- CHAPTER I -

SCOPE AND AIM OF THE THESIS

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Ketamine has been in use as an anaesthetic agent in equine medicine since the mid 70s.[1] Initially, ketamine was applied just as an induction agent, producing amnesia, loss of consciousness, analgesia and immobility. In later years, based on these properties, the application of ketamine in equine anaesthesia was extended by using it as an adjunct to inhalation anaesthesia,[2-4] in different total intravenous anaesthesia protocols[5-7] and for local analgesia.[8,9] Currently, studies focus on the antinociceptive effects of subanaesthetic ketamine continuous rate infusions (CRIs) in conscious horses[10] since in human medicine these low dose rate infusions have been proven to be sufficient for post-operative pain management.[11-13] Beside its anaesthetic and analgesic effects, ketamine has been reported to possess anti-inflammatory effects in rodents and humans. In various in vitro and in vivo studies, ketamine reduced the production of distinct inflammatory mediators.[14-17] These results suggest a beneficial role for ketamine in patients suffering from distinct inflammatory diseases. However, the species-specific response in production of inflammatory mediators and the corresponding underlying mechanisms impede the extrapolation of these previous results from either rodents or humans to horses. As yet, no data are available regarding the anti-inflammatory properties of ketamine in horses. Hence, this thesis presents the first experimental work regarding the anti-inflammatory effects of ketamine in both in vitro and in vivo equine models.

In **Chapter II**, a general overview is given regarding ketamine, the studied inflammatory mediators and the key mediators involved in the signal transduction cascade ultimately leading to their production. At the end of each subchapter, the influence of ketamine on the respective mediator in either rodent or human studies is described. Moreover, the presence or otherwise of this respective mediator in horses is depicted.

Chapter III presents the results of the primary in vitro study evaluating the anti-inflammatory effects of ketamine in equines. In this study, an equine macrophage cell line (also referred to as e-CAS cells), developed by Werners et al.,[18] was used, since macrophages play a pivotal role in the pathophysiology of many equine inflammatory disorders. Upon stimulation with lipopolysaccharides (LPS, outer-wall constituents of Gram-negative bacteria), the influence of ketamine on the pro-inflammatory cytokines tumour necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) was investigated.

In rodents and humans, ketamine has been demonstrated to reduce the LPS-induced cytokine response by inhibiting expression of the nuclear transcription factor nuclear factor-kappa B (NF-κB).[19-21] Next to NF-κB, other upstream key mediators like the intracellular mitogen-activated protein kinases (MAPKs), c-Jun N-terminal kinase (JNK), mitogen-activated protein kinase p38 (p38) and extracellular signal-related kinase (ERK), and the extracellular Toll-like receptor 4 (TLR4) can be involved in the LPS-induced cytokine response.[22] In **Chapter IV**, the influence of ketamine on TLR4, MAPK and NF-κB expression is investigated to identify the underlying molecular mechanism of the ketamine-induced reduction of pro-inflammatory cytokine production in LPS-treated e-CAS cells.

Upon stimulation by LPS, cells express inducible enzymes like inducible nitric oxide synthase (iNOS)[23] and cyclooxygenase-2 (COX-2)[24] which subsequently induce the production of nitric oxide (NO) and prostaglandin E_2 (PGE_2). Although generally involved in regulating physical homeostasis, overproduction of NO and PGE_2 is correlated with the pathogenesis of inflammatory diseases like arthritis, colitis, neurodegenerative diseases and septic shock.[25,26] In **Chapter V** and **Chapter VI**, the influence of ketamine on respectively iNOS expression and NO production and COX-2 expression and PGE_2 production is studied. Moreover, by inhibiting signalling key mediators like MAPK and NF-κB, the molecular mechanisms involved with iNOS and COX-2 expression in LPS-treated e-CAS cells are investigated.

Like other inflammatory mediators, reactive oxygen species (ROS) play a critical role in regulating homeostasis and represent a major mechanism of host defence against infection. However, under pathophysiologic conditions, oxidative stress may lead to an exaggerated production of ROS, which subsequently leads to cellular destruction and tissue injury.[27] In **Chapter VII**, the influence of ketamine on ROS production in e-CAS cells following exposure to LPS and phorbol myristate acetate (PMA) is described. Generally, cells possess several enzymatic anti-oxidants to protect them from 'home-made' ROS.[28] Glutathione peroxidase is such anti-oxidant which reduces ROS to water by using glutathione (GSH) as substrate. In this study, also the influence of ketamine on GSH-depleted ROS production in LPS/PMA-treated e-CAS cells was studied, to partly identify the mechanism by which ketamine reduces ROS production in stimulated e-CAS cells.

The anti-inflammatory effects of ketamine observed in previous experimental studies performed in rodents and humans, implies that ketamine might also have anti-inflammatory properties in vivo. To be clinically effective in conscious equine

patients in the postoperative period, long-term administration of subanaesthetic dose rates of ketamine seems to be required. Long-term infusions appear to be necessary, since ketamine displays a short elimination half-life,[29] while inflammatory mediators have been detected for hours after onset of inflammation. Moreover, subanaesthetic dose rates are preferred to avoid recumbency and side effects like excitation and catalepsia, regularly associated with ketamine administration. However, before studying the anti-inflammatory effectiveness of this infusion regimen in vivo, its influence on clinical and behavioural parameters in conscious horses was investigated. Hence, in **Chapter VIII**, the pharmacodynamic effects of a long-term subanaesthetic CRI of ketamine administered to healthy conscious horses are evaluated. Moreover, a pharmacokinetic profile of ketamine and its metabolites is described.

To study the influence of ketamine on LPS-induced inflammatory responses in vivo, tissue chamber modeling systems can be used.[30] Upon injection of LPS into the tissue chambers, a marked acute inflammatory response is produced which has been demonstrated to be valid for septic processes at soft tissue level.[31] Moreover, by applying the tissue chamber model, LPS-induced inflammatory responses will be confined largely to the tissue chamber, thereby minimising discomfort to experimental animals.[30] In **Chapter IX**, the anti-inflammatory effects of parenterally administered ketamine on a LPS-induced inflammatory response in tissue chambers of Shetland ponies is investigated. Both a bolus injection of ketamine and a subanaesthetic long-term continuous rate infusion were tested.

Finally, the main results of the aforementioned chapters are summarised and discussed in the concluding chapter of this thesis (**Chapter X**), in which also an outline of the clinical relevance of these results is given.

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