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Sodium Nitrite Alone Protects the Brain Microsomal Ca^{2+} -ATPase Against Potassium Cyanide-induced Neurotoxicity In Rats

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The effect of a short-term oral administration of potassium cyanide (KCN) (200 ppm in diet) with or without sodium nitrite (NaNO_2) pretreatment on rat brain microsomal Ca^{2+} -ATPase was investigated. The specific activity value of the enzyme significantly decreased ($p < 0.05$) by 50% compared with control and by 63% for KCN-treated rats compared with KCN-treated rats pretreated with NaNO_2 . There was no significant difference at the $p = 0.05$ level between the values obtained for the control and KCN-treated rats pretreated with NaNO_2 . These results show both that feeding lowers brain microsomal Ca^{2+} -ATPase activity and that NaNO_2 has a protective role (antidote function) in that respect.

KEY WORDS: Brain; microsomes; Ca^{2+} -ATPase; potassium cyanide; sodium nitrite.

INTRODUCTION

Many peasant communities in the developing countries fed on agricultural produce (plant and animal) that contain toxic substances even after processing. Among the staples in Nigerian diet for instance are cassava (*Manihata*) and bitter-leaf (*Amygdaline vernonia*). Cassava contains a cyanogenic glycoside-Linamarin, while bitter-leaf contains another cyanogenic glycoside-amygdaline.^{1,2} The consumption of cassava as the major source of carbohydrate has been associated with the cyanide-induced neurological disorder known as Tropical Ataxic Neuropathy.²

Toxin-induced cell death resulting from pathological increase in intracellular free calcium ion concentration is known to be caused by a number of mechanisms including direct interference with Ca^{2+} -transporter.⁵ The calcium transporter that plays a central role in the fine regulation of calcium ion concentration is the

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endoplasmic/sarcoplasmic reticulum Ca^{2+} -ATPase otherwise known as the microsomal Ca^{2+} -ATPase.³ It has been suggested that the Ca^{2+} -ATPase could be used as a target for toxins and drugs.^{4,5} In an earlier work,⁶ we observed that cyanide, specifically potassium cyanide (KCN), depresses the activity of the brain microsomal Ca^{2+} -ATPase in rats.

Sodium nitrite (NaNO_2) in combination with sodium thiosulphate is a widely used antidote for cyanide poisoning.⁷ Amazingly, even sodium nitrite itself is a toxicant.⁸ In this paper, we report that sodium nitrite alone protects the brain microsomal Ca^{2+} -ATPase against KCN-induced neurotoxicity.

MATERIALS AND METHODS

All chemicals used for these investigations were the purest quality available. Fifteen (15) male Wistar rats obtained from the animal house of the Faculty of Basic Medical Sciences, Ogun State University, Ago-Iwoye, Nigeria, were used for this experiment. All animals were fed on rat pellets (obtained from Pfizer Products Plc., Lagos) and maintained under laboratory conditions for a week before the experiment commenced. They were allowed feed and water *ad libitum* before and during the experiments.

The rats were divided into three groups based on the treatment given: group A (control), group B (KCN-treated) and group C (KCN/ NaNO_2 -treated), of five (5) animals each. Group A received no treatment and thus served as control. Animals in group B were given diet containing KCN (200 ppm). Group C animals were pretreated with water containing NaNO_2 (200 ppm) for seven (7) days and later received diet containing KCN (200 ppm). The allowable level of nitrite used for preservation of foods in the developing countries is around 200 ppm.¹¹ The three groups were fasted overnight and allowed their various diets and water for two days.

The rats were sacrificed at the end of the feeding period and their brains quickly removed and kept at 4°C in ice-cold buffer. Homogenates of the rat brains were prepared using potter-Elvehjem homogenizer. The post mitochondrial supernatant (PMS) was obtained as earlier described by Famulski and Carafoli (1982)⁹ except that centrifugation at $100,000 \times g$ for 1 hour was not necessary. The PMS was used for enzyme assay. Brain microsomal Ca^{2+} -ATPase activity was assayed as described by Adenuga *et al.* (1992).⁴

RESULTS

The brain microsomal Ca^{2+} -ATPase activities of rats after the administration of 200 ppm KCN in diet with and without nitrite pretreatment are shown in Tables 1–3. No significant difference was observed in the mean body weights of the animals in each of the three groups before and after treatments. The brain microsomal Ca^{2+} -ATPase activities of KCN-treated rats decreased significantly ($p < 0.05$) to about 50% that of the control group (Table 1). Also, significantly decreased Ca^{2+} -ATPase activities were observed for KCN-treated rats ($p < 0.05$)

Table 1. Effect of KCN on rat brain microsomal Ca²⁺-ATPase activity

Group	Body Weight (g)	Ca ²⁺ -ATPase activities (umoles Pi/mg Protein/hr.)
Control	160.3 ± 1.28	2.07 ± 0.21
KCN-treated	165.4 ± 1.43	1.03 ± 0.18*

* Significantly different from control at $p < 0.05$.

Table 2. Combined effect of KCN and NaNO₂ on rat brain microsomal Ca²⁺-ATPase

Group	Body weight (g)	Ca ²⁺ -ATPase activities (μmoles Pi/mg Protein/hr.)
Control	160.3 ± 1.28	2.07 ± 0.21
KCN/NaNO ₂ -treated	163.2 ± 1.41	1.63 ± 0.24

* Significantly different from control at $P < 0.05$.

Table 3. Effect of NaNO₂ pretreatment on KCN-induced inhibition of rat brain microsomal Ca²⁺-ATPase activity

Group	Body weight (g)	Ca ²⁺ -ATPase activities (μmoles Pi/mg Protein/hr.)
KCN-treated	165.4 ± 1.43	1.03 ± 0.18
KCN/NaNO ₂ -treated	163.2 ± 1.41	1.63 ± 0.24*

* Significantly different at $P < 0.05$.

in contrast to KCN-treated rats pretreated with NaNO₂ (Table 3). However, no significant difference was observed between the Ca²⁺-ATPase activities of KCN-treated animals pretreated with NaNO₂ and that of the control group ($p > 0.05$) (Table 2).

DISCUSSION

Cyanide is known to inhibit cytochrome oxidase at the active site and this blocks the terminal event in electron transport. This single site of action is responsible for the rapid and often fatal toxic effects of cyanide. As a result of cyanide inhibition, oxidative phosphorylation is compromised and there is an increased demand on glycolysis.¹⁰ This results in ATP depletion and the effects include: interference with membrane integrity, ion pumps, and protein synthesis, loss of cell function and perhaps cell death. Our earlier work⁶ showed that

cyanide disrupts Ca^{2+} homeostasis by inhibiting the microsomal Ca^{2+} -ATPase activity. However, it is yet to be investigated if this inhibition is by a direct interaction with the enzyme molecule or as a result of ATP depletion.

Sodium nitrite in combination with sodium thiosulphate is generally used as an antidote against cyanide poisoning because of its ability to mediate the oxidation of haemoglobin *in vivo* to form methaemoglobin. This haemoglobin variant is ineffective in carrying oxygen to tissues, but has a high affinity for cyanide and removes it from ferricytochrome oxidase enzyme that has been inhibited by binding of cyanide. Additional treatment with thiosulphate results in elimination of the cyanide.⁷

Interestingly, nitrite is known to inhibit platelet aggregation by virtue of its conversion to nitric oxide.¹⁰ Furthermore, it is believed that nitrites interact with transport proteins thereby interfering with normal receptor-ligand interactions.¹⁰ It however appears that nitrite reverses the effect of cyanide on the microsomal Ca^{2+} -ATPase by its ability to free ferricytochrome oxidase enzyme from cyanide inhibition. Perhaps as a result, adequate energy is generated and the pump resumes its normal function. It is possible though that the net positive effect of nitrite in the system overwhelms its seemingly toxic tendencies. It is not yet clear whether nitrite could ameliorate cyanide poisoning by influencing the enzyme-cyanide interaction, if at all there is any.

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