory disorders.[235,238] In contrast with COX-1, COX-2 is generally present in tissues at very low levels, but highly inducible by stimuli such as LPS, cytokines, mitogens and other growth and differentiation factors.[234,237] Upon stimulation, expression of COX-2 is modulated by an array of transcription factors, among which are NF-κB, AP-1, CREB and C/EBP.[237,238] In addition, all three MAPKs (i.e., JNK, p38 and ERK) have been demonstrated to be involved with COX-2 expression.[210,238,239] The inducible COX-2 is an absolute requirement for delayed prostaglandin synthesis, which lasts for several hours following stimulation.[236] Effective catalysis by COX-2 can proceed at low levels of arachidonic acid (≤ 2.5 μM) derived from endogenous sources, whereas COX-1 requires high levels (> 10 μM) derived from exogenous sources to sustain catalysis.[235,236]

Nowadays, classification of COX into constitutive and inducible isoforms is somewhat oversimplified, since COX-1 can be induced during early stages of acute inflammation and COX-2 is constitutively present in brain and kidney.[237,240] Moreover, data increasingly indicate that COX-2 also has an important role in normal physiological functions, such as regulation of the glomerular filtration rate and gastric mucosal healing.[237]

2.8.1. PGE_2

Prostaglandin H_2 (PGH_2), the end product of COX, does not play a significant role as an inflammatory mediator. Rather, it is isomerised to various bioactive prostanoids (e.g., thromboxane A_2 ($TX A_2$), PGD_2, PGE_2, PGF_2 and PGI_2) by the respective terminal prostanoid synthases.[236]

Prostaglandins act in numerous physiological and pathophysiological processes, such as maintaining gastric mucosal integrity, renal function, vascular homeostasis, thrombosis, mitogenesis, ovulation, parturition, pyresis, algesia and inflammation.[234] Upon liberation, prostaglandins exert their effects by binding to

G-protein-coupled receptors.[240] In vivo, prostaglandins are short-lived (with half-lives of seconds to minutes) and act in an autocrine or a paracrine, rather than an endocrine fashion.[234]

PGE_2 is the most prominent prostaglandin in the onset of inflammation.[235,237] It has been detected in almost all experimental models of inflammation and in many inflammatory disorders in humans.[240] The biologic effects are extremely wide-ranging, but four areas are particularly pertinent to the regulation of the inflammatory response: fever, pain, oedema and regulation of leukocyte function.[240-242]

PGE_2 is a key mediator of the febrile response.[242] Injection of PGE_2 causes an increase in body temperature in many species and levels of PGE_2 are increased in cerebrospinal fluid from febrile animals.[240] PGE_2 mediates fever by acting on the thermoregulatory centre in the anterior hypothalamus.[242] However, the exact moment at which PGE_2 gets involved in mediating the febrile response following LPS stimulation is still unclear.[242] Fever promptly develops following LPS exposure, but synthesis of PGE_2 by COX-2 is significantly delayed. Since a local production of PGE_2 in the hypothalamus could not be demonstrated immediately after LPS injection, Blatteis et al.[242] postulated that PGE_2 is mainly responsible for the late and sustained phase of fever and that other components (e.g., norepinephrine) initiate the early phase.

PGE_2 plays an important role in mediating the pain response. Monoclonal antibodies to PGE_2 inhibit the pain response by 80%.[240] PGE_2 lowers the pain threshold and thus induces hyperalgesia during tissue injury and inflammation. Nishihara et al.[241] demonstrated that PGE_2-induced hyperalgesia is mediated by the N-methyl-D-aspartate (NMDA) receptor, since PGE_2-induced hyperalgesia was inhibited by both competitive and non-competitive blockers of these receptors.

PGE_2 induces both vasoconstriction and vasodilation, depending on the specific vascular bed involved.[168] As part of these vascular effects, PGE_2 has profound influence on the occurrence of oedema associated with inflammation, since PGE_2 increases blood flow to the inflamed site by arteriolar dilation. Oedema is not induced by injection of PGE_2 alone, but in the presence of an agent that increases vascular permeability to plasma proteins (e.g., LPS), a synergistic increase in the extent of plasma leakage, and thus oedema formation, can be observed. In addition, treatment with anti-PGE_2 antibody significantly reduced carrageenan-induced oedema.[240]

PGE_2 also shows immunosuppressive effects by modulating leukocyte function. In vitro, PGE_2 inhibits neutrophil activation, as has been measured by reduced

chemotaxis and superoxide production.[243] Moreover, PGE_2 decreases both TNF-α and IL-1 production in LPS treated macrophages.[244,245] This effect occurs also in vivo, since administration of COX inhibitors to mice[246] and humans[247] enhances the release of TNF-α in response to LPS exposure.

2.8.2. COX-2 AND PGE_2: THE ROLE OF KETAMINE

In vitro, ketamine inhibited the LPS-induced PGE_2 production in primary cell cultures of rat glial cells.[266] In vivo, pretreatment with ketamine attenuated hepatic COX-2 expression in LPS-treated rats,[248] whereas no influence on gastric mucosal COX-2 expression was found in rats anaesthetised with the combination ketamine/xylazine.[249] Nishihara et al.[241] found that PGE_2-induced hyperalgesia is mediated by NMDA receptors, since the non-competitive NDMA receptor antagonist ketamine inhibited PGE_2-induced hyperalgesia, although only following higher doses of PGE_2.

2.8.3. COX-2 AND PGE_2 IN EQUINES

In vitro, COX-2 expression significantly increased in LPS-treated equine alveolar macrophages[227] and vascular smooth muscle cells[164,250] when compared to non-treated cells. Upon stimulation with LPS or IL-1β, equine chondrocytes,[251,252] peritoneal macrophages[229] and vascular smooth muscle cells[164] have been shown a significant increase in PGE_2 synthesis, compared to non-stimulated cells.

In experimental in vivo studies, increased plasma PGE_2 concentrations have been associated with colitis[253] and ischemia-reperfusion injury in the large colon of horses.[254] Increased levels of PGE_2 have also been detected in the synovial fluid of LPS-induced synovitis of the carpal joint.[255]

In vivo, significantly higher PGE_2 concentrations have been measured in synovial fluid collected from osteoarthritic joints[256] and joints with osteochondritis dissecans[257] than in synovial fluid obtained from non-affected joints.

2.9. ROS

Phagocytic cells like neutrophils, monocytes and macrophages, produce and release reactive oxygen species (ROS), such as superoxide anion ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2) and hydroxyl radical (·OH), in response to phagocytosis.[221,258,259] Under pathophysiological circumstances, cells other than phagocytes may produce ROS.[221] Upon stimulation by either endogenous factors (e.g., cytokines, platelet-activating factor (PAF), complement fragments, eicosanoids) or exogenous influences (LPS, bacterial peptides, UV light irradiation, toxic chemicals and

drugs), the multicomponent nicotinamide adenine dinucleotide phosphate (NADPH) reduced oxidase system is assembled and activated, consequently catalysing the reduction of oxygen to superoxide anion.[221,258,260-262] Next to the NADPH oxidase pathway, superoxide anion can be enzymatically generated by cytochrome P_{450s}, hypoxanthine/xanthine oxidase, lipoxygenase, cyclooxygenase and through the oxidation of catecholamines. Moreover, superoxide anion can be non-enzymatically generated as a side product of mitochondrial respiration where oxygen is reduced to water by the acceptance of four electrons.[221,254,258,261]

Normally, 1-2% of total oxygen consumption may be converted to superoxide anion.[261] Formation of superoxide anion leads to a cascade of other ROS. First, superoxide anion is rapidly converted by superoxide dismutase (SOD) to hydrogen peroxide, which is then, in a spontaneous reaction catalysed by Fe^{2+} (Fenton reaction), converted to the highly reactive hydroxyl radical.[221,254,258,259]

Superoxide is only moderately reactive with other biological molecules. However, when produced in combination with NO, superoxide anion interacts with NO to form peroxynitrite ($ONOO^-$). The peroxynitrite molecule itself is not very reactive, but its acid form, peroxynitrous acid (ONOOH) is a strongly oxidizing and cytotoxic product.[221,263] Thus, rapid conversion of superoxide anion to hydrogen peroxide is important.

Hydrogen peroxide is relative unreactive, although it can be converted by myeloperoxidase (MPO) (i.e., an oxidative enzyme in neutrophil azurophilic granules) to hypochlorous acid (HOCl), which is a strong oxidant that can chlorinate amino acids, peptides and proteins, and oxidise thiol functions of many proteins.[258,264] Reaction of HOCl with hydrogen peroxide yields another radical, singlet oxygen ($^1O_2$).[258]

The hydroxyl radical is so reactive that it will instantaneously extract a hydrogen atom from the closest molecule, producing water and another radical. This resulting radical reacts with a second molecule to continue the chain reaction. Polyunsaturated fatty acids are susceptible targets. So, extraction of a hydrogen atom from a polysaturated fatty acid may initiate the process of lipid peroxidation.[221,258]

Mammalian cells possess several enzymatic anti-oxidants to protect them from ROS formed during normal cellular metabolism. Superoxide dismutase catalyses the conversion of superoxide anion to hydrogen peroxide. Catalase catalyses the reduction of hydrogen peroxide to water and oxygen to prevent secondary generation of hydroxyl radicals. Glutathione peroxidase also reduces hydrogen peroxide to water, using glutathione as the substrate. In addition, since redox-

active metals, such as Fe^{2+}, catalyse formation of hydroxyl radicals, concentrations of these metal ions are kept low by binding to storage and transport proteins (e.g., ferritin, transferrin, lactoferrin).[221,254,258,261] Moreover, non-enzymatic antioxidants, such as α-tocopherol, ascorbate and beta carotene function to protect against oxidant injury.[221,254,261]

Under physiological conditions, the respiratory burst (i.e., ROS production due to transient consumption of oxygen) is kept in control and represents a major mechanism of host defence against infection. However, under pathophysiological conditions, oxidative stress, defined as an imbalance between radical-generating and radical-scavenging systems, may generate exaggerated amounts of ROS, which subsequently lead to cellular destruction and tissue injury.[221,258]

ROS-induced tissue injury can be mediated by both direct and indirect mechanisms.[221,254] An important direct mechanism of ROS involves the peroxidation of membrane phospholipids, which cause cell damage by way of disruption and lysis of cells and their organelles. Other direct mechanisms are hydroxyl-induced oxidation of nucleic acids and proteins which subsequently lead to DNA strand breaks and inactivation of enzymes.[221,254] Indirect tissue injury initiated by ROS involves disruption of intracellular calcium homeostasis. Calcium accumulates because of release from the endoplasmic reticulum and mitochondriae and from influx of extracellular calcium into the cytosol secondary to inactivation of cell membrane-associated calcium-ATP-ase. Increased cytosolic calcium concentrations activate phospholipase A_2, an enzyme which releases arachidonic acid from cell membrane phospholipids, thereby leading to further structural cell and tissue damage.[221,254] Ultimately, ROS-induced tissue injury may lead to multiple organ dysfunction and death.[254]

In humans, ROS have been implicated in various disorders such as arthritis,[265] asthma,[266] carcinogenesis, drug toxicity and inflammation.[267]

Next to their bactericidal function in host defence, ROS play an essential role in intracellular signal transduction pathways.[221,261] Exposure to ROS induces activation of MAPK cascades (i.e., JNK, p38 and ERK) and transcription factors like NF-κB and AP-1, albeit largely cell-specific and stimulus-dependent.[140,221,261,268] For example, NF-κB is not activated by hydrogen peroxide in monocytic cell lines and peripheral T cells. Moreover, hydrogen peroxide enhances NF-κB-mediated COX-2 expression and PGE_2 production in human primary monocytes only following priming or activation by LPS.[268] Chandel et al.[269] even found that LPS activates NF-

κB and TNF-α gene expression through a ROS-independent mechanism in murine macrophage J774.1 cells.
The mechanism by which ROS potentially modulates MAPKs and several transcription factors is still under extensive debate.

2.9.1. ROS: ROLE OF KETAMINE

Regarding the influence of ketamine on ROS production, conflicting results have been found. In human polymorphonuclear leukocytes (PMNs), ketamine significantly reduced N-formyl-methionyl-leucyl-phenylalanine (FMLP)/ opsonised zymosan[270] or FMLP/phorbol 12-myristate 13-acetate (PMA)-induced[89] ROS production in a dose-dependent manner. In contrast, no influence of ketamine on ROS synthesis in PMA-treated,[271] FMLP-treated[99] and opsonised zymosan-treated[272,273] human PMNs could be demonstrated.

2.9.2. ROS IN EQUINES

In vitro, both ROS produced by PMA-stimulated equine neutrophils, as well as ROS present in the supernatant of PMA-stimulated equine neutrophils, were highly cytotoxic to equine endothelial cells in culture.[274] This indicates that under pathological conditions with an important activation of neutrophils, damage induced by ROS can spread to cells and tissues away from the primary inflammatory focus.[274]
In vivo, ROS have been demonstrated to play a pivotal role in the pathogenesis of gastrointestinal ischemia-reperfusion injury in horses in both experimental and clinical studies.[254,275] Following one hour of intestinal ischemia, an increased xanthine oxidase (XO) concentration was detected in the equine small intestine, whether inconsistent results were found with respect to XO concentrations in the equine large colon.[254] In another study, Grülke et al.[275] found a significantly higher plasma level of the oxidative enzyme MPO in horses operated for strangulating obstruction of the large intestine compared with horses suffering from a non-strangulating displacement of the large intestine. These results indicate that PMN activation might play a major role in the pathogenesis of acute abdominal disease and endotoxaemic shock.

3. CONCLUSIONS

The presented literature review suggests that ketamine may exert an anti-inflammatory effect also in horses. As possible targets of its action, interference with pathogen-associated signalling pathways (represented by LPS), leading to the

release of pro-inflammatory mediators, could be identified. Further studies should address the effect of ketamine on the individual components of the inflammatory cascade.

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