

EFFECT OF FLUORIDE ON THE RESPIRATION RATE OF RAT CARDIAC MITOCHONDRIA

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SUMMARY: Fluoride by modifying the activity of mitochondrial enzymes may change their respiration rate. In this study NaF at concentrations 5, 10, and 50 μM had no effect on the State 3 respiration rate of rat cardiac mitochondria with succinate substrate. At a higher concentration of 7.5 mM, a significant ($p < 0.001$) decrease by as much as 120.4 ± 5.7 nmol [O]/min/mg protein occurred in the State 3 respiration rate with succinate. Further increase in NaF concentration (15 and 25 mM) resulted in a reproducible decrease in the oxidation rate of the succinate in dose-dependent manner.

Keywords: Cardiac mitochondria; Fluoride and mitochondria; Malate; Pyruvate; Respiration rate; Succinate.

INTRODUCTION

Despite its extensive clinical uses for the prevention of dental caries, ingestion of fluoride (F) not only affects teeth and bones but also other organs.¹ In the areas where water is fluoridated, elevated F concentrations accumulate in many soft tissues and organs, including the heart.¹ Experimental fluorosis in animals produces changes in electrocardiogram and severe pathological lesions in the myocardium.^{1,3}

Recent *in vitro* studies⁴ have shown that F can cause changes in the ultrastructure of hepatocytes, in submandibular gland cells, and in cells of the pancreas with the most pronounced changes occurring in mitochondrial cells.

In studies with sodium fluoride (NaF), mitochondrial oxidative phosphorylation was inhibited.⁵ Further studies showed that NaF reduces the activity of mitochondrial pyruvate dehydrogenase in the presence of ATP and oligomycin in cardiac mitochondria⁶ and also impairs fatty acid oxidation in liver mitochondria.⁷ The activity of the succinate dehydrogenase in liver and renal mitochondria also appear to be inhibited by F.^{8,9}

Since there are only a few reports on the effect of F mitochondria, our aim was to determine the possible effect of NaF on the respiration rate of cardiac mitochondria in oxidizing succinate or pyruvate and malate.

MATERIALS AND METHODS

Isolation of mitochondria: Mitochondria cells were isolated from male Wistar rat hearts by a differential centrifugation procedure. After decapitation of the rats, their hearts were excised and rinsed in ice-cold isolation medium, containing 220 mM mannitol, 70 mM sucrose, 5 mM TES, and 0.5 mM EGTA (pH 7.4, adjusted with Trizma base at 2°C). Mitochondria were isolated in the same medium

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supplemented with 2 mg bovine serum albumin/mL (BSA; fraction V, A4503, Sigma). The homogenate was centrifuged at 750 g for 5 min, then the supernatant was recentrifuged at 6740 g for 10 min, and the pellet was washed once in the isolation medium without BSA, suspended in it and kept on ice.

Assays: The mitochondrial protein concentration was determined by the biuret method.¹⁰ The final mitochondrial protein concentration in all experiments was 0.5 mg/mL. For the substrate, 10 mM of succinate (+5 μ M rotenone) was used. Mitochondrial oxygen consumption was recorded by means of the Clark-type electrode system in KCl medium (120 mM KCl, 5 mM KH_2PO_4 , 5 mM TES, and 1 mM MgCl_2 ; pH 7.4, adjusted with Trizma base at 37°C). The solubility of oxygen was taken to be 422 nmol [O]/mL at 37°C or 452 nmol [O]/mL at 25°C.¹¹ Respiration rates were expressed as nmol [O] atoms/min/mg of mitochondrial protein.

Statistical analysis of experimental data: Results are expressed as means \pm S.E. of 3 to 5 independent experiments. An analysis of variance (ANOVA) followed by Dunnet test was used for comparisons between experimental groups. A value of $p < 0.05$ was considered statistically significant.

Reagents: All chemicals were obtained from Sigma-Aldrich (St Louis, MO, USA).

RESULTS AND DISCUSSION

Since mitochondria are the main sites of cellular energy supply, modulation of their functional activity may be very important for preserving cell viability under normal conditions and during metabolic stress.¹² By modifying the activity of mitochondrial enzymes may change their respiration rate.

At the beginning of the experiment, mitochondrial State 2 respiration rate (V_0) was determined to be 71.4 ± 3.6 nmol [O] /min/mg protein. When ADP (at final concentration of 1 mM) was added to the medium, State 3 respiration rate (V_{ADP}) increased to 147.8 ± 5.9 nmol [O]/min/mg protein (Figure 1).

Our data showed that NaF at concentrations 5, 10, and 50 μ M had no effect on the State 3 respiration rate of mitochondria with succinate and other tested substrates (pyruvate + malate) (Fig. 1). Plasma F concentrations in humans with 0.6–0.8 ppm F in their drinking water and who use fluoridated dentifrices range from 0.44 to 0.54 $\mu\text{mol/L}$ ¹³ may rise as high as 10–25 μM after oral use or intake of NaF.¹⁴ According to our results, such low concentrations which may occur in plasma during F exposure will not probably have any impact on the respiration rate of mitochondria. However, fluoridation of drinking water with oral intake of F may lead to accumulation of elevated F concentrations in soft tissues. Thus the effect of the high F concentrations used in our experiments could conceivably be important for incurring acute toxic effects of F.

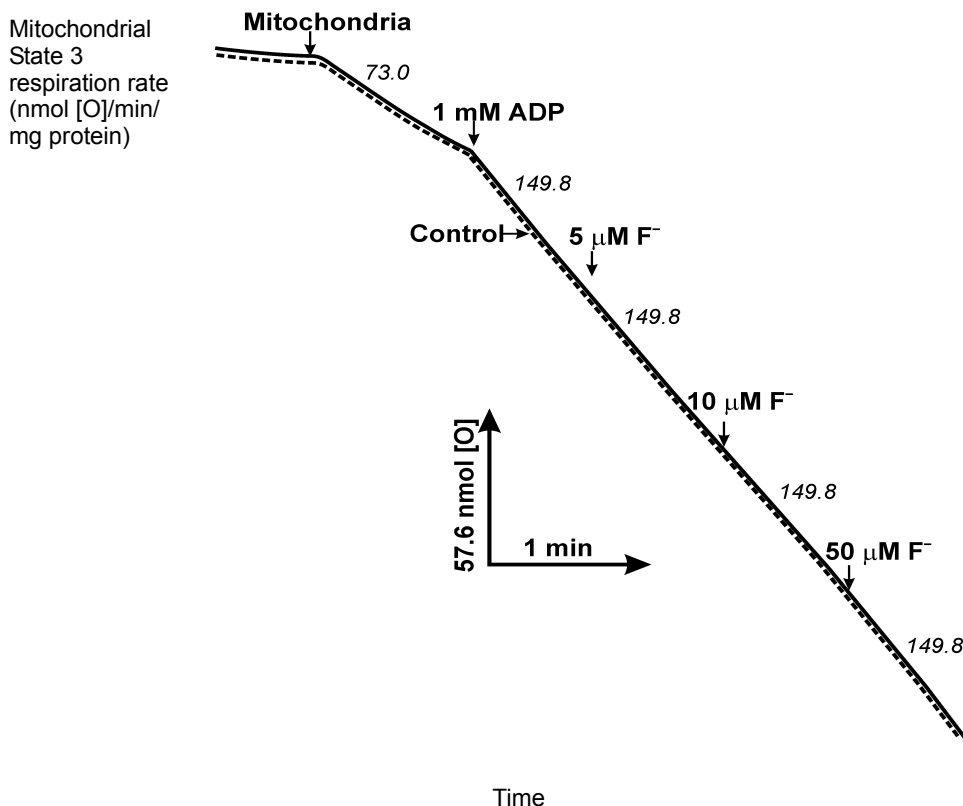


Figure 1. Influence of low concentrations of F ions on the mitochondrial State 3 respiration rate. Dashed line represents control, solid line: with F ions. Mitochondrial respiration rates, expressed as nmol [O] atoms per min per mg of mitochondrial protein, are shown in italics. Experiments were performed in KCl medium at 37°C, substrate: 10 mM of succinate (+5 μM rotenone) + 1 mM ADP; n=5.

In our study we therefore also tested the effect of 7.5, 15, and 25 mM NaF on the respiration rate of mitochondria for oxidizing succinate. Our results showed that at a concentration of 7.5 mM, NaF significantly ($p < 0.001$) decreased the State 3 respiration rate with succinate substrate to as much as 120.4 ± 5.7 nmol [O]/min/mg protein (Figure 2). Further increase in NaF concentration to 15 and 25 mM resulted in a reproducible decrease of oxidation rate of succinate in dose-dependent manner. Interestingly, NaF had no effect on the oxidation rate of other tested respiratory substrates such as pyruvate + malate (data not shown).

Owing to its affinity for magnesium and calcium ions, F ion can affect the activity of many enzymes, mainly oxy-reductases, transferases, hydrolases, enzymes of Krebs cycle, and ATP production.⁴ According to data of previous studies, F has been shown to inhibit succinate dehydrogenase isolated from pig renal cortex.⁹ Our results showing a decrease of succinate oxidation rate at elevated concentrations of F might be explained by this mechanism. Our data also

support results of our previous study showing that NaF at concentrations of 7.5, 15, and 25 mM can inhibit neutrophil function with a negative effect on their protective performance.¹⁵

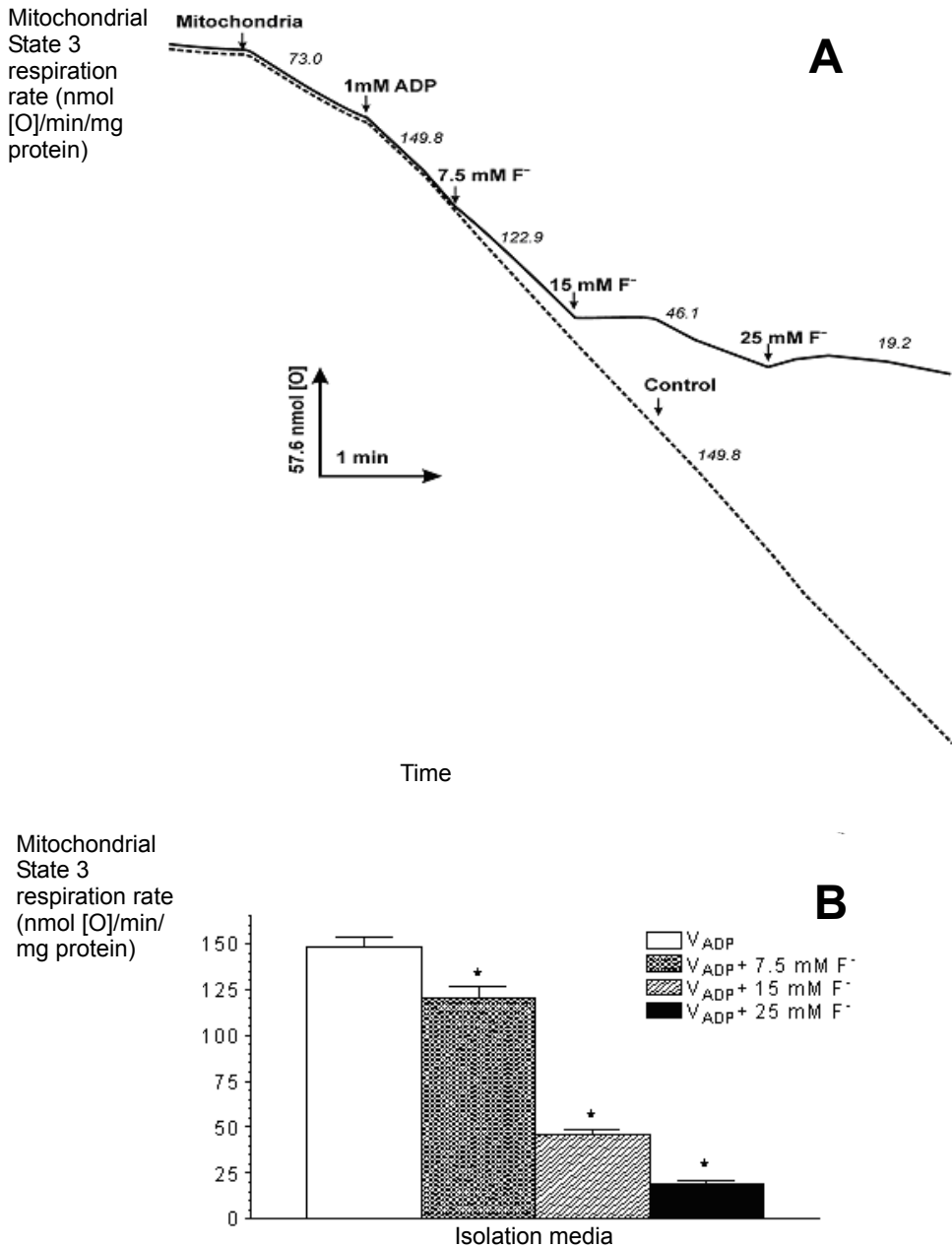


Figure 2. Effect of high concentrations of F ions on the mitochondrial State 3 respiration rate. (A) typical respiratory curves, dashed line represents control; (B) calculated data. Mitochondrial respiration rates are expressed as nmol [O] atoms per min per mg of mitochondrial protein. Experiments were performed in KCl medium at 37°C, substrate: 10 mM of succinate (+5 μM rotenone) + 1 mM ADP; n=5. *p<0.001, statistically significant effect of F ions compared to control. The results were analyzed with one-way analysis of variance (ANOVA) followed by Dunnet post test.

In summary, NaF at concentrations of 5, 10, and 50 μM had no effect on the State 3 respiration rate of mitochondria oxidizing succinate or pyruvate + malate. However, NaF concentrations of 7.5, 15, and 25 mM resulted in a reproducible decrease in the oxidation rate of the succinate in dose-dependent manner. Since topical application of F-containing dental products can lead to accumulation of F in the oral cavity, in some cases at fairly high concentrations,¹⁶ such procedures run the risk of possibly inhibiting mitochondrial function in cells of the oral cavity and may even be cardiotoxic as well.

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