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Stimulation of methemoglobin reduction by selenium: A comparative study with erythrocytes of various animals

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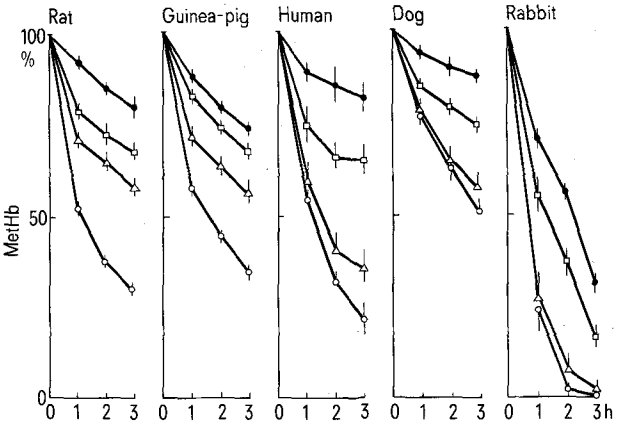
Summary. The extent of stimulation of methemoglobin (metHb) reduction by selenite depends upon the level of reduced glutathione (GSH) in the erythrocytes. The reason for the species difference in the effect of selenite was discussed with respect to species differences in the GSH levels in erythrocytes.

Recently, we reported that selenite stimulates metHb reduction in rat erythrocytes and that its action may be to catalyze the reduction of metHb by GSH[1,2]. Selenite was shown to accumulate in erythrocytes, where it was metabolized by GSH-dependent systems[3–6]. Thus, the GSH level in erythrocytes is thought to be closely related to the selenite effect. This paper is on the relationship between the effect of selenite on metHb reduction and the GSH level in the erythrocytes of various animals.

Materials and methods. Heparinized blood samples from male adult Sprague-Dawley strain rats, guinea-pigs, rabbits, dogs and humans were used. The methods used for isolation of erythrocytes and conversion of hemoglobin to metHb were as described previously[1]. Erythrocytes containing metHb were depleted of GSH by treatment with N-ethylmaleimide (NEM) using a modification of the method of Morell et al.[7]: namely, equal volumes of packed, metHb-containing cells and 4 or 8 mM NEM in isotonic phosphate buffered saline (pH 7.4) (PBS) containing 10 mM glucose were mixed and incubated for 20 min at 37 °C, and then the cells were washed 4 times with PBS. The basal mixture used to study the reduction of metHb consisted of 25% erythrocytes plus 10 mM glucose in PBS. MetHb was determined by the method of Evelyn and Malloy[8] and GSH by the method of Beutler et al.[9].

Results. The table shows the relationship between the endogenous GSH level in erythrocytes and the rate of enhancement of metHb reduction observed on adding selenite. The GSH level in the cells decreased on preincubation of the cells with NEM, the decrease depending on the concentration of NEM, and the GSH did not reappear during the 60-min incubation period. In cells with normal GSH levels, about 45% of the metHb was reduced on addition of selenite, whereas in cells with half the normal GSH level preincubated with 2.0 mM NEM, the effect of selenite was considerably less, and in cells completely depleted of GSH (preincubated with 4.0 mM NEM), selenite did not enhance metHb reduction at all. Jenkins and Hidiroglou[10] reported the NEM inhibited the uptake of



Species differences in enhancement by selenite of metHb reduction in erythrocytes from various animals. No addition, ●——●; Na_2SeO_3 10^{-5} M, O——O; 3×10^{-6} M, △——△; 10^{-6} M, □——□.
Means±SE of values in 5 experiments are shown.

Effect of GSH depletion on enhancement of metHb reduction by selenite in rat erythrocytes

Pretreatment	Addition	GSH (mM) Initial	After incubation	Reduced metHb (%)
None	None	2.22±0.16	2.14±0.04	5.2±1.9
None	Na_2SeO_3	2.22±0.16	1.45±0.08	44.5±3.6
NEM (2 mM)*	Na_2SeO_3	1.16±0.10	0.69±0.01	29.2±4.4
NEM (4 mM)*	Na_2SeO_3	0.28±0.06	0.16±0.04	3.4±1.9

Means±SE of values in 3 experiments are shown. Cells were incubated for 60 min at 37 °C with or without 10^{-5} M selenite.
* Final concentration.

selenite by erythrocytes, but we could not confirm this under our conditions. Thus, the present findings indicate that stimulation of metHb reduction by selenite depends upon the GSH level in the cells.

The figure shows the species differences in metHb reduction in erythrocytes from various species in the absence and presence of selenite. In the absence of selenite, the rate of metHb reduction was lowest in the erythrocytes of dogs and highest in those of rabbits, the rate in erythrocytes of humans, rats and guinea-pigs being intermediate. Selenite (especially at high concentration), stimulated the reduction most in rabbit erythrocytes and least in dog erythrocytes. The rate of metHb reduction in these erythrocytes in the presence of selenite decreased in the following order: rabbit > human, rat, guinea-pig > dog. Lower concentrations of selenite resulted in similar rates of metHb reduction in erythrocytes of the various species, except guinea-pigs whose rate was considerably lower than others.

Discussion. Reduction of metHb in erythrocytes is mainly enzymatic (i.e. due to metHb reductase)[11], and thus the reduction observed in the absence of selenite is probably due to metHb reductase. The species differences in the rates of reduction of metHb observed in the absence of selenite were similar to those reported previously[12]. As shown in the table, GSH was necessary for stimulation of metHb reduction by selenite. We determined the GSH levels in erythrocytes of various animals to be as follows: rabbit 3.01±0.07 mM, guinea-pig 2.83±0.15 mM, rat 2.16±0.11 mM, human 2.25±0.22 mM and dog 1.72±0.22 mM (means±SE of 5 determinations). The stimulation of metHb reduction by a high concentration of selenite was found to be high in rabbit erythrocytes, in which the GSH level is high, and lower in dog erythrocytes in which the GSH level is low. However, this correlation was not observed with lower concentrations of selenite, suggesting that a certain molar ratio of selenite to GSH is important for selenite-catalyzed reduction of metHb by GSH[2]. We also found that selenite did not influence NADH-metHb reductase activity in vitro, measured by the method of Hegesh et al.[13] (data not shown). Therefore, we conclude that the species difference in the stimulation of metHb reduction by selenite is related to a species difference in the level of GSH in erythrocytes.

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Plasma vasoactive intestinal polypeptide (VIP) levels and intestinal ischaemia

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Summary. Vasoactive intestinal polypeptide (VIP) is released into the portal circulation in large quantities by ischaemic bowel. In view of its known high concentration in the gut and potent vasoactive properties it may well be implicated in the pathogenesis of the serious haemodynamic changes produced by gut ischaemia.

Vasoactive intestinal polypeptide is a pharmacologically active pepetide with a broad biological activity[1] which has been isolated from both the gut and the brain[2]. It has as yet no proven physiological role and no concensus exists as to its release mechanisms though both calcium provocation[3] and vagal stimulation[4] have been demonstrated to produce a rise in plasma levels. The causative role in the pathogenesis of the watery diarrhoea syndrome has recently been directly established[5] but its relationship to any other pathological process has yet to be determined. Many of the clinical changes that follow ischaemia of the gut such as tachycardia, profound hypotension and watery diarrhoea could well be explained by the release of VIP.

Materials and methods. 6 English white pigs (20–25 kg) were anaesthetised with nitrous oxide and oxygen. The internal jugular vein, portal vein and femoral artery were cannulated for plasma sampling and monitoring of the haemodynamic status. Ischaemia was produced by clamping the superior mesenteric vascular pedicle for 2 periods of 15 min each with an intervening rest period of 30 min. Haemodynamic changes were continually recorded by an intra arterial pressure manometer, a central venous catheter and an electrocardiograph. Blood samples were taken at 5 min

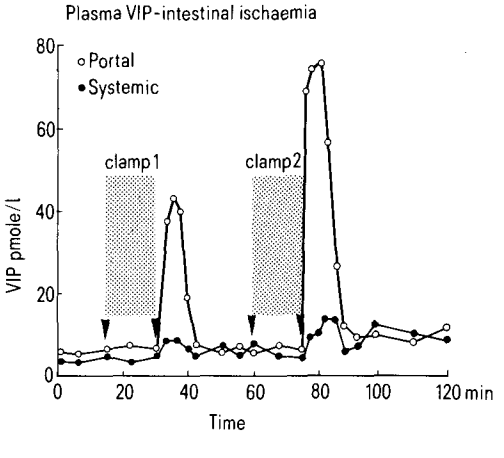

Fig. 1. The portal and plasma VIP levels during intestinal ischaemia in a single pig. The portal VIP levels are significantly higher than the systemic levels. The 2 15-min ischaemic periods are indicated by the shaded areas.