CHANGES IN POLYAMINE CONTENT OF RAT LIVER FOLLOWING HYPOPHYSECTOMY AND TREATMENT WITH GROWTH HORMONE*

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Received March 17, 1966

The injection of pituitary growth hormone into rats produces a series of events in the liver, the earliest of which are the stimulation of amino acid accumulation by the cells (Riggs and Walker, 1960) and the stimulation of nuclear RNA polymerase activity (Widnell and Tata, 1964). Then, hepatic RNA production (Talwar et al., 1964) increases, and eventually protein synthesis is stimulated. The latter event is correlated with an increase in the number of polysomes in the liver (Korner, 1964). Hypophysectomy, which eliminates growth hormone from the circulation, reduces the activity of hepatic nuclear RNA polymerase and diminishes the rates of RNA and protein synthesis.

Growth hormone may stimulate RNA and protein synthesis in the liver by increasing the intracellular concentration of some substance, which possesses the ability to enhance the activity of anabolic processes. The polyamines, spermidine and spermine, which are found in many tissues including the liver (Rosenthal and Tabor, 1956), do have the ability to influence RNA and protein synthesis. Both substances have stimulated the activity of DNA-dependent RNA polymerase

* Supported by grants from the U.S.P.H.S. (AM 04521) and from the Life Insurance Medical Research Fund (G-65-11).

isolated from A. vinelandii (Krakow, 1963) and from M. lysodeikticus (Fox and Weiss, 1964). Also, they have stimulated amino acid incorporation into protein in a ribosomal system prepared from S. typhimuruim (Martin and Ames, 1962) by increasing the number of functional or 100 S ribosomes in the system. Similarly, amino acid incorporation into the proteins of microsomal and ribosomal systems prepared from rat liver have been stimulated by the addition of spermidine and spermine (Hershko et al., 1961). Silman et al. (1965) have also shown that spermine caused ribosomes prepared from E. coli and mouse liver to aggregate. In addition, the concentration of spermidine, in particular, has been found to increase in the rat liver during regeneration (Dykstra and Herbst, 1965) and in the chick embryo during phases of development characterized by intense RNA and protein synthesis (Raina, 1963; Caldarera et al., 1965). Thus, it was of great interest to learn if hypophysectomy and treatment with growth hormone have any influence on the concentrations of spermidine and spermine in the rat liver.

Materials and Methods

Normal and hypophysectomized female rats were purchased from the Charles River Breeding Laboratories. Normal rats were used for experiments when they were 29 days of age and weighed 60-79 grams. The hypophysectomized rats were operated upon at 25 days of age and used when they were 36-62 days of age and weighed 50-80 grams. Bovine growth hormone (NIH-BGH-9)† was injected intraperitoneally in 0.5 ml of slightly alkaline 0.9% NaCl at various times before the animals were sacrificed. Control rats received comparable injections of 0.9% NaCl.

The rats were killed by cervical fracture, and the liver was rapidly

† A gift of the Endocrinology Study Section of the NIH, U.S.P.H.S.

excised and placed in a chilled beaker. Two livers were pooled for each determination. They were blotted, weighed and homogenized rapidly in 10 ml cold distilled water. Then 10 ml of 8% trichloroacetic acid was added and homogenization was completed. The homogenate was centrifuged, and the protein-free supernatant was used for determination of spermidine and spermine. Trichloroacetic acid was extracted from an aliquot (11-15 ml) of the supernatant with ether. The aliquot was then neutralized and placed on a 0.9 x 5 cm column Dowex 50 (K^+-form, 2% cross-linked, 100-200 mesh). Gradient elution was carried out with 2.5 N HCl; 275 ml of water were in the mixing vessel. The fractions (10 ml) were concentrated under reduced pressure at 60 C, and the amounts of spermidine and spermine present were estimated following their conversion to DNP-derivatives by the method of Rosenthal and Tabor (1956). The identity of spermidine and spermine in the fractions was confirmed by paper chromatography with two solvent systems (ethylene glycol monomethyl ether-propionic acid-water, 70:15:15, saturated with NaCl; n-propanol-triethylamine-water, 85:3:15), and by comparison of the absorption spectra of the DNP-derivatives with those of the authentic compounds under the conditions described by Dubin (1960).

Results and Discussion

The amount of spermidine in the livers of hypophysectomized rats was considerably less than that present in the livers of normal rats of comparable weight (compare the value for normal rats with those of hypophysectomized rats which received saline, Table I). While the mean concentration of spermidine varied among the groups of hypophysectomized rats studied (0.73-0.94 μmoles/g), the variation did not

appear to be correlated with age. The amount of spermine in the livers of hypophysectomized rats was somewhat greater than that present in normal rat livers. However, the combined polyamine concentration (spermidine + spermine) was still significantly less than normal in the livers of all groups of hypophysectomized rats studied.

When 1 mg of bovine growth hormone was administered to hypophysectomized rats 3 hours before the livers were removed, there was no

Table I

Amounts of Spermidine and Spermine in Rat Liver
(μmoles/g wet liver)

Rats	Spermidine	Spermine
Normal	1.37 ± 0.03‡ (8)	0.73 ± 0.04 (7)
Hypox. + Saline (3 hours)	0.73 ± 0.03 (6)	0.88 ± 0.05 (6)
Hypox. + BGH (3 hours)	0.71 ± 0.02 (4)	0.83 ± 0.05 (4)
Hypox. + Saline (24 hours)	0.94 ± 0.03 (5)	0.87 ± 0.01 (3)
Hypox. + BGH (24 hours)	1.23 ± 0.07 (5)	0.79 ± 0.03 (4)
Hypox. + Saline (4 days)	0.81 ± 0.03 (5)	0.95 ± 0.03 (5)
Hypox. + BGH (4 days)	1.28 ± 0.08 (6)	0.69 ± 0.05 (6)

‡Mean ± S.E., (n) = no. of observations, Hypox. = hypophysectomized.

effect on the amounts of spermidine or spermine in the liver. In contrast, when two 0.5 mg doses of growth hormone were given 24 and 14 hours before removal of the liver, the concentration of spermidine was increased 31%. The concentration of spermine was unaffected. A similar increase in the spermidine concentration 24 hours following growth hormone treatment was observed in another group of rats (hypox. = 0.65 ±

0.06; Hypox. + BGH = 0.82 ± 0.03; n = 6). It should be noted that it is during this same 24 hour period of exposure to growth hormone that alterations in hepatic RNA and protein metabolism are produced. Chronic treatment (1 mg/day/4 days) of hypophysectomized rats with growth hormone increased the concentration of spermidine 58% above that of saline-injected controls, bringing it within the range found for normal rats of similar weight. The amount of spermine in the livers of the hormone-treated rats was similar to that of normal rats and below that of the saline-injected controls. However, the combined polyamine (spermidine + spermine) level of the hormone-treated group was significantly greater than that of the controls. In the regenerating rat liver, Dykstra and Herbst (1965) have obtained essentially similar results, namely, that the spermidine concentration is elevated, but the spermine level is initially unaltered and then gradually decreases.

These experiments show then that following hypophysectomy, when hepatic protein synthetic activity is depressed, the concentration of spermidine in the liver is markedly reduced. Growth hormone, which stimulates hepatic RNA and protein synthesis, increases the amount of these substances in the cells. These experiments do not indicate if growth hormone increases the content of spermidine by increasing its rate of biosynthesis or by retarding its rate of metabolism. However, it is worth noting that an early action of growth hormone is to increase the ability of the liver to accumulate amino acids, which presumably serve as the biosynthetic precursors for spermidine biosynthesis (see Raina, 1963). Whether or not these changes in spermidine concentration in the liver actually cause some or all of the changes in RNA and protein metabolism that follow hypophysectomy and growth

hormone treatment remains to be established.

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