THE INHIBITION OF METHEMOGLOBIN FORMATION BY GLUTAMATE

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We have demonstrated previously [1] that glutamate administered to rats simultaneously with a lethal dose of sodium nitrite prevented the death of the animals and lowered the blood methemoglobin levels by about 40% compared to control values in animals given nitrite alone The effect of glutamate on methemoglobin formation was demonstrated even more clearly in isolated blood in vitro.

However, from the previous experiments with isolated blood it was not clear whether the actual effect of glutamate was to inhibit the nitrite-induced methemoglobin formation, or whether it simply accelerated the reversal of the process—the reduction of methemoglobin to hemoglobin. The present work was undertaken in order to elucidate this fact.

METHODS

White rats were given sodium nitrite subcutaneously, in doses of 100 mg/kg body weight. Blood samples were taken from the tail vein 20 min later for methemoglobin determination, and immediately some of the animals (experimental group) were given sodium glutamate (100 mg per 100 g weight) subcutaneously, while the remaining animals were similarly injected with normal saline. All animals were killed 20 min later by decapitation and blood methemoglobin in all samples was determined by the method of Horn, slightly modified by us [1].

RESULTS

Within 20 min of sodium nitrate administration to the rats, the methemoglobin content of the blood taken from the tail veins of 11 animals corresponded to a conversion of 47.2 % (mean values) of the total hemoglobin to methemoglobin. The results of the assay on the decapitated animals, 20 min after glutamate (experimental animals) or saline (controls) administration, are shown in Table 1.

It will be seen from the Table that in control animals the mean (of 10 experiments) methemoglobin level was 67 %, while in experimental animals the value was 60.5 %, i.e., only 9.7 % lower. It is thus obvious that glutamate, when administered to animals with high blood methemoglobin levels, failed to reduce it back to hemoglobin. Glutamate, apparently, is only capable of inhibition of nitrite-induced methemoglobin formation .

TABLE 1

Blood Methemoglobin Levels in Rats which Received Sodium Glutamate Subsequent to Injection of Nitrite

Experiment No.	Blood methemoglobin, %		Fall in methemoglobin levels in experimentals, %
	controls	expt	
1	71,8	66,2	7,7
2	64,0	57,5	11,2
3	74,0	62,2	15,3
4	77,1	67,0	13,3
5	65,7	57,5	12,5
6	59,4	52,4	11,9
7	69,4	62,8	9,8
8	63,7	57,8	9,6
9	61,1	57,0	7,2
10	63,6	63,4	0,5
Mean	67,0	60,5	9,7

It was of interest to observe the protective effect of glutamate when administered prior to the sodium nitrite. Sodium nitrite in these experiments was therefore given to rats 30 min after glutamate injection, and the blood methemoglobin levels were assayed, as before, 20 and 40 min later. Ten rats, given nitrite alone, served as controls. At the two times of assay, methemoglobin levels in experimental (protected) rats were 28.2% and 22% lower, respectively, than in the control rats. Thus, glutamate given 30 min before nitrite administration also exerted an inhibitory effect on methemoglobin formation, although not as efficiently as when administered together with the poison, as was demonstrated earlier [1].

Intravenously introduced glutamate is known [2] to disappear rapidly from the blood stream. It may therefore be assumed that the inhibition of methemoglobin

TABLE 2

The Induction of Methemoglobin Formation in Blood in Vivo by Nitrite in the Presence of Various Amino Acids (exposure time — 2 hr at 18°; each value represents a mean of 8 experiments)

Blood methemoglobin (%) with nitrite and amino acids					
nitrite (control)	glycine	serine	cysteine	leucine	glutamate
85,2	85,6	88,7	87,8	87,1	2,0

TABLE 3

The Effect of Glutamate on Methemoglobin Formation in Isolated Blood Treated with Ferricyanide (exposure— 2 hr at 18°; all values are means of 8 experiment)

Blood methemoglobin (%) after addition of :	
potassium ferricyanide	ferricyanide + + glutamate
70,5	38,7

formation was due to glutamate itself, and not to some product of its catabolism.

It is difficult at present to speculate on the possible mechanism through which glutamate inhibits the nitrite-induced methemoglobin formation. A direct chemical interaction between glutamate and nitrite, resulting in decreased blood levels of the latter, is hardly possible. Although nitrous acid is known to react with amino acids to liberate the amino nitrogen as a free gas, this reaction takes place only in strongly acid environments which can be excluded under in vivo conditions. Should such a reaction be possible, it would be expected to apply in the case of amino acids other than glutamate, as well. This is not actually the case. Under the present experimental conditions, the in vitro addition, to blood treated with sodium nitrite, of corresponding amounts of serine, glycine, cysteine or leucine, had no effect on the nitrite-induced methemoglobin formation, while glutamate, added in the same concentration, almost entirely abolished it (see Table 2).

Glutamate was also found to inhibit considerably the methemoglobin formation in blood treated with ferricyanide (Table 3).

The suppression of methemoglobin therefore appears to be specifically a property of glutamate, which can be demonstrated in the presence of various methemoglobin-producing stimulants.

SUMMARY

Glutamate has been shown to suppress considerably methemoglobin formation in rats in vivo and in isolated rat blood. The mechanism did not involve reduction of methemoglobin to hemoglobin. The effect was specific to glutamate, other amino acids tested (serine, glycine, leucine, cysteine) being ineffective. The protective effect of glutamate also applied to ferricyanide-induced methemoglobin formation. The protective effect of glutamate was maximal when the amino acid was administered simultaneously with the poison.

LITERATURE CITED

1. A. M. Genkin and M. S. Volkov, Byull. Éksp. Biol. i Med. 47, 50 (1959).*
2. V. Klingmüller and K. Z. Vogelgesang, Physiol. Chemie 300, 97 (1955).

* Original Russian pagination. See C.B. Translation.