REDUCTION OF METHEMOGLOBIN BY MEANS OF GLUTAMIC ACID

A. M. Genkin and M. S. Volkov

From the Department of Biochemistry (Head – Prof. S. A. Brailovskii), Sverdlovsk Medical Institute (Director – Prof. A. F. Zverev)

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The use of a number of reducing substances has been proposed for the conversion to hemoglobin of methemoglobin, which can arise in the human organism by the action of various industrial poisons (potassium chlorate, nitrites, arsine, etc.) and of drugs (amyl nitrite, sulfanilamides), as well as in toxemias of pregnancy.

TABLE 1

Effect of Sodium Glutamate on the Methemoglobin-Producing Action of Sodium Nitrite

No. of experiment	Time elapsed between injection of sodium nitrite and taking of blood sample	Blood methemoglobin content (%)		% Diminution in methemoglobin content in the experimental group of rats
		control group	experimental group	
1	120	56,8	31,4	44,8
2	85	67,3	45,8	32,0
3	52	67,1	44,4	33,8
4	52	62,7	40,5	35,5
5	65	75,8	47,6	37,2
6	40	80,6	39,3	51,3
7	65	77,4	46,4	40,0
8	33	82,6	49,6	40,0
9	33	74,6	40,9	45,3
10	45	77,0	46,4	39,7
Mean	—	72,2	43,0	40,0

Of the earlier drugs used for the treatment of methemoglobinemia, methylene blue has been the most widely applied, both in experimental work and clinically [2, 6, 9, 13]. Although it rapidly reduces methemoglobin to hemoglobin, methylene blue is not without its drawbacks, since it also gives rise to methemoglobin formation, forming, according to different authors, from 8 to 24% of methemoglobin [6, 10].

Lactic and ascorbic acids are well known to be active reductants of methemoglobin in vitro [1, 12], but these substances were found to be without action in animal experiments [7, 11, 13]. It has recently been shown [3] that glutamic acid greatly raises the resistance of organisms to deficiency of oxygen in the atmosphere. An analogous effect is found in hypoxic conditions, due to administration of sodium nitrite [4]. Furthermore, simultaneous administration of glutamic acid protects rats from the effects of a lethal dose of sodium nitrite.

These results suggested that glutamic acid may affect the process of methemoglobin formation in blood. The present paper is devoted to an investigation of this possibility. We have been unable to find any references in the literature to the action of glutamic acid on blood methemoglobin.

EXPERIMENTAL METHODS

The animal material consisted of adult rats. Methemoglobinemia was induced by subcutaneous injections of sodium nitrite, at a dosage level of 10 mg per 100 g body weight. Our experiments showed that this dose invariably caused the death of the animals. The control rats were decapitated when they displayed preterminal convulsions, and blood methemoglobin was determined. Sodium glutamate (100 mg per 100 g bodyweight) was injected together with sodium nitrite into the experimental rats.

TABLE 2

Effect of Adding Sodium Nitrite and Sodium Glutamate to Blood on Methemoglobin Formation

No. of experiment	methemoglobin content of blood (%) after adding to it	
	sodium nitrite	sodium nitrite + sodium glutamate
1	70,1	19,8
2	74,7	28,1
3	52,4	20,3
4	60,5	25,7
5	66,0	22,7
6	68,8	18,6
7	65,8	20,3
8	67,0	17,2
9	49,3	9,4
10	58,3	16,0
Mean	63,3	19,8

Methemoglobin was determined by a modification of Gorn's method [7]. We used a photoelectric colorimeter with a red filter. We did not use 0.25% ammonia solution for hemolysis and dilution of blood as recommended by Gorn, because according to our findings and to literature references [5] conversion of methemoglobin into hemoglobin is accelerated in alkaline solution (pH > 8), which also transform some of the methemoglobin into alkaline hematin. We effected hemolysis with water alone, and then added sufficient phosphate buffer (pH = 7.4) to bring the concentration of the blood in the solution to 2.66%, as recommended by Gorn.

RESULTS OF EXPERIMENTS

Injection of sodium nitrite into the control animals (10 rats) was followed by uneasiness and then somnolence, the breathing became superficial, and convulsions heralded the onset of death. This took place within 33-120 minutes after injection. The methemoglobin content of blood taken during the agonal period ranged from 56.8 to 82.6%, mean value 72.2%.

The animals of the experimental group given glutamate together with sodium nitrite showed no symptoms, except, possibly slight drowsiness in some of them. Not one of the ten rats went into convulsions, Their blood methemoglobin ranged from 31.4 to 49.6%, mean value 43%, which was 40% lower than in the control rats.

Thus the methemoglobin-producing action of sodium nitrite is very considerably reduced by simultaneous injection of sodium glutamate (Table 1).

We thought it would be of interest to ascertain whether this effect of glutamic acid was exerted only in the

whole animal, or whether it could also be shown in blood withdrawn from animals. For this purpose blood taken from normal rats was hemolyzed and diluted, as before. To 7.5 ml of diluted hemolyzate we added 4 mg of sodium nitrite (control), and to another portion we also added 20 mg of sodium glutamate (experiment). Methemoglobin was determined in both solutions after two hours. The results are presented in Table 2.

It is clearly evident from the data of Table 2 that the methemoglobin-producing effect of sodium nitrite is to a considerable extent inhibited in the presence of sodium glutamate. It may be supposed that this action of glutamic acid, exerted both in the whole animal and in shed blood, should be ascribed to promotion of the reverse process of reduction of methemoglobin, rather than to inhibition of conversion of hemoglobin into methemoglobin by action of nitrite. This question will be the subject of a future research.

SUMMARY

Simultaneous administration of sodium glutamate counteracts the effect of a lethal dose of sodium nitrite given to rats. The methemoglobin content of the blood is 40% lower than in unprotected animals in the terminal stage of nitrite poisoning. Addition of sodium nitrite to blood in vitro gives 63.3% conversion to methemoglobin, as compared with 19.8% when sodium glutamate is added simultaneously.

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