Nitrate transport in salivary glands with implications for NO homeostasis

Jon O. Lundberg[1]

Department of Physiology and Pharmacology, Karolinska Institutet, S-171 77 Stockholm, Sweden

In humans and other mammals, about one-quarter of all circulating inorganic nitrate (NO_3^-), derived from diet or oxidation of endogenous nitric oxide (NO), is actively taken up by the salivary glands and excreted in saliva (1, 2). As a result, salivary nitrate levels are 10–20 times higher than those levels found in plasma (3). The mechanism behind this massive nitrate accumulation in saliva has remained elusive. In PNAS, the work by Qin et al. (4) reports that the protein sialin can function as an effective nitrate transporter. These results are of considerable interest, especially in light of recent research suggesting a role for salivary nitrate transport and metabolism in physiological regulation of systemic NO homeostasis (5, 6).

Historically, the fact that nitrate and nitrite are present in human saliva has received little attention, because no one could attribute any kind of function to these anions. However, this lack of interest ceased in the 1970s, when researchers formulated a pathophysiological model for gastric cancer based on the accumulation of nitrate in saliva (7). Commensal bacteria in the mouth reduce parts of the salivary-derived nitrate to nitrite (NO_2^-), and when swallowed into the acidic stomach, this nitrite yields reactive intermediates that can react with dietary compounds to promote formation of *N*-nitrosamines (a versatile class of carcinogens in rodents) (8). With the emergence of this theory, nitrate immediately fell into deep disgrace, and ever since that time, authorities worldwide have put strict regulations on allowable levels of nitrate in our food and drinking water (9).

NO Generation from Inorganic Nitrate

In the 1990s, research on nitrate took an unexpected turn when two research groups independently showed that salivary nitrate was a substrate for the formation of NO (10, 11), a newly identified and highly potent biological messenger that was receiving enormous attention at the time. A few years earlier, researchers had shown that NO is generated endogenously in mammals from the amino acid L-arginine by specific enzymes (the NO synthases). It was now being revealed that NO was playing a key role in virtually every aspect of human physiology, including regulation of cardiovascular function, cellular energetics, immune function, neurotransmission, and more (12, 13). The newly described alternative means of NO generation from nitrate was fundamentally different from the NO synthase pathway; it did not use arginine as a substrate, and it was independent of NO synthases. After the discovery that nitrate could be a substrate for the formation of a potentially beneficial biological messenger, the interest in nitrate shifted away from only being focused on carcinogenesis, and instead, researchers started to study potential NO-like physiological effects of this anion. From intense research performed during the past 15 y, it is now clear that administration nitrate or nitrite has robust NO-like effects in humans and other mammals (5). These effects include vasodilation (14, 15), reduction in blood pressure (16), protection against experimental ischemia reperfusion injury (17), reduction in cellular oxygen consumption (18, 19), reversal of metabolic syndrome (20), reduction in oxidative stress (21), stimulation of mucosal blood flow and mucus formation in the gastrointestinal tract (22), and more. Intriguingly, most of these nitrate effects occur at dietary doses easily achievable through a normal diet rich in vegetables. Bioactivation of nitrate requires initial reduction to the more reactive nitrite anion, and this reaction is mainly carried out by commensal bacteria in the oral cavity (23) and to a lesser degree, the tissues by mammalian enzymes (24). Salivary-derived nitrite is partly reduced to NO in the acidic stomach as described above, but much nitrite also survives gastric passage and enters the systemic circulation, which is evident from the marked nitrite increase in plasma seen after ingestion of nitrate (3). In blood and tissues, nitrite can undergo additional metabolism to form NO and other bioactive nitrogen oxides, including S-nitrosothiols. A number of enzymes and proteins have been shown to act as nitrite reductases, including deoxygenated hemoglobin, myoglobin, xanthine oxidase, mitochondrial respiratory chain enzymes, and more (25).

The work by Qin et al. identifies sialin as a major transporter of inorganic nitrate in the salivary glands.

Sialin Is a Nitrate Transporter

After the identification of this entire alternative nitrate-dependent NO pathway with possible physiological and pathophysiological implications, it has become increasingly important to try to characterize the mechanism by which nitrate is transported in cells and how it is accumulated in saliva. The work by Qin et al. (4) takes a major step by identifying sialin as an effective nitrate transporter. The work by Qin et al. (4) shows that sialin functions as an electrogenic NO_3^-/H^+ transporter in the plasma membrane of salivary acinar cells. Nitrate transport is reduced by knockdown of sialin expression. Mutations in the sialin gene cause Salla disease and infantile sialic acid storage disorder, which are serious autosomal recessive lysosomal storage disorders characterized by early physical impairment and mental impairment. Fibroblasts from patients with infantile sialic acid storage disorder show a lower nitrate transport activity compared with healthy controls. The work by Qin et al. (4) also tests the importance of sialin for nitrate transport in the pig in vivo. Interestingly, adenovirus-dependent expression of a sialin mutant vector (sialinH183R) in the salivary gland decreases NO_3^- secretion in saliva after ingestion of a nitrate-rich diet compared with control animals.

Sialin is expressed not only in the salivary glands but also in the brain, heart, lung, kidney, and liver, although seemingly at lower levels. The functional importance of nitrate transport into cells in these tissues would be of interest to study. In this context, it is interesting to note that nitrate metabolism does, indeed, occur in mammalian cells, although to a much lesser degree than in bacteria. The work by Jansson et al. (24) reported on a functional mammalian nitrate reductase in

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[1]E-mail: jon.lundberg@ki.se.

numerous tissues, including liver, kidney, and intestines. Xanthine oxido reductase was identified as the major mammalian nitrate reductase, but the study indicated the presence of other unidentified nitrate reductases as well (24).

The work by Qin et al. (4) proposes that sialin functions as the major NO_3^- uptake system in salivary gland cells; however, a remaining question is how this nitrate is further transported to saliva through the apical portion of the cells. Sialin seems to be a versatile anion transporter that also mediates H^+-dependent transport of NO_2^-, aspartate, and glutamate. Previously, antagonism between nitrate, perchlorate, iodine, and thiocyanate for secretion in human saliva was shown (26), but in the work by Qin et al. (4), these anions are not studied. It will be of interest to study if sialin also transports these anions. Definitive evidence for a functional role of sialin in nitrate transport and systemic nitrite/NO homeostasis in humans is lacking, but with the identification of this protein as an important nitrate transporter, it now seems possible to study this area. One approach could be to study the nitrate–nitrite–NO pathway in genetically engineered mice or perhaps, patients with Salla disease. Are salivary and plasma levels of nitrate/nitrite different in these patients? Do these animals or the patients exhibit any signs of systemic NO deficiency, including increased blood pressure, altered blood flow responses, different cellular energetics, or others? In the case that NO homeostasis is disturbed in Salla disease, would the patients benefit from substitution with nitrite? By giving nitrite instead of nitrate, one could bypass the initial nitrate transport step that might be disturbed in these patients, and NO and other bioactive nitrogen oxides would form directly from nitrite in blood and tissues. Interestingly, this therapeutic approach was recently successfully tested in another genetic disorder involving a disturbed NO homeostasis (27). Another approach could be to study the proposed negative consequences of nitrate transport. If salivary nitrate transport promotes nitrosamine formation, which has been believed for a long time, are nitrosamine levels and occurrence of gastric malignancies lower in subjects lacking the transporter?

In summary, the work by Qin et al. (4) identifies sialin as a major transporter of inorganic nitrate in the salivary glands. Active transport of nitrate from blood to saliva represents the central first step in a recently described sequential event eventually leading to systemic formation of bioactive nitrogen oxides, including NO, and there are possible physiological regulatory roles in the cardiovascular system and elsewhere.

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