DEPRESSION OF GLUTATHIONE FORMATION BY INSULIN IN THE RAT LIVER AND ITS PREVENTION BY SELENIUM

Ts. M. Shtutman and R. V. Chagovets

UDC 615.31:546.23].015.45:616.36.36-008.939.626

The effect of insulin on the utilization of cysteine and methionine for glutathione biosynthesis in the rat liver and the possibility of preventing these changes by vitamin E and selenium were investigated. The decrease in the glutathione concentration under the influence of insulin was accompanied by a decrease in the incorporation of glycine-2-C^{14}, administered concurrently with L-cysteine, and of sulfur of methionine-S^{35} into glutathione. Vitamin E had no effect on this action of insulin. Sodium selenite prevented both the decrease in the glutathione level and the inhibition of incorporation of glycine-2-C^{14}, administered together with L-cysteine, and sulfur of methionine-S^{35} into glutathione.

Selenium in the form of sodium selenite stimulates incorporation of methionine-S^{35} into glutathione in the liver of rats [4, 5] and chickens [13] and of glycine-2-C^{14} and formate-C^{14} in the rat liver [6].

The increase in the glutathione concentration under the influence of selenium in animals receiving both DL-methionine and L-cysteine is evidence of its direct or indirect effect on the enzyme system of glutathione biosynthesis. Vitamin E stimulates incorporation of methionine-S^{35} and glycine-2-C^{14} into glutathione in the rat liver, but if these substances are administered together with ballast methionine and cysteine, characteristic differences from the action of selenium are observed [6, 7].

To continue the study of the connection between selenium and vitamin E with glutathione metabolism, insulin was used, for insulin rapidly lowers the glutathione level in the liver [9]. The object of the present investigation was to determine whether the decrease produced by insulin in the glutathione concentration in the liver of rats is accompanied by changes in the incorporation of glycine-2-C^{14}, administered along with L-cysteine, and sulfur of methionine-S^{35} into glutathione and the extent to which vitamin E and selenium counteract the insulin effect.

EXPERIMENTAL METHOD

Experiments were carried out on albino rats weighing 100 ± 20 g. The animals were starved for 17 h before the experiment. Vitamin E (10 or 20 mg/100 g body weight) was given by gastric tube 60 min before administration of the water-soluble compound in the form of DL-α-tocopheryl acetate. Insulin (4 units), selenium as sodium selenite (75 μg), methionine-S^{35} (50 μCi), glycine-2-C^{14} (50 μCi), and L-cysteine (16 mg) were injected subcutaneously (doses are given per 100 g body weight). The animals were decapitated 2 h later, and liver homogenates were prepared in 0.02 M Trilon B solution (1:5) on ice. The glutathione concentration was determined from the level of free SH-groups in a protein-free extract [11] in the modification of Seblak and Lindsay [16].

Part of the homogenate was precipitated with ethanol and the protein-free extract after evaporation on a water bath was subjected to paper chromatography in a system of butanol—pyridine—water (1:1:1) [15]. The chromatograms were treated with ninhydrin (0.25% solution in acetone). The radioactivity of the

Kiev Institute of Biochemistry, Academy of Sciences of the Ukrainian SSR. (Presented by Academician of the Academy of Medical Sciences of the USSR S. E. Severin.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 74, No. 8, pp. 36-40, August, 1972. Original article submitted February 1, 1971.

TABLE 1. Effect of Vitamin E and Selenium on Incorporation of Glycine-2-C^{14} Administered Together with L-Cysteine into Liver Tissue Components of Rats Treated with Insulin (mean results of 4-6 experiments)

Substance administered	Statistical index	Free SH-groups (in μg/g)	Radioactivity (pulses/min/mg tissue)			Glutathione, chromatogram of extract of 20 mg tissue (pulses/min
			homogenate	protein-free extract	protein	
Control	$M \pm m$	205±18	217±20	112±6,3	86±4,3	260±47
Insulin	$M \pm m$ P	128±13 <0,01	188±17 1,0	110±7,6 1,0	77±11,5 1,0	70±12,8 <0,01
Insulin + vitamin E (20 mg/100 g body weight)	$M \pm m$ P	122±7,2 <0,01	201±8,5 1,0	118±8,5 1,0	83±2,2 1,0	87±13 <0,01
Insulin + Na_2SeO_3 (75 μg/100 g body weight)	$M \pm m$ P P_{ins}	179±10 1,0 <0,05	250±9 <0,2 <0,02	171±3,5 <0,001 <0,001	78±9,8 1,0 1,0	229±12,3 1,0 <0,001

TABLE 2. Effect of Vitamin E and Selenium on Incorporation of Methionine-S^{35} into Liver Tissue Components of Rats Treated with Insulin (mean results of 4-7 experiments)

Substance administered	Statistical index	Free SH-groups (in μg/g)	Radioactivity (pulses/min/mg tissue)			Glutathione, chromatogram of extract of 20 mg tissue (pulses/min)
			homogenate	protein-free extract	protein	
Control	$M \pm m$	145±8,3	446±21,9	190±21,0	256±14,2	730±32
Insulin	$M \pm m$ P	75±4,0 <0,001	334±26 <0,001	152±20,6 >0,2	182±7,5 <0,001	100±12 <0,001
Insulin + vitamin E (20 mg/100 g body weight)	$M \pm m$ P	64±4,3 <0,001	379±10,2 <0,02	193±15 1,0	186±13,5 <0,01	114±15,5 <0,001
Insulin + Na_2SeO_3 (75 μg/100 g body weight)	$M \pm m$ P P_{ins}	102±6,9 <0,02 <0,05	370±17,4 <0,02 1,0	213±28,2 1,0 >0,1	157±18,2 <0,001 1,0	577±37 <0,02 <0,001

spot corresponding to glutathione [8] was measured with a BFL-T-25 counter. A parallel chromatogram was kept in contact with RM-1 x-ray film for 10 days.

The total radioactivity and the radioactivity of the protein-free extract of 1-g tissue also were determined and the radioactivity of the proteins was obtained from the difference.

EXPERIMENTAL RESULTS AND DISCUSSION

Preliminary experiments revealed a substantial decrease in the concentration of free SH-groups in the liver of the rats after administration of insulin, as shown previously [9], and principally in SH-groups belonging to glutathione [8]. Under these conditions incorporation of glycine-2-C^{14}, administered together with L-cysteine, into glutathione was restricted. It is worth noting that insulin did not significantly change the intake of glycine-2-C^{14} into the liver or its distribution between the proteins and the tissue protein-free extract, from which it follows that insulin restricted glutathione formation. In the next series of experiments the experimental animals received vitamin E or sodium selenite. Vitamin E did not alter the effect of insulin. By contrast, sodium selenite apparently prevented the decrease in the concentration and radioactivity of glutathione produced by insulin (Table 1). The activation of glutathione synthesis by selenium previously described [6] was thus confirmed under new experimental conditions.

In the next experiments the effect of insulin on the conversion of methionine by demethylation and trans-sulfonation was studied. In these experiments the animals received methionine-S^{35}. Unlike in the

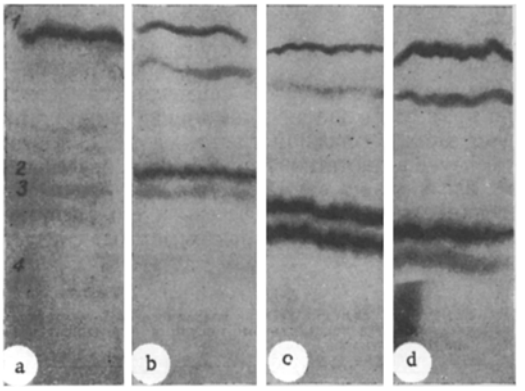

Fig. 1. Autoradiographs of chromatograms of protein-free extracts of rat liver after administration of methionine-S^{35}: a) control, b) insulin given; c) vitamin E + insulin given; d) sodium selenite + insulin given. 1) Glutathione; 2, 3) unidentified sulfur-containing metabolites; 4) site of localization of methionine.

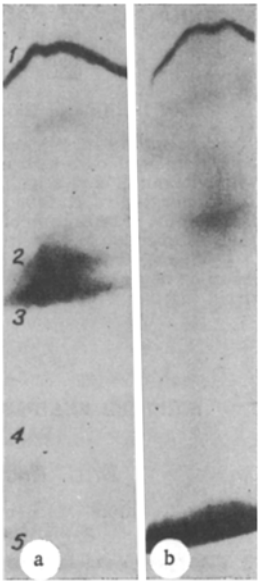

Fig. 2. Autoradiographs of chromatograms of protein-free extracts of liver from rats treated with insulin: a) methionine-S^{35} given; b) methionine-$C^{14}H_3$ given. 1) Glutathione, 2, 3) unidentified sulfur-containing metabolites; 4) site of localization of methionine; 5) end point of chromatograms.

experiments with glycine-2-C^{14}, insulin reduced the intake of labeled methionine into the liver tissue ($P < 0.01$), and under these circumstances incorporation into protein was reduced with no significant decrease in the radioactivity of the protein-free extract. The radioactivity of the glutathione under these conditions was reduced by 7 times ($P < 0.001$). Administration of vitamin E did not change the effect of insulin, while selenium largely prevented both the decrease in the concentration of free SH-groups and the radioactivity of the glutathione (Table 2).

Radioactive sulfur-containing metabolites of methionine-S^{35} accumulating under the influence of insulin (Fig. 1) consisted mainly of its demethylated products. This was shown by comparing autoradiographs of the chromatograms obtained from the protein-free extracts of the liver of rats receiving methionine-S^{35} and methionine-$C^{14}H_3$ together with insulin (Fig. 2). In the latter case most of the radioactivity was localized at the end point of the chromatogram and none was present where it had been found after administration of methionine-S^{35}. It thus follows that insulin diminishes the incorporation of sulfur of methionine-S^{35} into glutathione after demethylation of the methionine.

Insulin evidently inhibits the utilization of cysteine for glutathione biosynthesis, while selenium, as sodium selenite, removes this block. The increase in the concentration of serine in the rat liver and in its radioactivity after administration of glycine-2-C^{14} and formate-C^{14} under the influence of selenium [3, 6], the increase in glutathione biosynthesis by selenium after administration of methionine and cysteine in normal animals [6] and after its inhibition by insulin, as shown in the present investigation, and the increase in incorporation of cysteine-S^{35} into coenzyme A in the liver of rats receiving a necrogenic diet [17] all suggest that one of the manifestations of the biological action of selenium may be protection of serine and cysteine against degradation. This hypothesis is based on the fact that cysteine desulfhydrase and serine dehydratase catalyze reactions of a general type [1, 2], and the latter has been shown to be inhibited by selenocystine [14]. Although the possibility of formation of selenium analogs of sulfur-containing amino acids has been denied in rabbits [10] and

chickens [12], inhibition of these enzymes by other selenium compounds in animals cannot be ruled out. This hypothesis requires experimental verification.

This marked action of selenium and the complete absence of effect of vitamin E after administration of insulin emphasize the difference between the mechanisms of their biological action in the animal body.

LITERATURE CITED

1. A. E. Braunshtein, Uspekhi Sovr. Biol., 35, 27 (1953).
2. E. V. Goryachenkova, in: Advances in Biological Chemistry [in Russian], Vol. 10, Moscow (1969), p. 64.
3. L. N. Kuznetsova and Ts. M. Shtutman, Vopr. Pitaniya, No. 1, 41 (1967).
4. R. V. Chagovets and Ts. M. Shtutman, in: Collection in Memory of Emanuilov [in Russian], Bulgarian Academy of Sciences, Sofia (1969), p. 289.
5. Ts. M. Shtutman, R. V. Chagovets, A. M. Sukharevskaya, et al., Vopr. Pitaniya, No. 2, 3 (1968).
6. Ts. M. Shtutman and V. P. Artyukh, Ukr. Biokhim. Zh., No. 6, 747 (1970).
7. Ts. M. Shtutman, R. V. Chagovets, and V. P. Artyukh, Ukr. Biokhim. Zh., No. 2, 210 (1971).
8. Ts. M. Shtutman, R. V. Chagovets, and T. I. Kashleva, Ukr. Biokhim. Zh., 39, No. 1, 55 (1967).
9. F. Binkley, G. M. Christensen, et al., J. Biol. Chem., 186, 159 (1950).
10. L. M. Cummins and J. L. Martin, Biochemistry, 6, 3162 (1967).
11. G. L. Ellman, Arch. Biochem., 82, 70 (1959).
12. K. J. Jenkins, Canad. J. Biochem., 46, 1417 (1968).
13. J. Martin and J. Spalholz, Fed. Proc., 29, 499 (1970).
14. H. Nakagawa and H. Kimura, J. Biochem. (Tokyo), 66, 669 (1969).
15. P. J. Peterson and G. W. Butler, J. Chromatogr., 8, 70 (1962).
16. J. Seblak and R. H. Lindsay, Analyt. Biochem., 25, 192 (1968).
17. C. S. Yang, G. H. Dialameh, and R. E. Olson, Red. Proc., 18, 553 (1959).