Biochimica et Biophysica Acta, 679 (1982) 343–346
Elsevier Biomedical Press

BBA Report

BBA 40000

RELEASE OF MITOCHONDRIAL RESPIRATORY CONTROL BY CYANATE SALTS

WENDY CAMMER

The Saul R. Korey Department of Neurology and the Department of Neuroscience, Albert Einstein College of Medicine, 1300 Morris Park Avenue, F-140, Bronx, NY 10461 (USA)

(Received August 19th, 1981)

Key words: Cyanate; Oxidative phosphorylation; Uncoupler; Demyelination; Respiration; (Mitochondria)

Mitochondrial respiration is stimulated by 5–40 mM potassium cyanate in the presence or absence of oligomycin. When the cyanate concentration is increased over 40 mM, the mitochondria respire at progressively lower rates. In these respects, although at relatively high concentrations, cyanate behaves as an uncoupler of oxidative phosphorylation.

When administered to members of susceptible animal species, compounds belonging to a class of neurotoxins induce the formation of vacuoles in the myelin sheath. The vacuoles contain fluid and salts and can, frequently, be observed, at early stages of intoxication, in the absence of any apparent injury to axons or neuronal or glial cell bodies [1-7]. A common biochemical property of several of these toxins is the ability to release mitochondrial respiratory control in vitro; i.e., they act as 'uncouplers' of oxidative phosphorylation [8–12]. Evidence has also been presented that at least two of these toxins exert direct destructive effects on myelin [7,13], and that uncoupling agents increase the permeability of mitochondrial membranes and other membranes to protons [14] and/or cations [15]. The latter is still controversial [14]. In view of such evidence, it has been proposed that primary demyelination, characterized by vacuole formation, is initiated when a toxin provokes the influx of ions through the myelin membranes [11,12], in a manner similar to its effects on the permeability of mitochondrial membranes. We began to inquire about the effects of cyanate on mitochondrial respiration after sodium cyanate, which had undergone clinical trials in the treatment of sickle cell disease, was found to produce vacuolation of myelin in vivo [16–18].

Liver mitochondria and a crude fraction of brain mitochondria (no density gradient centrifugation step) were prepared from the tissues of Sprague-Dawley rats [19]. Oligomycin, adenosine-5′-diphosphate (ADP), Trizma base, potassium thiocyanate and substrates were purchased from the Sigma Chemical Co. (St. Louis, MO), and the substrates were titrated to pH 7.4 before use. Potassium cyanate was obtained from Fisher (Fair Lawn, NJ), and a sample of recrystallized sodium cyanate, from Dr. R. Nagel. Cyanuric acid was purchased from Eastman (Rochester, NY). All other reagents were reagent grade. Respiration measurements were performed in the Gilson oxygen polarograph [20], and protein concentrations were determined by the method of Lowry et al. [21].

During utilization of the NAD-linked substrates, glutamate and malate, by liver mitochondria, potassium cyanate stimulated the rate of respiration in a concentration-dependent manner (Fig. 1a). The similar effect of adding recrystallized sodium cyanate suggested that the cyanate ion, rather than an impurity, was capable of stimulating mitochondrial respiration (Fig. 1a). Similarly, cyanate released mitochondrial

respiratory control when succinate served as substrate (Fig. 1b). Like a typical uncoupling agent, cyanate, at an appropriate concentration (40 mM), stimulated respiration to the state 3 rate (ADP, no cyanate), and the rate of respiration dropped below the ADP-stimulated rate at higher cyanate concentrations (Fig. 1b).

An additional parameter in which cyanate resembled classical uncoupling agents was its ability to stimulate respiration in the presence of oligomycin (Fig. 2, trace A). It has been reported that, after the addition of two somewhat atypical uncoupling agents, triethyl-tin and acetylethyltetramethyltetralin, to mitochondria, the increase in the respiration rate occurs gradually [12]. Trace A also shows that, unlike those two compounds, and like most uncoupling agents, cyanate stimulated mitochonsrial respiration immediately. Potassium cyanate also released the respiratory control of brain mitochondria (Fig. 2, trace B).

Most uncoupling agents are weakly acidic [14]; thus, a mechanism of uncoupling depicts the anion picking up a proton on one side of the membrane and carrying it to the other side of the membrane, where the proton is discharged [14]. Cyanic acid is moderately acidic, with a pK of 3.2 [22]. The relatively polar structure, compared to the more lipophilic nature of most uncoupling agents, and the slightly lower pK of cyanic acid, probably account for the high concentrations of cyanate required to stimulate respiration. A potential contaminant,

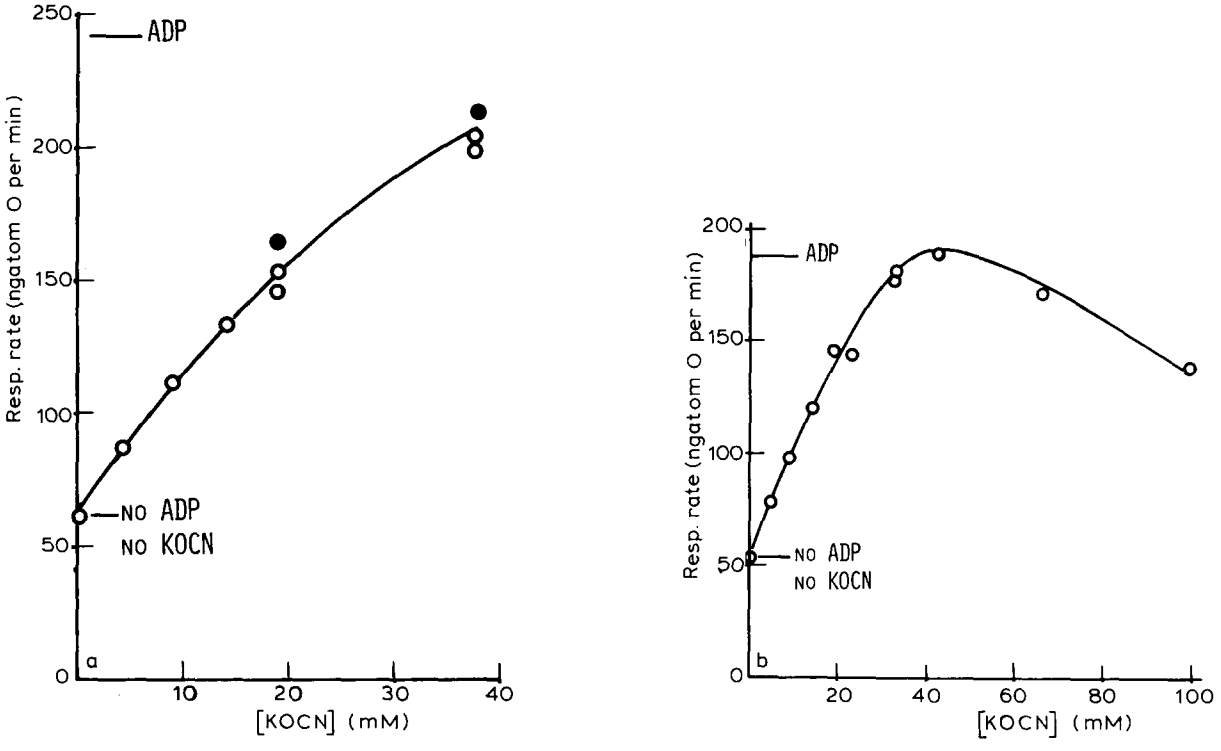

Fig. 1. a. The effect of potassium cyanate on the respiration of rat liver mitochondria utilizing glutamate plus malate as substrates. The respiration medium comprised 0.25 M sucrose/19 mM Tris-HCl (pH 7.4)/15 mM KCl/10 mM NaCl/5 mM $MgCl_2$/and 15 mM sodium phosphate buffer, pH 7.5. Mitochondria (4.1 mg protein) and substrates (6.3 mM glutamate plus 6.3 mM malate) were added to 1.6 ml of respiration medium in the oxygen electrode chamber. After the rate of respiration had been measured for 1–2 min, either 0.120 mM ADP or a freshly prepared solution of potassium cyanate, to obtain one the the final concentrations shown (open circles), was added, and the measurement of respiration was continued. Closed circles, sodium cyanate b. The effect of potassium cyanate on the respiration of rat liver mitochondria utilizing succinate as substrate. The experimental conditions were similar to those described for (a) except that the substrate was 6.3 mM succinate. The addition of potassium cyanate, at the highest concentration shown, did not change the pH of the reaction mixture.

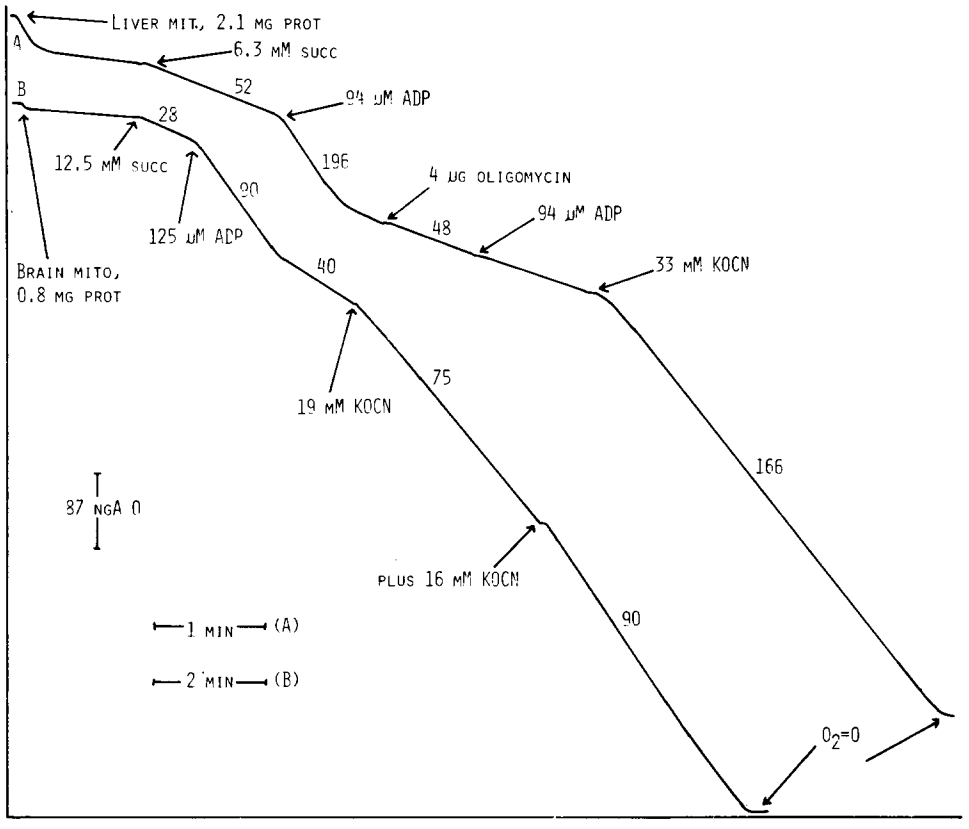

Fig. 2. Stimulation of mitochondrial respiration by cyanate in the presence of oligomycin, and the effect of cyanate on the respiration of brain mitochondria. Substrates and cyanate were added, as shown, to liver or brain mitochondria suspended in the respiration medium described in the legend to Fig. 1a, and the rates of respiration were recorded as shown. The numbers above the traces are respiration rates in ngatom (ngA) O per min. Mito, mitochondria; SUCC, succinate; PROT, protein.

cyanuric acid, at concentrations up to 39 mM, neither stimulated respiration in the absence of ADP nor inhibited oxygen uptake in the presence of ADP (data not shown). Furthermore, potassium thiocyanate, at concentrations up to 19 mM, did not mimic the effects of cyanate. This is not surprising, since thiocyanic acid is strongly dissociated [22]. An appropriate conclusion is that cyanate ion and cyanic acid, at a total concentration of 10–40 mM, and not a contaminant, can uncouple oxidative phosphorylation. It is not suggested that the uncoupling of oxidative phosphorylation necessarily contributes to the pathogenesis of cyanate neuropathy, but, rather, that cyanate may be capable of altering the proton and/or cation permeability of myelin membranes as well as releasing mitochondrial respiratory control.

This work was supported by USPHS Grants NS12890 and NS03356 and by National Multiple Sclerosis Society Grant 1089.

References

1 Aleu, F.P., Katzman, R. and Terry, R.D. (1963) J. Neuropath. Exp. Neurol. 22, 403–413
2 Kimbrough, R.D. and Gaines, T.B. (1971) Arch. Env. Health 23, 114–118
3 Towfighi, J. (1980) in Experimental and Clinical Neurotoxicology (Spencer, P.S. and Schaumburg, H.H., eds.), pp. 440–455, Williams and Wilkins, Baltimore, MD
4 Spencer, P.S., Sterman, A.B., Haroupian, D.S. and Foulds, M.M. (1979) Science 204, 633–635
5 Kurtz, S.M., Schardein, J.L., Fitzgerald, J.E. and Kaump, D.H. (1969) Toxic. Appl. Pharmacol. 14, 652
6 Lock, E.A. (1977) Trans. Int. Soc. Neurochem. 6, 401

7 Jacobs, J.M., Cremer, J.E. and Cavanagh, J.B. (1977) Neuropath. Appl. Neurobiol. 3, 169–181
8 Aldridge, W.N. and Street, B.W. (1964) Biochem. J. 91, 287–297
9 Cammer, W. and Moore, C.L. (1972) Biochem. Biophys. Res. Commun. 46, 1887–1894
10 Wilson, D.F. (1969) Biochemistry 8, 2475–2481
11 Cammer, W. (1980) in Experimental and Clinical Neurotoxicology (Spencer, P.S. and Schaumburg, H.H., eds.) pp. 239–256, Williams and Wilkins, Baltimore, MD
12 Cammer, W. (1980) Biochem. Pharm. 29, 1531–1535
13 Sterman, A.B. and Spencer, P.S. (1981) J. Neuropath. Exp. Neurol. 40, 112–122
14 McLaughlin, S.G.A. and Dilger, J.P. (1980) Physiolog. Rev. 60, 825–863
15 Green, D.E. and VandeZande, H. (1981) Biochem. Biophys. Res. Commun. 100, 1017–1024
16 Peterson, C.M., Tsairis, P., Ohnishi, A., Lee, Y.S., Grady, R., Cerami, A. and Dyck, P.S. (1974) Ann. Int. Med. 81, 152–158
17 Tellez-Nagel, I., Korthals, J.K., Vlassara, H.V. and Cerami, A (1977) J. Neuropath. Exp. Neurol. 36, 351–363
18 Tellez, I., Johnson, D., Nagel, R.A. and Cerami, A. (1979) Acta Neuropathol. (Berl.) 47, 75–79
19 Johnson, D. and Lardy, H. (1967) Methods Enzymol. 10, 94–96
20 Estabrook, R.W. (1967) Methods Enzymol. 10, 41–47
21 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275
22 Windholz, M., Budavari, S., Strountsos, L.Y. and Fertig, M.N. (1976) The Merck Index, 9th edn., pp. 350 and 1204, Merck, Rahway, NJ