SUBCELLULAR DISTRIBUTION OF GLUTATHIONE REDUCTASE AND SULFHYDRYL GROUPS IN ADRENAL TISSUE

A. S. Mikosha and A. Ya. Mestechkina

UDC 612.451.015

Glutathione reductase activity and the content of SH groups were studied in subcellular fractions of bovine and canine adrenals. Glutathione reductase (GR) was determined by the reaction of NADP · H_2 oxidation in the presence of oxidized glutathione. The content of SH groups was determined by amperometric titration with $AgNO_3$. The distribution of the enzyme among the fractions was similar in both species of animals, but the GR activity was higher in cattle than in dogs. Activity was highest in the cytoplasm, lower in the mitochondria, and lower still in the microsomes. Destruction of the mitochondria by repeated freezing leads to a marked increase in glutathione reductase activity. The content of Sh groups, calculated as protein, is identical in the adrenal fractions of cattle and dogs. The distribution of GR, but differences in the concentrations of SH-groups in the fractions are less marked.

The adrenals are among the organs richest in glutathione [6]. The hypothesis that glutathione may participate in the reactions of corticosteroid biosynthesis [8] has not been confirmed and the role of glutathione and its metabolism in the adrenals have received little study. Nevertheless the possibility cannot be ruled out that glutathione reductase (GR; 1.6.4.2), an enzyme of glutathione metabolism, may participate in the oxido-reduction conversions of NADP, the chief cofactor of steroid formation. Evidence of activation of GR in the adrenals of rats by ACTH [10] and of a decrease in its activity after hypophysectomy [5] has been described. In the existing view GR may participate in the reduction of protein disulfide bonds [11]. In turn, the interconversion of sulfhydryl and disulfide bonds influences the tertiary configuration of proteins [1], the activity of certain enzymes [2], the permeability of the membranes [9], and oxidative phosphorylation [4].

The object of the investigation described below was to determine GR activity and the level of sulfhydryl (SH) groups in the subcellular fractions of bovine and canine adrenal tissue.

EXPERIMENTAL METHOD

Fresh bovine adrenals were freed from fat and cut into slices. The cortex was removed with scissors and homogenized in 0.25 M sucrose containing 0.05 M tris-HCl buffer, pH 7.4. The adrenal tissue of dogs was homogenized without separation of the medulla. The concentration of the homogenate was 6% for the bovine and 2% for the canine adrenals. Tissue fibers and intact cells were precipitated from the homogenate by centrifugation at 1500 g for 5 min. The mitochondria were separated by centrifugation at 27,000 g for 10 min, and the microsomes at 103,000 g for 1 h. The supernatant obtained after the last centrifugation was used as a cytoplasmic fraction. To disintegrate the mitochondria and obtain soluble mitochondrial proteins a suspension of mitochondria was frozen five times with liquid nitrogen. After the last freezing the preparation was centrifuged for 1 h at 103,000 g and the supernatant was used. All the isolation procedures were carried out at 4°C.

Research Institute of Endocrinology and Metabolism, Kiev. (Presented by Academician of the Academy of Medical Sciences of the USSR N. A. Yudaev.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 74, No. 7, pp. 44-46, July, 1972. Original article submitted July 6, 1971.

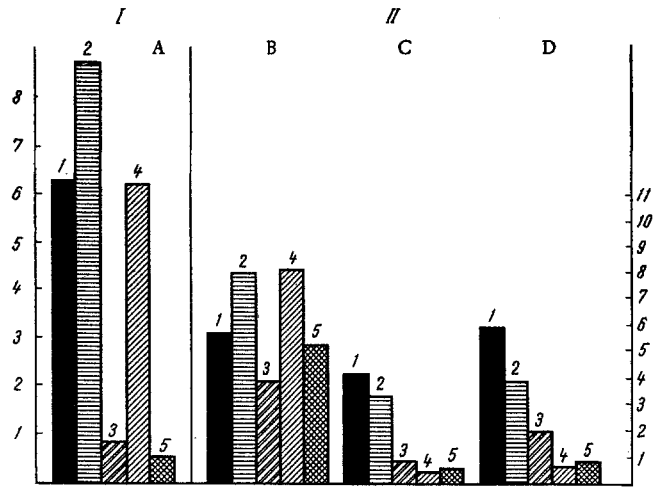

Fig. 1. Distribution of GR and SH-groups in subcellular fractions of bovine adrenals: I) GR activity; II) content of SH groups and protein; A) GR (in nmoles NADP . H_2/min per mg protein); B) SH-groups (in μmoles per 100 mg protein); C) SH-groups (in μmoles per gram fresh tissue); D) protein (in %); 1) homogenate; 2) cytoplasm; 3) intact mitochondria; 4) disintegrated mitochondria; 5) microsomes.

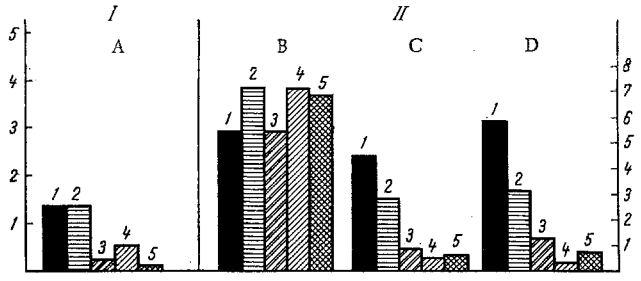

Fig. 2. Distribution of GR and of SH-groups in subcellular fractions of canine adrenals. Legend as in Fig. 1.

The incubation mixture for GR estimation contained 10^{-3} M oxidized glutathione, 6×10^{-5} M NADP·H_2, and 0.05 M tris-HCl, pH 7.4 [5]. The control samples did not contain glutathione. The change in optical density at 340 nm during the 6 min after addition of the adrenal fractions were recorded with an EP-3T Hitachi spectrophotometer at 26°C. The region in which the decrease in optical density was linear was used to calculate the rate of oxidation of NADP · H_2. The protein content was determined by Lowry's method after preliminary extraction of the lipids [7] and also by the biuret method using Benedict's reagent [3]. Sulfhydryl groups were determined by amperometric titration with 0.01 M $AgNO_3$ solution in ammoniacal buffer, pH 9.2, using a revolving platinum electrode.

EXPERIMENTAL RESULTS AND DISCUSSION

Estimation of the GR activity in the adrenal cortical tissue of bovine origin showed that the highest enzyme concentration was present in the cytoplasmic fraction (Fig. 1A). Gr activity per milligram protein in the cytoplasm was about 10 times higher than in the intact mitochondria ($P = 0.02$) and 17 times higher than in the microsomal fraction ($P < 0.02$). After disintegration of the mitochondria the enzyme activity was greatly increased ($P < 0.02$).

The SH groups were distributed in the fractions, calculated per 100 mg protein, similarly to the GR activity (Fig. 1B). The content of SH groups in the cytoplasm was higher than their content in the intact mitochondria and microsomes ($P < 0.001$ and $P < 0.01$, respectively). Disintegration of the mitochondria led to an increase in the content of SH groups calculated per 100 mg protein ($P < 0.001$). The distribution of SH groups calculated per gram tissue was largely determined by the protein content in the corresponding fractions.

The enzyme activity in the adrenal tissue of dogs was investigated without content in the corresponding fractions.

The enzyme activity in the adrenal tissue of dogs was investigated without separation of the cortex and medulla. The most active fraction of the homogenate was the cytoplasm (Fig. 2A). GR activity in the intact mitochondria and microsomes was much lower than in the cytoplasm ($P<0.01$). Determination of the content of SH groups in the subcellular fractions of the dog's adrenals showed no difference when calculated per 100 mg protein (Fig. 2B). The distribution of protein (in %) and SH-groups (in μmoles/g) in the canine adrenal tissue was closely similar to the characteristic values for the bovine adrenal cortex (Fig. 2B, C).

Comparison of the GR activity in the subcellular fractions of the bovine adrenal homogenate with that of the canine homogenate shows that the enzyme distribution was similar, although GR activity in the bovine adrenals was higher than in the canine. The distribution of GR among the fractions of the rat adrenals resembles its distribution in the adrenals of cattle and dogs: the concentration of the enzyme was highest in the cytoplasm, lower in the mitochondria, and lowest in the microsomes [5]. As a result of disintegration of the mitochondria the GR activity was increased. Presumably the enzyme detected in the mitochondrial suspension was intramitochondrial and not the cytoplasmic enzyme adsorbed on the mitochondria.

The character of distribution of SH-groups in the subcellular fractions of the bovine adrenals, calculated relative to protein, corresponds to some extent to the distribution of GR activity. Whereas GR activity in the adrenals of cattle is much higher than its activity in dogs, the content of SH-groups in the adrenal proteins is identical in cattle and dogs.

LITERATURE CITED

1. Yu.M. Torchinskii, Sulfhydryl and Disulfide Groups of Proteins [in Russian], Moscow (1971).
2. Yu.M. Torchinskii, in: Enzymes [in Russian], Moscow (1964), p. 124.
3. J.L. Bailey, Techniques in Protein Chemistry, Amsterdam (1962), p. 294.
4. D.C. Gautherou, Bull. Soc. Chim. Biol. (Paris), 52, 499 (1970).
5. B.W. Harding and D. H. Nelson, Endocrinology, 75, 506 (1964).
6. C. Long, Biochemist's Handbook, London (1961).
7. O.H. Lowry, N. Rosebrough, J. Farr, et al., J. Biol. Chem., 193, 265 (1951).
8. C. Matthyssen and G.W. Goldziher, Biochim. Biophys. Acta, 60, 20 (1962).
9. V.R. Michael and A. L. Lehninger, J. Biol. Chem., 239, 2083 (1964).
10. N.A. Schor and D. Glick, J. Histochem. Cytochem., 16, 185 (1968).
11. F. Tietze, Arch. Biochem., 138, 177 (1970).