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INHIBITION OF LIVER AMYLASE SYNTHESIS BY CYANIDE

PROTECTIVE EFFECTS OF METHEMOGLOBIN AND THIOSULFATE*

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Abstract—It has previously been demonstrated, in experiments using the isolated perfused rat liver, that amylase is synthesized by the liver and then secreted into the plasma of the perfusing blood. Under normal conditions, production of amylase is about 30–40 units per g of liver per 4-hr perfusion period. Addition of 1–2 mg potassium cyanide (2–4 × 10^{-4}M final concentration) to the perfusing blood had relatively little effect on amylase production. Addition of 5 mg KCN (10^{-3} M) reduced amylase production to 11·5 units/g of liver and addition of 10 mg resulted in a net loss of amylase in the 4-hr perfusion. Since 2 mg KCN is enough to kill a rat of the size from which these livers were taken, the amounts of cyanide necessary to inhibit amylase production are surprisingly high. It is postulated that the high rhodanese concentration in liver serves as a protective mechanism against the cyanide toxicity. Production of methemoglobin or the presence of thiosulfate in the perfusing blood protected against the inhibiting action of cyanide on amylase production.

EXPERIMENTS in this[1, 2] and other[3, 4] laboratories have shown that amylase is synthesized by the isolated, perfused rat liver and secreted into the plasma of the perfusing blood. This synthesis may be inhibited in varying degrees by anoxia or previous damage to the liver,[2] puromycin[4, 5] and dinitrophenol.[6]

The experiments described in this paper illustrate the inhibitory effect of cyanide on liver amylase synthesis as well as the protection against such inhibition provided by thiosulfate and by transforming some of the hemoglobin of the perfusing blood into methomoglobin. A somewhat surprising feature of these findings was the relatively high level of cyanide required for effective inhibition.

EXPERIMENTAL

Materials. The livers used in these experiments were obtained from male, Sprague–Dawley, white rats, weight 400–500 g.

The chemicals used were as follows: potassium cyanide, Merck & Company, reagent grade; sodium nitrate, J. T. Baker Company, "Baker's Analyzed" reagent; sodium thiosulfate, General Chemical Company, reagent grade.

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Liver perfusion. The liver perfusion apparatus was identical to that designed by Despopoulos[7] and the procedures used were those previously described.[1] The perfusing fluid was 55–60 ml of heparinized rat blood from normal, fed donors, diluted to 75–80 ml with 0·9% NaCl (plasma volume, 45–50 ml). Potassium cyanide or other compounds to be added were dissolved in 5 ml of 0·9% NaCl. Unless otherwise indicated, the compounds were added in the following manner: two-fifths of the dose at zero time and one-fifth each at 1, 2 and 3 hr. Perfusion was usually continued for 4 hr, withdrawing small samples of blood at 0, 1, 2, 3 and 4 hr and minor lobes of liver at 0, 2 and 4 hr for amylase determinations. Bile was collected continuously, measuring the volumes at hourly intervals as a rough measure of liver function.

Analytical procedures. Amylase was determined in plasma and liver samples by Van Loon's[8] amyloclastic method following procedures previously described.[9] Liver samples were always frozen, kept overnight at −18° and thawed before making homogenates for analysis. The homogenates were centrifuged at 12,000 *g* for 15 min in the SS-1 Servall centrifuge and the supernatant fluids were used in the analyses. Amylase levels are expressed as units per 100 ml of plasma or 100 g of tissue. Van Loon amylase units are numerically equal to Somogyi units.

Formation of methemoglobin was induced by addition of sodium nitrite solution to the circulating perfusing blood and was measured by a modification of the method of Evelyn and Malloy.[10]

In experiments testing the protective effect of methemoglobin formation or cyanide conversion to thiocyanate, $NaNO_2$ or $Na_2S_2O_3$ was added 5 min after the addition of the KCN, unless otherwise noted.

RESULTS

The inhibiting effect of potassium cyanide on the synthesis of amylase by the liver is clearly evident from the results given in Table 1. However, it is also evident that relatively large amounts are required for significant inhibition. In our trials, whereas

TABLE 1. EFFECTS OF CYANIDE ON LIVER AMYLASE PRODUCTION*

Potassium cyanide added (mg)	KCN concn. in system (M)	Amylase production† (units/g liver)	Bile flow‡ (ml/hr)
0		+38·7	0·8 → 0·5
1	2×10^{-4}	+26·9	0·6 → 0·4
2	4×10^{-4}	+30·7	0·4 → 0·2
5	10^{-3}	+11·5	0·6 → 0·2
5 (without O_2)		+ 1·9	0·2 → 0
10	2×10^{-3}	− 3·9	0·3 → 0·05
10 (without O_2)		− 7·0	0·2 → 0
10 (one dose)		−15·0	0·1 → 0

* The results given here are the average of three or four trials in each case; all determinations were carried out in duplicate.

† Amylase production is calculated from both the increase (if any) in plasma amylase and the decrease in liver amylase (cf. ref. 1). It represents the sum of both and so is a net value, which on occasion may be negative.

‡ The values given for bile flow represent those observed during the first and last hours of the perfusion in each case and thus are indications of the changes produced by cyanide addition.

five out of five 400–500 g white rats were killed by the intraperitoneal injection of 2 mg potassium cyanide, addition of a like amount to the perfused liver system had relatively little effect on the liver's ability to synthesize amylase. The lethal effect of the cyanide and the liver's ability to produce amylase are obviously not related.

When 5–10 mg (10^{-3} to 2×10^{-3} M) amounts of cyanide were added, the amylase synthesis was markedly or even completely inhibited. Lack of oxygenation[2] along with addition of cyanide further inhibited amylase synthesis. Addition of 10 mg cyanide, all in a single dose, at zero time was quite toxic to the liver and gave even lower rates of amylase synthesis than when added in divided doses.

In Table 1 it will be noted that at higher cyanide concentrations some values for amylase production are negative. This is explained when one realizes that amylase production is the net result calculated from changes in both plasma and liver amylases.[1] When amylase production in the liver is completely blocked by cyanide, there is little or no increase in plasma amylase. However, the catabolic processes resulting in amylase breakdown in the liver are not inhibited and therefore the liver amylase shows a decrease. Thus the net amylase production calculated from these values is a negative number.

The inhibiting effects of cyanide on amylase synthesis could be prevented by the addition of sodium nitrite to the system (Table 2) and this protective effect was

TABLE 2. PROTECTIVE EFFECT OF METHEMOGLOBIN FORMATION ON CYANIDE INHIBITION OF AMYLASE SYNTHESIS

Potassium cyanide added* (mg)	Sodium nitrate (mg)	Amylase production (units/g liver)	Bile flow (ml/hr)
		38·7	0·8 → 0·5
5		11·5	0·6 → 0·2
	5	22·6	1·0 → 0·6
5	5	21·7	0·8 → 0·3
10		− 3·9	0·3 → 0·05
	20	24·1	0·5 → 0·3
10	20	29·5	1·0 → 0·8

* In these experiments, KCN was added in divided doses (see Experimental) followed at 5-min intervals by addition of $NaNO_2$.

undoubtedly due to the formation of methemoglobin.[11] Control experiments indicated that $NaNO_2$ alone had only a very small inhibiting effect. The addition of 20 mg $NaNO_2$ produced levels of methemoglobin of 2·1 to 2·5 g per 100 ml within 1 hr. Addition of 5 mg $NaNO_2$ produced slightly lower levels, 1·2 to 1·4 g per 100 ml within 1 hr.

Table 3 indicates that the addition of $Na_2S_2O_3$ to the perfused system will afford protection against the toxic effects of cyanide. When 10 mg cyanide alone was added in a single dose at zero time (see Table 1), amylase synthesis was completely inhibited (−15·0 units/g of liver), but the addition of 10 mg thiosulfate 5 min after 10 mg cyanide led to restoration of bile formation and finally to increase in plasma amylase levels. Control experiments showed no marked inhibitory effects of thiosulfate alone.

TABLE 3. REVERSAL OF CYANIDE TOXICITY BY THIOSULFATE*

Hours	With thiosulfate			Without thiosulfate		
	Amylase levels (units/100 ml or 100 g)		Bile flow (ml/hr)	Amylase levels (units/100 ml or 100 g)		Bile flow (ml/hr)
	Plasma	Liver		Plasma	Liver	
0	1210	2830		1440	2650	
1	1070		0·33	1230		0·10
2	1070	2860	0·00	1350	1940	0·00
3	1150		0·18	1300		0·00
4	1790	2990	0·56	1350	1820	0·00

* In these experiments, 10 mg KCN was given in a single dose at zero time. In the first case, this was followed by 10 mg $Na_2S_2O_3$ 5 min later. Net amylase synthesis with thiosulfate was 27·6 units/g of liver; without thiosulfate, it was −15·0 units/g of liver.

DISCUSSION

Hokin[12] demonstrated that amylase synthesis in pigeon pancreas slices was completely inhibited by 10^{-4} M KCN and 70 per cent inhibited by 10^{-5} M. Bdolah *et al.*[13] found that cyanide inhibited amylase secretion from rat parotid slices.

One might have predicted than that cyanide would effectively inhibit amylase synthesis in the isolated perfused liver, but it was somewhat surprising at first to note how little effect the addition of 1–2 mg KCN ($2–4 \times 10^{-4}$ M) produced. Only at levels of about 10^{-3} M or higher did cyanide very effectively inhibit liver amylase synthesis. It is known, however, that the mechanism for cyanide detoxification in mammalian tissues is the conversion of cyanide to thiocyanate.[14, 15] There is present in rat liver, in relatively high concentration, an enzyme system capable of catalyzing the reaction of sulfhydryl compounds with cyanide to form thiocyanate. In addition, there is an enzyme, rhodanese (thiosulfate: cyanide sulfurtransferase EC 2.8.1.1), present in liver[16] in higher concentrations than in other tissues. In light of these findings, the liver's ability to withstand cyanide is more easily understood. In this investigation, the ability of thiosulfate (Table 3) to block effectively the inhibiting action on amylase synthesis of an otherwise overwhelming dose (10 mg) of KCN was demonstrated.

It has also been shown previously that the presence of methemoglobin[11] can protect against the toxic effects of cyanide, apparently because of the stable nature of the cyanmethemoglobin complex formed. We have shown that addition of sodium nitrite to the perfused liver system does lead to the formation of methemoglobin[11] and protection against otherwise inhibiting doses of KCN.

In previous experiments in which dinitrophenol was added to the perfused liver system,[6] accumulations of amylase in liver were noted and it was speculated that this might indicate that the transport of amylase out of the liver was dependent on oxidative phosphorylation. In our experiments with cyanide, however, we have observed no accumulation of amylase in the liver.

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