SUMMARY

Asymmetric dimethylarginine (ADMA) is an inhibitor of nitric oxide synthase (NOS). ADMA is also a potent cardiovascular risk factor, with elevated ADMA levels associated with various cardiovascular diseases. Regulation of NOS activity is essential as nitric oxide (NO) is a signalling molecule involved in various metabolic pathways. In addition, diseased conditions affecting both the brain and lungs, which have high NOS expression, have been attributed to unregulated ADMA levels and, thus, NO levels, making regulation of both ADMA and NO a key interest for the development of targeted therapeutics. Dimethylarginine dimethylaminohydrolase 1 (DDAH1) is a crucial regulator of circulating ADMA levels. The enzymatic activity of DDAH1 towards ADMA has been well described and tested in various models. However, contrasting evidence has been published regarding the role of the sister isoform, DDAH2, in the regulation of ADMA levels. Since its discovery, DDAH2 has been thought to have a similar role as DDAH1 as it carries 50% homology to the sister isoform. However, other published studies have shown no correlation between DDAH2 and ADMA metabolism, preventing further progress in understanding the biochemical role of DDAH2. With the lack of extensive characterisation of the DDAHs expression, especially in the brain and lungs, there is a significant gap in the current knowledge on both DDAH1 and DDAH2 proteins.

The data presented in this thesis addresses two research aims: to clarify the controversial role of DDAH2 and to characterise the DDAH expression in the brain and lungs. In order to address the biochemical role of DDAH2 towards ADMA, various *DDAH* deficient cell and animal models were used in the study. Protein lysates from the cell and animal tissues were used in a DDAH activity assay, using stable isotope labelled ADMA as the reaction substrate. Additionally, various immunohistochemistry techniques were applied to validate DDAH1 and DDAH2 specific antibodies, which were later used in the DDAH proteins localisation study in the brain and lungs.

This work has shown that DDAH2 is inactive against ADMA metabolism. The use of labelled ADMA allowed the degradation to be traced through the production of labelled citrulline products, identifying DDAH1 as the sole isoform responsible for ADMA metabolism. Furthermore, the antibody validation test identified candidate antibodies that were used in the localisation studies of the specific DDAH isoforms in the brain and lung. DDAH1 and DDAH2 appeared to be distinctly localised within the brain, with no overlapping signal between the isoforms. DDAH1 expression was limited to the bronchial region but less so in the alveolar network of the lung.

In summary, the data presented in this thesis has not only resolved the long-standing controversy regarding the physiological role of DDAH2 but also provided novel insights into the distinct expression patterns of DDAH1 and DDAH2 in the brain and lung. These findings could provide the foundation for further studies on their biological functions and the development of targeted therapy against disorders or diseases, with the potential to impact the fields of biochemistry, pharmacology, and neuroscience.