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Investigation of methaemoglobin reduction by extracellular NADH in mammalian erythrocytes

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Abstract

The effect of extracellular NADH on the rate of reduction of nitrite-induced methaemoglobin in erythrocytes from man, cattle, dog, horse, grey kangaroo, pig and sheep was investigated. Extracellular NADH was found to enhance the rate of methaemoglobin reduction in man, dog, pig and kangaroo erythrocytes, but had essentially no effect on the rate of methaemoglobin reduction in erythrocytes from cattle, horse and sheep. In erythrocytes of those animals affected by extracellular NADH the rate of reduction of metHb in the presence of NADH was the same or greater than that observed in the presence of nutrients such as glucose and inosine. The combination of nutrient and NADH produced a more profound increase in the rate of methaemoglobin reduction. The rate of methaemoglobin reduction in all cases was significantly less than that observed with methylene blue, the standard treatment of methaemoglobinaemia. Extracellular NADH was found to indirectly increase the intracellular NADH concentration through displacement of the pseudo-equilibrium of the intracellular LDH reaction and relied upon the presence of sufficient LDH activity released into the extracellular medium through haemolysis. The lack of response of cattle, horse and sheep RBCs to extracellular NADH was found to derive mainly from their low extracellular LDH activity, but also correlated with their lower NADH-methaemoglobin reductase activity compared to the other species.
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Keywords: Methaemoglobin; NADH; Mammalian erythrocytes; PMOR

Abbreviations: GAPDH, glyceraldehyde-3-phosphate dehydrogenase; Hb, haemoglobin; LDH, lactate dehydrogenase; metHb, methaemoglobin; NADH-MR, NADH-methaemoglobin reductase; PMOR, plasma membrane oxidoreductase; RBC, erythrocyte

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1. Introduction

Methaemoglobin (metHb) is a form of haemoglobin in which the haem iron is oxidised from the ferrous (Fe^{2+}) state to the ferric (Fe^{3+}) state, and the sixth coordination site of the haem iron is liganded to a water molecule. In human erythrocytes (RBCs) approximately 3% of total haemoglobin is cycled to the metHb state every day, mainly through slow reaction of haemoglobin with molecular oxygen (Jaffé, 1981); however, due to metHb reduction mechanisms the steady-state level of metHb is ∼1% (Giardina, Messana, Scatena, & Castagnola, 1995). In vivo, metHb is predominately reduced by the NADH-cytochrome b_5-metHb reductase system (NADH-MR), which requires the cofactors NADH and cytochrome b_5 (Jaffé, 1981). MetHb may also be reduced through minor pathways such as the NADPH-dependent metHb reductase and direct reduction by intracellular antioxidants such as ascorbate and glutathione (Jaffé, 1981).

Methaemoglobinaemia is the accumulation of metHb above steady-state levels. It can be induced by the ingestion of nitrates (i.e. in well water, some foods, and fertilisers), and many medications (Matteucci, Reed, & Tanen, 2003). Nitrates are converted by intestinal and oral bacterial flora to nitrites, which can lead to the formation of metHb (Wright, Lewander, & Woolf, 1999). The prevalence of nitrates in rural areas through the use of fertilisers, suggests that methaemoglobinaemia may also be a problem for domesticated farm animals such as cattle and sheep.

The standard treatment of methaemoglobinaemia in humans is through the intravenous delivery (1 mg/kg over 5 min) of methylene blue (Jaffé & Hultquist, 1995). Methylene blue rapidly reduces metHb by the NADPH-dependent metHb reductase pathway (Lukens, 1993), which requires a functioning oxidative pentose phosphate pathway to recycle NADPH. Hence, patients with glucose-6-phosphate dehydrogenase deficiency cannot be treated this way. Methylene blue can also cause complications in young children through its oxidative capacity (Wright et al., 1999), and can inhibit guanylate cyclase activity (Jin, Webb, & D'Alecy, 1995); hence alternatives to methylene blue with similar efficacy are desirable.

Ascorbate can reduce metHb directly; however, in vitro studies have shown that at least 10 mM ascorbate is required to accelerate metHb reduction, making ascorbate an impractical alternative (Dotsch et al., 1998). *N*-Acetylcysteine has been shown to reduce metHb in vitro in human RBCs and in vivo in cat RBCs (Wright, Magnani, Shannon, & Woolf, 1996), however it is ineffective in reducing metHb when supplied intravenously to humans (Tanen, LoVecchio, & Curry, 2000). The reducing agent sodium thiosulfate has also been postulated as a potential replacement for methylene blue; it however failed to reduce nitrite-induced methaemoglobinaemia in vitro (Matteucci et al., 2003).

As the main in vivo mechanism for reducing metHb under normal conditions is via the NADH-dependent NADH-MR system, enhancing the intracellular NADH concentration increases the rate of metHb reduction (Hsieh & Jaffé, 1975; Zerez, Lachant, & Tanaka, 1990). We have previously observed that incubating RBCs with extracellular NADH leads to an apparent increase in intracellular NADH concentrations (unpublished data). It was proposed that this increase was due to the transfer of reducing equivalents through a plasma membrane oxidoreductase (PMOR) from extracellular NADH allowing the formation of intracellular NADH.

The aim of this study was to investigate the effect of extracellular NADH on metHb reduction rates in human RBCs. Extracellular NADH was shown to be effective in reducing metHb and the mechanism of the enhanced reduction was elucidated. MetHb formation and reduction rates show considerable species variation (Smith & Beutler, 1966) and RBCs of some animals (horses and pigs) are insensitive to methylene blue and show limited metHb reduction in its presence (Smith & Beutler, 1966). This study also investigated whether extracellular NADH had a differential effect on metHb reduction for RBCs from animal species known to respond differently to methylene blue.

2. Materials and methods

2.1. Materials

[*Adenyl*-2,8-^{3}H]NAD^+ was obtained from Perkin-Elmer Life Sciences (Boston, MA, USA) and converted to [*adenyl*-2,8-^{3}H]NADH using the method of Ottolina, Riva, Carrea, Danieli, and Buckmann (1989).

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