Skin Acidic Glycosaminoglycans in Alloxan Diabetic Rats

J. A. Kofoed, Ph.D., C. E. Bozzini, Ph.D., and R. M. Alippi, D.D.S.,
Buenos Aires

SUMMARY

Rats made diabetic by administration of alloxan showed a 36 per cent reduction in total GAG concentration. The concentration of hyaluronic acid was 59 per cent and that of chondroitin-4-sulfate 40 per cent decreased. Heparin concentration was 51 per cent increased. The other sulfated fractions did not appear to be affected. The administration of insulin almost entirely restored the level of the affected GAG to normal values, with the only exception of heparin. DIABETES *19*:732-33, October, 1970.

Previous studies in this and other laboratories have shown that the endocrine system influences both the distribution and synthesis of the acidic glycosaminoglycans (GAG).[1-6] Since both the uronic acid and hexosamine moieties of GAG derive from glucose, and since insulin regulates the utilization of glucose, Schiller and Dorfman[7,8] have postulated and confirmed a role for insulin in the biosynthesis of the GAG. In this paper, the results of experiments investigating the distribution of the GAG in the skin of alloxan diabetic, partially fasted, and insulin treated diabetic rats by using new methods of fractionation[10] are described.

Forty male rats of the Wistar strain were divided into four groups of equal size. One group for control purposes. The second group was sampled twenty-five days after the administration of 160 mg./kg. body weight of alloxan monohydrate. The third group of normal animals was pair-fed with the group of alloxan diabetic rats during the experiment. The fourth group was treated as was the second group, but twenty days after alloxan administration the diabetic animals of this group were injected daily for five days with 30 U. of insulin (Lilly) per kg. of body weight.

The rats were killed and as much shaved skin from the ventral region as possible was obtained. Tissues freed of blood were defatted and dehydrated for thirty-six hours with two changes of ether-acetone. The dry, defatted tissue was digested with papain[9] during twenty-four hours. Following digestion, 10 per cent trichloroacetic acid was added to precipitate any residual protein. Total crude GAG was precipitated by adding four volumes of 5 per cent potassium acetate in ethanol. This extract was then fractionated on cellulose microcolumns by the technic of Svejcar and Robertson.[10] Uronic acid was determined on each fraction using the method of Bitter and Muir.[11] Identification of GAG fractions was accomplished as described by Svejcar and Robertson.[10] Blood glucose was determined by the method of Nelson.[12]

From the Càtedra de Fisiologìa, Facultad de Odontologìa, Universidad de Buenos Aires; and from the Departamento de Radiobiologìa, Comisiòn Nacional de Energìa Atòmica, Buenos Aires, Argentina.

RESULTS

Before sacrifice, the blood glucose levels were as follows: control rats, 28 to 44 mg./100 ml.; pair-fed rats, 22 to 38 mg./100 ml.; untreated diabetic rats, 387 to 534 mg./100 ml.; and insulin treated diabetic rats, 34 to 51 mg./100 ml. The total concentration of the GAG, based on 40 per cent uronic acid content, and the concentration of each GAG fraction in the skin are shown in the table. Total GAG concentration in the untreated diabetic rats was 36 per cent below normal values; the concentration of hyaluronic acid was 59 per cent ($p < 0.001$) and that of chondroitin-4-sulfate 40 per cent ($p < 0.05$) decreased when compared to normal control values. It should be noted that while one N-amino sulfated GAG (heparitin sulfate) was not significantly affected in the untreated diabetic rats, the other (heparin) was significantly increased (51 per cent, $p < 0.01$). This particular behavior of heparin was also observed by Schiller and Dorfman[8] and is not readily explainable. The concentrations of the other sulfated fractions, chondroitin-6-sulfate and dermatan sulfate, did not appear to be importantly affected. Essentially no differences were found in normal and pair-fed rats concerning the distribution of GAG, although the latter presented a 50 per cent reduction in body weight. A similar reduction in body weight was also seen in the untreated diabetic rats. The administration of insulin almost entirely restored the level of the affected GAG to normal values, with the only exception of heparin, which remained unexpectedly high.

DISCUSSION

The present findings confirm and extend those previously reported by Schiller and Dorfman.[7,8] These workers studied only three fractions of GAG, hyaluronic acid, chondroitin sulfuric acid and heparin, because of a lack of reliable methods for a more complete fractionation. Within the GAG

TABLE 1

Concentration of AGAG in skin of normal and diabetic rats

Experimental group	Total AGAG*	HA	HS	C-4-S	C-6-S	DS	H
				μg./gm. dry weight			
Normal	445.2	226.8	21.6	29.2	27.0	78.8	28.3
n = 10	(48.6)	(21.3)	(3.9)	(4.7)	(5.6)	(11.8)	(5.3)
Pair-fed	435.3	194.3	20.8	28.4	27.2	76.8	32.4
n = 10	(36.9)	(18.4)	(3.7)	(4.8)	(5.7)	(11.6)	(7.8)
Diabetic	282.4	92.0	18.L	17.6	22.4	71.8	42.7
n = 10	(16.2)	(14.6)	(3.7)	(4.1)	(4.0)	(9.8)	(7.3)
Insulin-treated	417.6	187.2	18.8	27.6	26.8	76.4	43.2
diabetic, n = 10	(51.3)	(23.6)	(5.4)	(6.8)	(3.7)	(11.1)	(9.8)

*Quantity of AGAG is based on 40 per cent uronic acid content.
Numbers in parentheses denote standard error of the mean.
AGAG = acidic glycosaminoglycans; HA = hyaluronic acid; HS = heparitin sulfate; C-4-S = chondritin-4-sulfate; C-6-S = chondroitin-6-sulfate; DS = dermatan sulfate; H = heparin.

forming the "chondroitin sulfuric acid" of Schiller and Dorfman only the chondroitin-4-sulfate is significantly affected by diabetes, while the chondroitin-6-sulfate and the dermatan sulfate are almost unaffected.

Our findings and those of Schiller and Dorfman present convincing evidence that insulin participates in the metabolism of acidic GAG, although its mechanism of action remains uncertain.

ACKNOWLEDGMENT

This paper was partially supported by Research Grant No. 2710/68 from the Consejo Nacional de Investigaciones Cientificas y Técnicas de la Repùblica Argentina.

REFERENCES

[1] Schiller, S., and Dorfman, A.: The metabolism of mucopolysaccharides in animals. The effect of cortisone and hydrocortisone. Endocrinology. 60:376, 1957.

[2] Asboe-Hansen, G.: The effect of hormones on turnover of mucopolysaccharides in connective tissue. Physiol. Rev. 38:446, 1958.

[3] Dziewiatkowski, D. D.: Hormonal effects on connective tissue, in "Connective tissue: intercellular macromolecules," Boston, Little, Brown and Co. 1964, p. 215.

[4] Kofoed, J. A., Bozzini, C. E., and Tocci, A. A.: Hydrocortisone and the synthesis of glycosaminoglycans in skin and trachea of rats. Acta Physiol. Latinoam. 18:327, 1968.

[5] Kofoed, J. A., Bozzini, C. E., and Tocci, A. A.: The distribution of acidic glycosaminoglycans in tracheal cartilage of radiothyroidectomized rats: the effect of isomeres of triiodothyronine. J. Endocr. (in press).

[6] Kofoed, J. A., and Bozzini, C. E.: The concentration and synthesis of glycosaminoglycans in skin and tracheal cartilage of normal and orchidectomized rats injected with testosterone. Acta Endocr. (in press).

[7] Schiller, S., and Dorfman, A.: The metabolism of mucopolysaccharides in animals. IV. The influence of insulin. J. Biol. Chem. 219:625, 1957.

[8] Schiller, S., and Dorfman, A.: The distribution of acid mucopolysaccharides in skin of diabetic rats. Biochim. Acta 78:371, 1963.

[9] Schiller, S., Slover, G. A., and Dorfman, A.: A method for the separation of acid mucopolysaccharides: its application to the isolation of heparin from the skin of rats. J. Biol. Chem. 236:983, 1961.

[10] Svejcar, J., and Robertson, W. van B.: Microseparation and determination of mammalian acidic glycosaminoglycans (mucopolysaccharides). Analyt. Biochem. 18:333, 1967.

[11] Bitter, T., and Muir, H. N.: The determination of uronic acids in organic fluids. Analyt. Biochem. 4:330, 1962.

[12] Nelson, N.: Photometric adaptation of Somogyi method for the determination of glucose. J. Biol. Chem. 153:375, 1944.