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LETHALITY OF CYANIDE IN THE ABSENCE OF INHIBITION OF LIVER CYTOCHROME OXIDASE

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The predominant toxicologic effect of cyanide has been attributed to the inhibition of the terminal respiratory enzyme, cytochrome oxidase both *in vitro* and *in vivo* [1-4]. Moreover, the degree of cyanide intoxication and the efficacy of various cyanide antagonists have been evaluated by assessing the enzymatic activity of cytochrome oxidase [4-6]. The report herein indicates that cyanide lethality in the presence of the classic cyanide antidotes can occur with no apparent inhibition of liver cytochrome oxidase activity.

The inhibition of liver and brain cytochrome oxidase was compared after administration of lethal doses of cyanide in animals receiving no prior treatment and animals pretreated with the classic cyanide antidotal combination of sodium nitrite and sodium thiosulfate [7].

Swiss-Webster male mice (18-21 g) were divided into three groups of four or more animals, and the following treatments were administered. Control animals were treated with saline (i.p.), group two received KCN (10 mg/kg, i.p.) and group three was treated with sodium nitrite (100 mg/kg, s.c.) and sodium thiosulfate (1 g/kg, i.p.) 45 and 15 min, respectively, before KCN (70 mg/kg, i.p.). Three min after KCN, the animals were sacrificed and the brains and livers were rapidly removed and homogenized in Tris buffer (pH 7.0; 30 mM). The 0.2% (w/v) crude homogenates of both tissues were used to assay for cytochrome oxidase activity. Enzymatic activity was determined by the spectrophotometric method of Cooperstein and Lazarow [8] with minor modifications. The procedure entails the addition of an aliquot of a tissue homogenate to a standardized reduced cytochrome C solution and observing the change in optical density over a 5 min period at 550 nm. Cytochrome oxidase activity was expressed as the first-order rate constant [9], based on milligrams of protein. In order to compare the cytochrome oxidase activity of each treatment, the enzymatic activity was expressed as per cent inhibition of control value.

Since the possibility existed that rhodanese may metabolize cyanide in the tissue homogenates, resulting in the reactivation of cyanide-inhibited cytochrome oxidase, 10 mM sodium sulfite, a rhodanese inhibitor [10], was added to the homogenates. The results

obtained with and without sodium sulfite were quite similar; therefore, the role of cell membrane disruption and subsequent release of rhodanese into the incubation mixture to re-activate cyanide-inhibited cytochrome oxidase was greatly minimized.

In animals receiving no antidotes, a lethal dose of potassium cyanide (10 mg/kg) greatly depressed the enzymatic activity of cytochrome oxidase from liver and brain tissues (Fig. 1). The cytochrome oxidase activity from liver was not inhibited to a much greater degree than it was from brain. When animals were pretreated with the classic antidotal combination of sodium

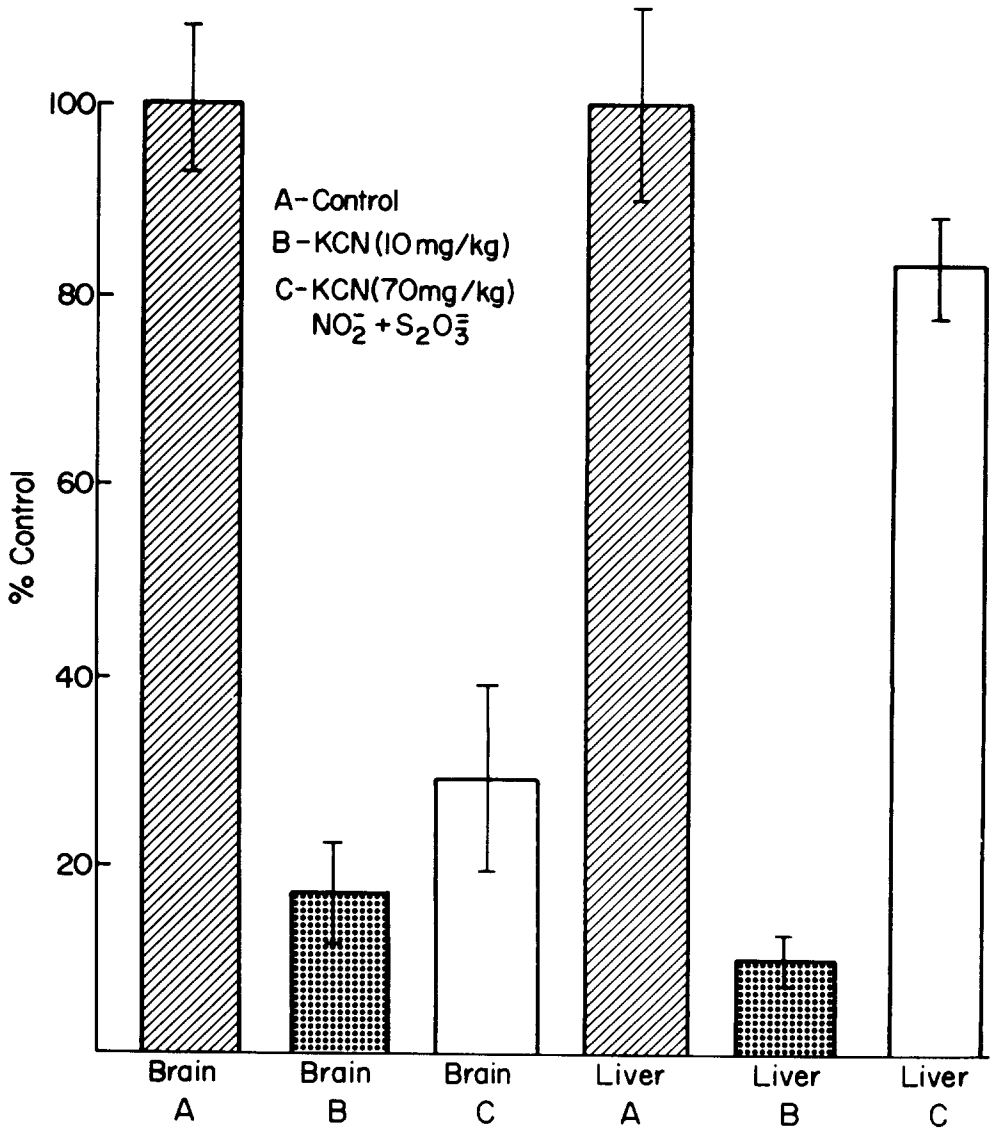

Fig. 1. Liver and brain cytochrome oxidase activity determined in the presence of 10 mM sodium sulfite and measured 3 min after the administration of lethal doses of cyanide. A - control (administered only saline); B - KCN (10 mg/kg); C - KCN (70 mg/kg) administered to mice pretreated with sodium nitrite (100 mg/kg) and sodium thiosulfate (1 g/kg). Each value represents the average ± S.E.M. from four or more animals.

thiosulfate and sodium nitrite [7], a lethal dose of potassium cyanide (70 mg/kg) inhibited brain cytochrome oxidase activity to approximately the same extent as the unantagonized animals. However, it was quite surprising to observe that the liver cytochrome oxidase activity of animals pretreated with sodium nitrite and sodium thiosulfate was not appreciably affected, in spite of the fact that these animals were dead. Whereas the liver enzymatic activity in the unantagonized animals was the most greatly inhibited, no appreciable enzymic inhibition was noted in liver cytochrome oxidase activity in the antagonized animals.

The inability of a lethal dose of cyanide to inhibit liver cytochrome oxidase activity to any appreciable extent in antagonized animals may be attributed to various factors. First, the disposition of cyanide to sites of liver cytochrome oxidase localization may be limited due to the high content and turnover number in the liver of rhodanese, the enzyme which detoxifies cyanide to thiocyanate [10]. Second, in the case where cyanide has inhibited liver cytochrome oxidase, this cyanide-cytochrome oxidase complex is, in the presence of rhodanese and sulfur donors, rapidly reactivated due to cyanide metabolism. Third, the physiological disposition of the active form of the cyanide antidotes, sodium thiosulfate and nitrite-generated methemoglobin, has a more limited distribution to brain than liver. This would result in a higher sensitivity of brain tissues to cyanide.

In a similar experimental design, the blood cyanide level in antagonized animals was considerably higher than in unantagonized animals.

The present studies demonstrate that, in animals pretreated with the classic cyanide antidotal combination, a lethal dose of cyanide does not inhibit liver cytochrome oxidase. In view of these studies, reports that reactivation of liver cytochrome oxidase takes place rapidly after sodium thiosulfate treatment and the use of liver cytochrome oxidase activity as a measurement of the degree of cyanide intoxication may not be warranted. On the other hand, brain cytochrome oxidase is maximally inhibited in antagonized and unantagonized animals which is in agreement with Estler [5,6]. These results suggest tissue distribution of cyanide, thiosulfate or rhodanese may play an important role in determining the degree of intoxication and the efficacy of antidotal treatment.

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