Karolinska Institutet

**Department of Physiology and Pharmacology**

# NITRIC OXIDE FORMATION FROM INORGANIC NITRATE AND NITRITE

## Contribution from eukaryotic and prokaryotic pathways

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# ABSTRACT

Nitric oxide (NO) is an essential signaling molecule that plays a central role in a broad range of physiological functions. Classically, NO is synthesized from L-arginine and molecular oxygen by NO synthases. Once formed, it is rapidly oxidized to nitrite and nitrate. These two inorganic anions were previously considered to be inert end products but this view is now being seriously challenged by research revealing that nitrite can be physiological reduced to again generate NO. The reduction of nitrite *in vivo* seems particularly enhanced during hypoxia and acidosis; conditions when the oxygen-dependent NO-synthase pathway is dysfunctional. Besides the endogenous formation of nitrate and nitrite by NO synthase, these anions are also ingested naturally via the diet. The first step in bioactivation of nitrate is formation of the more reactive nitrite anion; a reaction suggested to involve oral nitrate reducing bacteria. It has been generally viewed that mammalian cells cannot metabolize the stable nitrate anion.

In the present thesis, we intended to further characterize NO generation from the nitrate-nitrite-NO pathway. In particular we have studied the importance of commensal bacteria in nitrate metabolism and attempted to explore if mammalian tissues are also capable of nitrate reduction. We also studied possible interactions between the classical NO synthase pathway and the nitrate-nitrite-NO pathway.

We show that bacteria in the gastrointestinal tract play an interesting role in mammalian NO biology. Besides the bioactivation of nitrate in the oral cavity to form nitrite, bacteria in the small and large intestine can catalyze the same reaction and also the subsequent reduction of nitrite to form NO. NO formation in the gut can be stimulated *in vivo* by supplementation with dietary nitrate and probiotic bacteria.

In further studies involving also germ-free mice, we surprisingly find that inorganic nitrate is enzymatically reduced to nitrite in tissues and we identify the enzyme xanthine oxidoreductase (XOR) as the dominant nitrate reductase. Mammalian nitrate reductase activity is enhanced during hypoxic conditions but is also active during normoxia. The functional consequences of this nitrate reductase activity were studied after nitrate administration *in vivo*. Nitrate attenuated the increased blood pressure caused by an NO synthase inhibitor and prevented the severe decline in blood flow during post-ischemic reperfusion.

The expression of XOR is enhanced in tissues of germ free mice, which may reflect a feedback response to the absence of bacterial nitrate reduction in these animals. Such crosstalk is further supported in a study of long-term dietary nitrate supplementation in rats, in which expression of phosphorylated eNOS in aortic tissue and eNOS activity was down-regulated after nitrate supplementation. All together these data suggest a crosstalk between NOS-independent and NOS-dependent pathways in control of NO vascular homeostasis.

In summary, the present thesis helps to draw a new picture of mammalian NO gene-ration which occurs by serial reductions of the supposedly inert anions nitrate and nitrite. In this pathway both eukaryotic and prokaryotic pathways contribute to formation of NO and maintenance of homeostasis. Intriguingly, NO formation from nitrate in the gastrointestinal tract, the cardiovascular system and elsewhere, can be controlled by simple dietary interventions with resulting physiological effects.