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Effects of hemoglobin concentration and temperature on the in vitro release of cyanide from sodium nitroprusside

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Abstract

Purpose. This in vitro study was performed to determine whether changes in hemoglobin (Hb) concentration and temperature influenced the amount of cyanide (CN^-) released from sodium nitroprusside (SNP).

Methods. Canine whole blood with a Hb concentration of 8.5 to 18.9 g·dl (5.3 to 18.9 mM) was equilibrated with SNP at either 37°C or 25°C, and CN^- levels in plasma and red blood cells (RBC) were measured using the microdiffusion method.

Results. Changes in Hb concentration and temperature did not have any statistically significant effect on the CN^- released from SNP in plasma. On the other hand, CN^- levels in RBC decreased with increasing Hb concentrations. CN^- levels in RBC were significantly lower at 25°C than at 37°C.

Conclusion. Though the Hb concentration and temperature changed the amount of CN^- released from SNP in RBC, the change observed was not clinically significant.

Key words: blood, hemoglobin, hypothermia, metabolism, sodium nitroprusside, toxicity, cyanide

Introduction

The first step of the metabolic breakdown of sodium nitroprusside (SNP) to cyanide (CN^-) involves a nonenzymatic reaction with hemoglobin (Hb). Both free and intracellular Hb can react directly with SNP, yielding cyanmethemoglobin, a nontoxic compound, and the unstable nitroprusside radical [1]. The CN^- level in red blood cells (RBC) may depend on the Hb content. It is also known that metabolic activity, in general, is reduced at lower temperatures. This in vitro study was undertaken independently of the influence of circulation and metabolism to determine whether changes in Hb concentration influenced the amount of CN^- released from SNP and whether there was an influence of temperature on the release of CN^- from SNP.

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Methods

Fresh, heparinized canine whole blood containing 8 μg·ml^{-1} of SNP was equilibrated in a temperature-controlled bubble tonometer [2] utilizing an oxygen-nitrogen mixture to maintain the PO_2 of the sample above 150 mmHg. The entire chamber was maintained at the desired temperature by a stirring pump in a temperature-controlled bath [3]. The tonometer was wrapped with aluminum foil to protect SNP from light. CN^- levels in the gas phase were not measured in the succeeding experiments.

Hb concentration was measured by an IL CO-Oximeter 282 (Instrumentation Laboratories, Lexington, MA, USA). Twenty samples whose Hb concentrations ranged from 8.5 g·dl (5.3 mM) to 18.9 g·dl (11.8 mM) were equilibrated at 25°C and 21 samples with Hb concentrations of 10.5 to 18.9 g·dl at 37°C to determine what effect temperature and hemoglobin concentration had on the release of CN^-. Whole blood samples were centrifuged and separated into plasma and RBC components. The RBC components were further washed with isotonic saline and centrifuged three times. Cyanide levels were determined immediately after SNP was injected into the tonometer (0 h), and at 0.5, 1, 2, 3, and 4 h equilibration. CN^- levels in plasma and RBC were measured by the Orion ion-specific electrode (Orion Research, Cambridge, MA, USA) using the microdiffusion method [4,5]. To minimize the possibility of degradation by light during the measurements, the samples were covered with aluminum foil.

Multiple regression was employed to assess the relationship of Hb concentration and CN^- release, controlling for equilibrium time. A repeated-measures

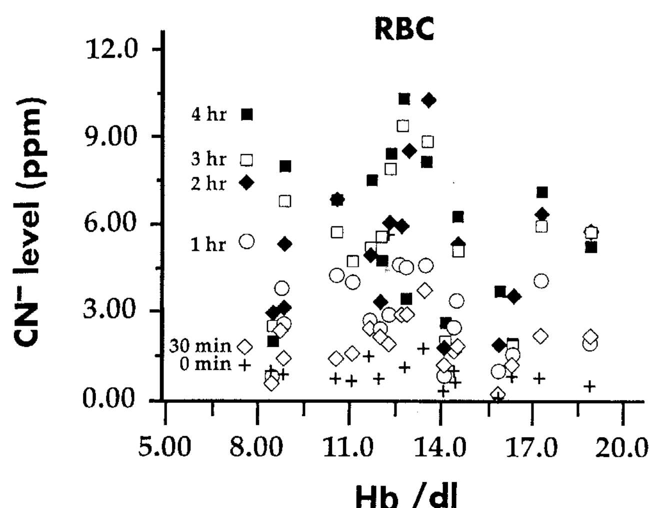

Fig. 1. Relationship of cyanide (CN^-) levels in RBC to time with hemoglobin (Hb) concentrations of 8.5 and 18.9 $g \cdot dl^{-1}$ at 37°C, and 10.5 and 18.9 $g \cdot dl^{-1}$ at 25°C

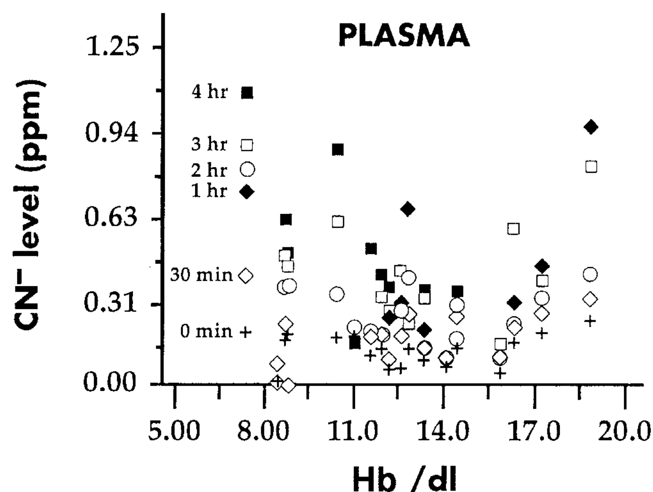

Fig. 2. Relationship of CN^- levels in RBC to Hb concentration at times 0.5 and 4 h

Table 1. Cyanide concentrations (ppm) in RBC and plasma at 25°C and 37°C

Temperature	Control	0.5 h	1 h	2 h	3 h	4 h
			RBC			
25°C	0.987	1.445*	1.758*	3.279*	3.594*	4.144*
	±0.483	±0.584	±0.577	±1.223	±1.175	±1.562
37°C	0.966	2.092*	3.229*	5.333*	5.837*	6.643*
	±0.604	±0.903	±1.424	±2.564	±3.200	±3.846
			Plasma			
25°C	0.229	0.246	0.281*	0.370*	0.382*	0.426*
	±0.125	±0.133	±0.176	±0.210	±0.183	±0.165
37°C	0.172	0.228	0.298*	0.390*	0.454*	0.644*
	±0.086	±0.111	±0.180	±0.290	±0.321	±0.489

Results are mean ± SD.
* Statistically significant difference ($P < 0.05$) compared with the control value.

analysis of variance was used to examine changes in CN^- release over time at 25°C and 37°C. Kruskal-Wallis post hoc tests were used to compare control values of CN^- with those at each equilibrium time. $P < 0.05$ was considered to be statistically significant.

Results

The results are shown in Table 1 and Figs. 1 and 2. CN^- in RBC decreased with increasing Hb concentration and increased with temperature and time significantly after 30 min of equilibration (Table 1 and Fig. 1). CN^- in RBC can be expressed as

$$CN^-_{RBC}\ (ppm) = -0.0815\ Hb + 0.0795\ temperature + 1.13\ time - 0.0336\ (r^2 = 0.564)$$

where CN^- (ppm) is the predicted CN^- level in RBC.

There were no statistically significant differences in plasma CN^- with respect to either Hb concentration changes or temperatures (25°C *vs* 37°C). (Table 1 and Fig. 2). CN^- in plasma increased significantly after 1 h equilibration at 37°C as well as at 25°C. Thus, the CN^- level in plasma is a function of time only, and is expressed as

$$CN^-_{plasma}\ (ppm) = 0.0852\ time + 0.175\ (r^2 = 0.245)$$

where CN^- plasma is the predicted plasma cyanide concentration in ppm.

Less than 1 ppm of CN^- was measured in the control samples. There are several possible sources of CN^- contamination in solution [5]. Since we could not rule out any of the possibilities in this study, we did not subtract CN^- levels obtained as a background ion leakage from those at each measurement.

Discussion

CN^- can be released from SNP when the latter is incubated with a variety of biological materials. The most active biological preparation tested is blood. The first and most rapid pathway for metabolic breakdown of SNP involves a nonenzymatic reaction with Hb. RBC are far more active than plasma in releasing CN^- [1]. Several factors might influence CN^- release from SNP following incubation with whole blood: Hb concentration and oxygen saturation, SNP concentration used, species differences, and temperature [6]. Trace amounts of CN^- were thought to escape via the gas flowing through the bubble tonometer. However, no CN^- could be detected by the method reported by Boxer and Richards [7] in the first few experiments, so the directly measured CN^- levels were reported in this study.

Our results show a negative correlation between Hb concentration and the amount of CN^- in RBC released from SNP. Smith and Kruszyna [1] found that the CN^--releasing activity of intact RBC might be due to their Hb concentration. Their results, however, may not be well founded in light of their use of three different Hb concentrations of human (3.2, 8.0, and 8.9mM) and two of rat (8.0 and 8.9mM) whole blood containing mostly unsaturated Hb that was incubated with SNP for 60min. Increased CN^- levels at 60min incubation with both human and rat whole blood were reported with increasing Hb concentration. These data were limited, however, to single values from representative experiments without statistical analysis, which does not permit comparison with our results.

Our results show CN^- levels in both RBC and plasma increased with time. Arnold et al. [8] suggested that Hb did not remove CN^- from SNP molecules when they observed the lack of increase in the CN^- levels of blood samples stored in the air at room temperature for 4h. Smith and Kruszyna used high concentrations of SNP (400mM or $119 \mu g \cdot ml^{-1}$) compared with ours ($8 \mu g \cdot ml^{-1}$ of whole blood samples) and Arnold's (continuous infusion of 2 to $8 \mu g \cdot kg^{-1} \cdot min^{-1}$ for 2h) [1,5,8]. In the latter experiments, the molar quantity of Hb is much larger than that of SNP. Since there is enough Hb to react with SNP, we would expect a minimal effect of changing Hb concentration. Moore et al. [6] reported a significant increase in CN^- in RBC in six patients with rectal temperatures of 25°C during hypothermic extracorporeal circulation. Changes in plasma CN^- levels, however, were minimal during both normothermia and hypothermia. They concluded that the nonenzymatic release of CN^- from SNP was not inhibited by hypothermia, whereas the enzymatic detoxification of CN^- to thiocyanate may have been suppressed. In their study, renal and hepatic blood flow might have been compromised by phenylephrine, which was administered to maintain blood pressure during 20min of SNP infusion. Phenylephrine-induced vasoconstriction could impair the rhodanase enzymatic system. The CN^- levels in their study might have been lower at 25°C without phenylephrine administration. It is difficult to compare the results obtained in vivo by Moore and colleagues [6] with those obtained in vitro, not only because of the differences in metabolism, excretion by the liver and/or kidney, and absence or presence of circulation, but also because of the differences in temperature, duration of observation, and probable light exposure to SNP during extracorporeal circulation. Moore et al. [6] did not report changes in CN^- levels when SNP was given during normothermia. It is not possible to compare their in vivo results with our in vitro results. In our study, the effect of RBC on the release of CN^- from SNP is excluded from the enzymatic system.

In conclusion, varying the level of Hb had a statistically significant effect on the amount of CN^- released from SNP when whole blood was incubated with SNP for 4h in vitro. Though our results showed statistically significant changes, it is fair to say that we do not have to be overly concerned with changes in Hb concentration over wide clinical ranges, since the changes in CN^- levels observed in the experiment appear to be clinically insignificant. Hypothermia, per se, decreased the CN^- in RBC released from SNP in the absence of the rhodanase enzymatic system. This finding does not necessarily imply that hypothermia protects from CN^- toxicity. Plasma CN^- levels during hypothermia were not different from those during normothermia.

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