Age- and Growth Hormone-Induced Alterations in Renal Sulfate Transport

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ABSTRACT

The effects of growth hormone (GH) treatment on renal sodium sulfate cotransport (NaSi-1) were studied in adult (9–10 months) and old (22–23 months) male Fischer 344 rats. All animals received twice-daily s.c. injections of recombinant human GH (hGH; 4 mg/kg) for up to 6 days. Animals were sacrificed by exsanguination on days 0, 1, 2, 3, 4, 5, and 6. Kidneys were removed, and kidney cortex was trimmed immediately and used for RNA and membrane preparations. Plasma hGH concentrations were significantly lower in old rats during the hGH treatment (P < .05). Insulin-like growth factor-I (IGF-I) levels were significantly increased and remained stable after day 2 of hGH treatment in both age groups (P < .05). There was no significant difference in plasma IGF-I levels between age groups. Plasma IGF-I concentrations were significantly lower in old rats during the hGH treatment (P < .05). Insulin-like growth factor-I (IGF-I) levels were significantly increased and remained stable after day 2 of hGH treatment in both age groups (P < .05). There was no significant difference in plasma IGF-I levels between age groups. Plasma IGF-I binding protein 3 (IGFBP-3) concentrations were significantly higher in 9- to 10-month-old rats compared with that in 22- to 23-month-old animals (P < .001). The NaSi-1 mRNA levels were significantly increased on days 2 and 3 of hGH treatment (P < .05) and then gradually decreased to the control value. The NaSi-1 protein levels in old animals (22–23 months) were also significantly lower than that of 9- to 10-month-old animals and were significantly increased from day 2 of hGH treatment, reaching a maximum level on day 3 or 4 and then returning to the baseline level in both age groups. From these results, it was concluded that 1) NaSi-1 mRNA and protein levels are lower in old animals and increase in both adult and aged rats after hGH treatment, 2) plasma IGF-I levels are similar in adult and aged rats and increase after hGH treatment, and 3) plasma IGFBP-3 levels are lower in old rats and remain unchanged after hGH treatment.

This increased reabsorption of sulfate in young animals is due to an increased capacity for sodium-dependent sulfate cotransport in renal BBM (Pena and Neiberg, 1992). Aged male Fischer 344 rats (22–23 months) have a lower urinary excretion rate of sulfate than do adult rats (4–5 months), but there is no change in serum sulfate concentrations (Bakhtian et al., 1993). This lower urinary excretion of sulfate is greater than can be expected by altered renal function in these aged rats because there is only a modest (19%) decrease in GFR. Additionally, aged rats exhibit a defect in the active tubular reabsorption of sulfate under conditions of sulfate depletion (Bakhtian et al., 1993).

The renal regulation of sulfate has not been extensively investigated. Studies in animals have demonstrated alterations in the renal reabsorption of sulfate after pharmacological doses or deficiencies of thyroid hormone (Tenenhouse et al., 1991; Sagawa et al., 1999), dexamethasone (Renfro et al., 1998), or glucocorticoids (Tenenhouse et al., 1991). This study investigated the role of growth hormone (GH) in the regulation of renal sulfate reabsorption in adult and aged rats.

Homeostasis of inorganic sulfate is maintained by its renal reabsorption. Renal tubular reabsorption of sulfate involves two transport systems. Inorganic sulfate crosses the basolateral membrane (BLM) of proximal tubular cells via sodium-dependent sulfate cotransport (NaSi-1). Sulfate exits from the cells by an anion exchange process in the renal basolateral membrane (BLM). The renal reabsorption of sulfate is greater in young animals and decreases as animals approach adulthood (Neiberg, 1992; Lee and Morris, 1995).

ABBREVIATIONS: BBM, brush border membrane; GH, growth hormone; hGH, human growth hormone; rGH, rat growth hormone; GFR, glomerular filtration rate; IGF-I, insulin-like growth factor-I; IGFBP-3, insulin-like growth factor binding protein 3; RT, reverse transcription; PCR, polymerase chain reaction; SSC, standard saline citrate; MDCK, Madin-Darby canine kidney; NaSi-1, sodium-dependent sulfate cotransport.
pared in the two different age groups. 

Effect of Age and GH on NaSi-1 Cotransport

Materials and Methods

Animals. Adult (9–10 months) and old (22–23 months) male Fischer 344 rats were obtained from specific pathogen-free breeding colonies maintained for the National Institute of Aging by Harlan Sprague-Dawley (Indianapolis, IN) and housed in sterilized cages containing sterilized bedding. Temperature, humidity, and lighting cycles were kept constant. After 1 week of acclimation, all animals were randomly divided into seven groups (n = 4–9), and they received twice-daily (8:00–9:00 AM and 5:00–6:00 PM) s.c. injections of hGH (4 mg/kg) for 0 to 6 days. All animals were sacrificed by exsanguination under light methoxyflurane (Metofen; Pitman-Moore, Mundelein, IL) anesthesia on days 0, 1, 2, 3, 4, 5, and 6 between 10:00 AM and 12:00 noon to control for the diurnal effect of hormones. Kidneys were removed from the animals, and kidney cortex was trimmed immediately. Tissue samples were frozen immediately in liquid nitrogen and stored at −80°C until used for RNA and membrane preparations.

GH, IGF-I, and IGFBP-3 Analysis. Plasma hGH concentrations and plasma rat GH (rGH) concentrations were measured using separate radioimmunoassay kits (Diagnostic Systems Laboratories, Webster, TX, and Amersham Life Sciences, Clearbrook, IL, respectively) according to the manufacturer’s instructions. IGF-I was separated from the binding proteins by acid/ethanol extraction, and the plasma IGF-I concentrations were measured using a rat IGF-I radioimmunoassay kit obtained from Diagnostic Systems Laboratories according to the manufacturer’s procedure. Plasma IGFBP-3 levels were measured using human IGFBP-3 immunoradiometric assay kit (Diagnostic Systems Laboratories) according to the manufacturer’s instructions. The cross-reactivity of the human IGFBP-3 immunoradiometric assay against rat IGFBP-3 could not be determined because pure rat IGFBP-3 was not available.

Tissue RNA Preparation. Total RNA was prepared from rat kidney cortex using TRIzol reagent (Life Technologies, Gaithersburg, MD) according to the manufacturer’s instructions. Tissue obtained from the animals at the same time point was pooled. Final RNA concentrations in samples were determined by absorbance at 260 nm.

Reverse Transcription-Polymerase Chain Reaction (RT-PCR). The levels of NaSi-1 mRNA were measured in total RNA isolated from kidney cortex as described previously (Sagawa et al., 1998a). The size of amplified NaSi-1 mRNA was 700 bp. Amplification efficiency, loading, and transfer efficiencies were corrected with externally added deletion standard cRNA (600 bp), which was prepared by deleting 100 bp of native cDNA located in the middle of the sequence. For the reverse transcriptase reaction, 10 ng of tissue total RNA and 700 fg of deletion standard cRNA were coamplified using SuperScript (Promega, Madison, WI) at 42°C for 45 min. After the reverse transcriptase reaction, additional reactants for PCR, including U/72°C, were directly added to the same tubes. After first heating at 95°C for 1 min, 25 cycles were run as follows: 95°C for 1 min, 65°C for 1 min, and 72°C for 1 min. Final extension was at 72°C for 7 min, and the samples were kept at 4°C.

Southern Hybridization. The RT-PCR products were size separated on 1.5% agarose gel and transferred to hybridization matrices (Duralon-UV; Stratagene, La Jolla, CA). The RT-PCR products were loaded onto the gel in duplicate. The hybridization probe was 300-bp NaSi-1 cDNA (positions 492–792). The random primer labeling reaction was prepared using a random primer labeling kit (Prime-It; Stratagene). Matrices were prehybridized for a minimum of 4 h and hybridized with the probe overnight in hybridizing solution (5× standard saline citrate (SSC), 1% SDS, 5× Denhardt’s 50% formamide, 100 ng/ml sheared salmon sperm DNA) at 42°C. Matrices were washed five times in 2× SSC and 0.1% SDS at room temperature and then twice in 0.1× SSC and 0.1% SDS at room temperature followed by 0.1× SSC and 0.1% SDS at 65°C until the radioactivity was decreased to the background levels. Hybridization signals were visualized and analyzed using a PhosphorImager (Molecular Dynamics, Sunnyvale, CA). The RT-PCR results were expressed as a ratio between amplified NaSi-1 mRNA and amplified deletion standard cRNA, added as an external standard, and were normalized by the amount of total RNA.

Crude Membrane Preparation for Enzyme-Linked Immunosorbent Assay. Crude membrane fractions were prepared from kidney cortex to determine the protein expression levels in the tissue. Approximately 0.50 g of ground tissue powder was homogenized in the homogenizing buffer (250 mM sucrose, 10 mM triethanolamine-HCl, pH 7.6) and centrifuged at 1250g for 10 min at 4°C. The supernatant was further centrifuged at 100,000g for 30 min at 4°C (Thomas and McNamme, 1990). The pellet containing crude membrane fractions was resuspended in 2.5% Triton-X in 1× PBS (sample buffer) to gently extract proteins. Protein concentrations were measured according to the method of Lowry et al. (1951).

Sandwich-Type Enzyme-Linked Immunosorbent Assay Procedure. NaSi-1 polyclonal and monoclonal antibodies were raised against rabbits and mice, respectively (Sagawa et al., 1997). The NaSi-1 protein abundance in the crude membrane preparations was measured as previously described (Sagawa et al., 1998b). Wells were incubated with 500 µg/well concentration of samples at 4°C overnight. The amounts of NaSi-1 in the tissue were calculated with a constructed standard curve using serial dilution of the NaSi-1 standard protein (6.58–164 fmol).
Statistical Analysis. All results are expressed as mean ± S.D. The differences in plasma hGH, IGF-I, and IGFBP-3 concentrations and NaSi-1 mRNA and protein levels between study groups and days of treatment were compared using two-way ANOVA.

Results

Plasma GH, IGF-I, and IGFBP-3 Concentrations. Plasma hGH, IGF-I, and IGFBP-3 concentrations were measured on days 0, 1, 2, 3, 4, 5, and 6 of hGH treatment in both age groups. Animals received hGH administration at 8:00 AM and 5:00 PM, and the blood samples were taken at 10:00 AM. Plasma hGH concentrations were not detectable on day 0, and the levels remained constant after day 1 of the hGH treatment in both adult and aged rats (Fig. 1). Average plasma hGH concentrations were significantly lower in adult rats compared with that in aged rats (3510 ± 1210 and 4440 ± 1250 ng/ml; n = 27–29, respectively; P < .05). There were no significant differences in endogenous rGH levels without hGH treatment between adult and aged rats (7.91 ± 7.30 and 8.46 ± 5.39 ng/ml; n = 9–10, respectively). Plasma IGF-I concentrations were significantly increased and remained constant after day 2 of hGH treatment in both age groups (P < .05; Fig. 2). There were no significant differences in plasma IGF-I levels between age groups. In contrast, plasma IGFBP-3 concentrations were significantly lower in the 22- to 23-month-old rats compared with that in the 9- to 10-month-old rats (P < .001; Fig. 3). There were no significant differences in plasma IGFBP-3 concentrations among days of hGH treatment.

NaSi-1 mRNA Level and Protein Abundance. Kidney cortex from the animals treated with hGH for 0, 1, 2, 3, 4, 5, and 6 days was harvested. Total RNA was isolated from the tissue pool, and NaSi-1 mRNA was analyzed in duplicate for each sample. The NaSi-1 mRNA level was significantly lower in 22- to 23-month-old rats compared with that in 9- to 10-month-old animals (P < .001; Fig. 4). The change in NaSi-1 mRNA levels after hGH treatment followed the same pattern for both age groups. The NaSi-1 mRNA level increased from day 2 of hGH treatment in both age groups (P < .05). The level gradually decreased to the day 0 value after reaching a maximum level on day 3.

NaSi-1 transport protein abundance was measured in the crude membrane fraction isolated from the kidney cortex. The NaSi-1 protein level in old animals (22–23 months) was significantly lower than that in 9- to 10-month-old animals (Fig. 5). The profile of NaSi-1 transport protein throughout the hGH treatment period was similar to that of NaSi-1 mRNA. The NaSi-1 protein levels significantly increased from day 3 of hGH treatment compared with the day 0 value (P < .05) and reached a maximum level on day 4 in the 9- to 10-month-old animals and on day 3 in the 22- to 23-month-old animals. The NaSi-1 protein levels after hGH treatment returned to the day 0 value on day 5. There were no significant differences in NaSi-1 protein abundance on days 1, 5, and 6 compared with the day-0 value.

Discussion

In the current investigation, we evaluated the age-dependent effect of hGH treatment on plasma IGF-I and IGFBP-3 concentrations and on age-dependent hGH-induced alter-
ations of renal NaSi-1 mRNA and protein expression levels in rats. We found no significant differences in pretreatment plasma IGF-I levels between 9- to 10-month-old and 22- to 23-month-old rats. This contrasts with studies in humans that reported lower GH and IGF-I levels in elderly subjects compared with that in young adults (Porch et al., 1997; Marcus and Hoffman, 1998). After twice-daily hGH injections, hGH levels remained high throughout the 6-day study period and were significantly greater in old rats than in the adult rats. Plasma IGF-I concentrations in both adult and old rats increased by day 1 of hGH treatment and remained elevated (149% of baseline) until the end of the 6-day study period. These findings are similar to that previously reported in humans (Verhagen et al., 1995) and monkeys (Sun et al., 1999). There were no significant differences between adult and old animals in IGF-I plasma concentrations after hGH treatment.

IGF-I circulates with binding proteins, with IGFBP-3 representing the major IGF carrier protein. A half-life of free IGF-I is 16 to 22 min in healthy volunteers, whereas protein-bound IGF-I has a half-life approximately 50-fold longer. IGFBP-3 levels are increased along with IGF-I levels after GH treatment in monkeys (Johnson et al., 1996). Froger-Gaillard et al. (1989) reported that IGFBP-3 synthesis was increased in the presence of IGF and suggested that the IGFs were involved in controlling the synthesis of their binding proteins. Our results demonstrated that there were no significant changes in plasma IGFBP-3 concentrations during hGH treatment in both age groups. However, we found that the plasma IGFBP-3 levels were significantly lower in 22- to 23-month-old rats compared with that in 9- to 10-month-old animals. These results suggest that IGF-I and IGFBP-3 are not coregulated and that less IGF-I would be bound to IGFBP-3 in the plasma of old rats, suggesting that both the clearance and distribution of IGF-I would differ in adult and old rats.
Inorganic sulfate is a physiological anion that is used in sulfation reactions, involving both endogenous and exogenous substrates. Numerous structural components of membranes and tissues are sulfate conjugates: sulfated glycosaminoglycans are components of cartilage and other tissues, and cerebroside sulfate is a constituent of the myelin membrane in the brain (DeMeio, 1975; Dietrich et al., 1977). Thus, sulfation represents a key metabolic process in organ differentiation, growth, and development. Suckling rats that received \([^{35}S]\)sulfate incorporated radiosulfate into cartilage as chondroitin sulfate (Dziewiatowski, 1951). Moreover, studies have shown that radiosulfate uptake in cartilage is controlled by GH in mice (McKern, 1985) and eels (Duan and Inui, 1990) or by IGF-I in tadpoles (Schneider and Hanke, 1997) and salmon (McCormick et al., 1992). McKern (1985) also suggested that GH might regulate skeletal growth by inhibiting degradation of sulfated glycosaminoglycans in cartilage. Renal reabsorption of sulfate is greater in young animals than in adults (Lee and Morris, 1995); however, little is known about sulfate renal reabsorption in old animals, although Bakhtian et al. (1993) reported that the urinary excretion of sulfate in old Fischer 344 rats is decreased, without significant changes in the serum concentrations of sulfate. We hypothesized that renal sulfate reabsorption may be decreased in old animals due to decreased levels of plasma GH and that the decreased renal sulfate reabsorption may be reversed by the administration of pharmacological doses of GH. Our results demonstrated that the levels of NaSi-1 mRNA and protein expression in the kidney cortex were significantly lower in old animals. Moreover, the NaSi-1 mRNA and protein expression levels in the kidney increased after hGH treatment in both old and adult animals. These results suggest that NaSi-1 may be at least in part regulated by circulating GH concentrations. However, we did not observe significant differences in baseline plasma rGH or IGF-I levels between age groups, which suggest that lower levels of NaSi-1 mRNA and protein in old animals might not be associated with decreased levels of circulating GH or IGF-I.

The findings of our investigation are similar to those previously reported for renal phosphate reabsorption. Aging in humans and in rats is associated with a decreased renal tubular reabsorption of phosphate (Levi and Rowe, 1992) and decreased abundance of sodium/phosphate cotransporter (NaPi-2) mRNA and protein (Sorribas et al., 1996). Additionally, Levi et al. (1989) demonstrated that the age-related decrease in BBM sodium/phosphate cotransport is associated with an age-related increase in renal BBM cholesterol content and that alterations of BBM cholesterol can produce decreases in BBM sodium/phosphate cotransport. We also examined the effects of cholesterol content on sodium/sulfate uptake in MDCK/NaSi-1 cells (MDCK cells stably transfected with NaSi-1). The addition of cholesterol to the incubation buffer resulted in decreased sulfate uptake with no significant effect of sodium-independent sulfate uptake. The magnitude of the effect was dependent on the cholesterol concentration, with 0.2 mM reducing the \(V_{\text{max}}\) value for sodium-dependent sulfate uptake by an average of 26%. Thus, it is possible that lower levels of steady-state NaSi-1 mRNA in old rats are due to a decreased transcriptional rate or a decreased stability of the mRNA due to an increased cholesterol level or some other physiological alterations in old animals.

In summary, the current investigation demonstrated that 1) NaSi-1 mRNA and protein levels were lower in aged (22–23 months) Fischer 344 rats and transiently increase in both adult (9–10 months) and aged rats after hGH treatment, 2) plasma IGF-I levels are similar in adult and aged rats and increase after hGH treatment, and 3) plasma IGF-BP-3 levels are significantly lower in old rats and remain unchanged after hGH treatment.

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