Effect of Heparin Administration on Plasma Growth-Hormone Concentrations[1] (34629)

TOSHIO TSUSHIMA, FUKASHI MATSUZAKI, AND MINORU IRIE
(Introduced by M. S. Raben)

The Third Department of Internal Medicine, University of Tokyo, Faculty of Medicine, Hongo, Tokyo, Japan, and the Department of Endocrinology, Toranomon Hospital, Akasaka, Minatoku, Tokyo, Japan

Recently, much information has been accumulated about the factors which influence the secretion of human growth hormone (HGH). We reported previously that the acute reduction of plasma free fatty acids (FFA) concentration by the administration of nicotinic acid can stimulate HGH secretion (1). This finding is interesting, because one of the important roles of growth hormone is lipid mobilization. The present paper describes the effect of heparin administration on plasma FFA and HGH in normal subjects, and the subsequent fall of plasma FFA is demonstrated to be accompanied by a marked rise of plasma HGH level.

Materials and Methods. All subjects studied were normal young male volunteers, their ages ranging from 20 to 25 years old. They had no family history of diabetes mellitus or other metabolic disorder. Obese subjects were not included. After overnight fast, they were kept at bed rest for at least 30 min before starting the tests, and avoided sleep during the tests. An indwelling catheter was placed into an antecubital vein and kept patent by a slow infusion of saline. All the blood samplings and injections were done through this catheter. Blood was drawn into heparinized syringes and centrifuged immediately. Plasma was stored at —20° until plasma concentrations of HGH, FFA, glucose, and α-amino nitrogen were determined.

Plasma HGH was measured by the two antibody radioimmunoassay method of Schalch and Parker (2). Wilhelmi's highly purified HGH(HS 1032B) was used as a standard. Minimum detectable dose of plasma HGH in our laboratory was 0.1 ng/ml using 1 : 10 diluted plasma. Plasma concentrations of glucose, FFA, and α-amino nitrogen were determined by glucose oxidase method (3), Dole's method (4), and ninhydrin method (5), respectively.

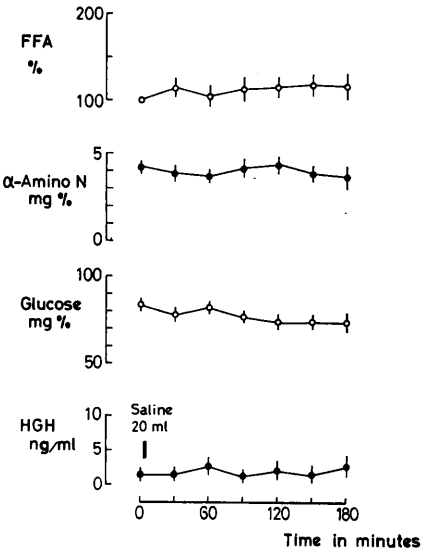

FIG. 1. Plasma FFA (described as percentage of the starting value), α-amino nitrogen, glucose, and HGH levels after intravenous administration of 20 ml of saline at 0 time in 10 subjects (mean±SEM).

Results. Group 1 (controls). Ten subjects were given 20 ml of saline intravenously as controls. Figure 1 shows the plasma concentrations of FFA (described as percentage of the starting value), glucose , α-amino nitrogen, and HGH at 30-min intervals after saline administration. No significant changes were observed during 3 hr in these values. Plasma HGH value at 0 time was 1.44±0.65 ng/ml (mean±standard error of the mean), and FFA, 586±55 μeq/liter.

[1] This study was partly supported by U.S. Public Health Service Grant 5R01-AM 10138.

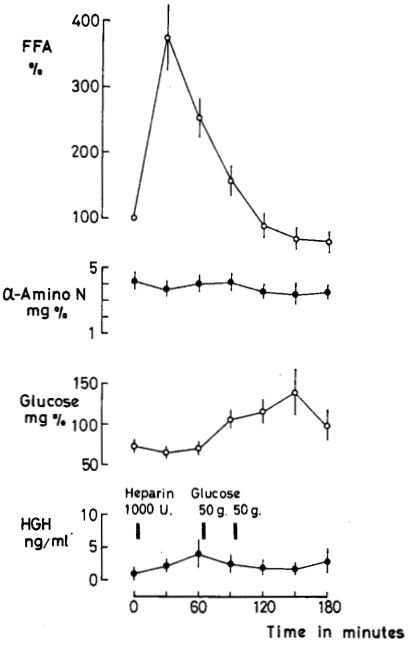

FIG. 2. Plasma FFA, α-amino nitrogen, glucose, and HGH levels after intravenous administration of 1000 units of heparin at 0 time in seven subjects (mean±SEM).

Group 2. In seven subjects, 1000 units of heparin sodium were injected intravenously at 0 time. Basal plasma HGH and FFA values were 2.51±0.77 ng/ml, 557±62 μeq/liter, respectively. These values were statistically not different from the values of the control group. After heparin injection, the plasma FFA level increased rapidly to about 300% of the starting value at 30 min and decreased thereafter as shown in Fig. 2. In association with the fall of plasma FFA from the peak value, the plasma HGH level increased gradually in all cases and reached to 12.18±2.35 ng/ml at 150 min. The HGH values at 120, 150, and 180 min were significantly higher than the respective control values ($p<.01$). There were no significant changes in plasma glucose and α-amino nitrogen levels.

Group 3. In six subjects, 1000 units heparin were administered intravenously at 0 time, and further injections of 500 units at 60 and 90 min were made to prevent the fall of plasma FFA. As demonstrated in Fig. 3, plasma FFA level was kept elevated above 250% of the starting value during the first 2 hr and then declined, but the value at 180 min remained high at 200%. Under this condition, the rise of HGH was not observed, and the concentrations of plasma glucose and α-amino nitrogen remain unchanged throughout the period.

Group. 4. Five subjects were given 50 g of glucose orally at 60 and 90 min, combined with intravenous administration of 1000 units of heparin at 0 time. In this group the rise of HGH was suppressed, though there was a marked fall of plasma FFA. The results are shown in Fig. 4.

Discussion. As we have reported (1), the lowering of plasma FFA by nicotinic acid administration was followed by a marked rise of plasma HGH without changes in plasma glucose levels. This finding was confirmed thereafter in a total of 17 cases. When nicotinic acid was given with heparin, the reduction of plasma FFA was prevented, and the HGH rise could not be demonstrated. It appears that a negative feedback mechanism operates between HGH secretion and plasma FFA level so far as these experiments are concerned. In the present report, the rise of

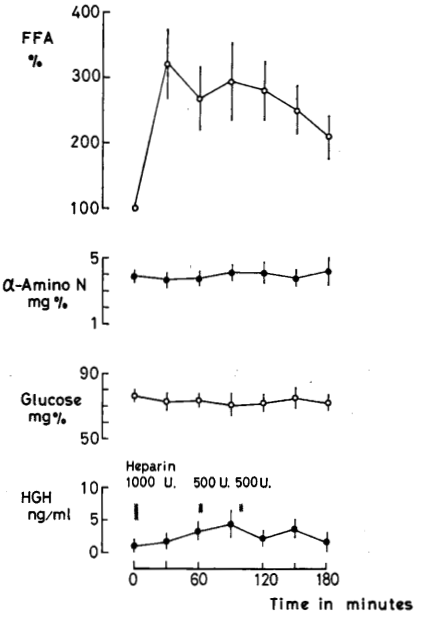

FIG. 3. Plasma FFA, α-amino nitrogen, glucose, and HGH levels after intravenous administration of 1000 units of heparin at 0 time, and 500 units at 60 and 90 min in six subjects (mean±SEM).

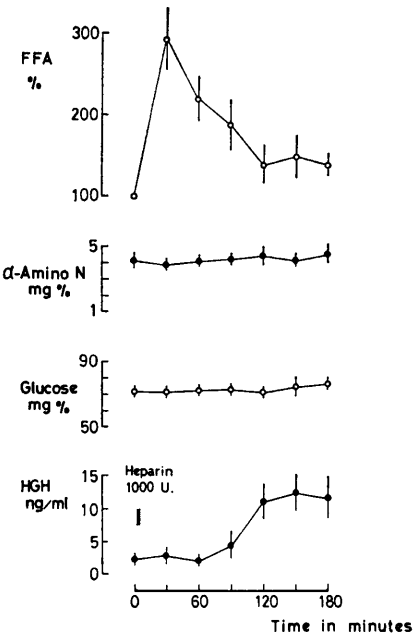

FIG. 4. Plasma FFA, α-amino, nitrogen, glucose, and HGH levels after intravenous administration of 1000 units of heparin at 0 time, combined with oral administration of 50 g of glucose at 60 and 90 min in five subjects (mean±SEM).

HGH was observed in association with the fall of plasma FFA, whereas it did not occur when plasma FFA was kept elevated above 200% of the basal level.

It is well recognized that insulin-induced hypoglycemia (6), falling blood glucose values (7, 8), stress (9), and infusion of amino acids (10) can stimulate HGH secretion.

In our present study (Group 2) plasma glucose levels showed no significant changes. The rise of plasma HGH, therefore, cannot be ascribed to changes in plasma glucose level. It differs from the stimulation of HGH secretion by amino acid infusion because there occurred no elevation in plasma α-amino nitrogen, and immunoreactive insulin levels (data not shown). Stress or direct effect of heparin can be ruled out, since neither control studies with saline injection (Group 1), nor repeated injections of heparin (Group 3) resulted in the elevation of plasma HGH.

These results suggest that reduction or fall of plasma FFA can be one of the stimuli to HGH secretion comparable to hypoglycemia and falling blood glucose values. Schalch and Kipnis (11) have reported that plasma HGH remained unchanged after the fat meal–heparin regimen. However, it was only 60 min observation after heparin administration, and in our experiment there were no significant changes of plasma HGH level during the initial 60 min.

It is not clear why the fall of plasma FFA is linked with the augmentation of HGH secretion. The secretion of HGH is well documented to be under the control of the hypothalamus. Many investigations show that the brain is mainly dependent upon glucose as an energy fuel. But other substrates have been demonstrated to serve as an energy source. Recently Owen *et al.* (12) reported that beta-hydroxybutyrate and acetoacetate replaced glucose as the predominant fuel for brain metabolism during starvation in obese subjects. Furthermore, the experiment with the use of palmitate-C-14 indicated that rat brain preparation can oxidize fatty acids to CO_2, although the rate of conversion is lower than that observed in other tissues (13).

It is conceivable, therefore, that the fluctuation of plasma FFA has some effect on brain metabolism. Thus the rise of plasma HGH concentration after the fall of plasma FFA could well be explained in the same manner as in the case of falling blood glucose values, both of them being induced by the acute reduction of energy fuel for the hypothalamus. In Group 4 HGH secretion was suppressed by glucose administration in spite of the marked fall of plasma FFA. This fact could be considered to show that plasma glucose and FFA can be replaced by each other as an energy substrate for the brain tissue.

From these observations, it would be reasonable to state that growth hormone is secreted when energy substrate, whether glucose or FFA, is acutely deprived, and thus secreted growth hormone supplies FFA for energy requirement, which contributes to sparing of glucose and protein. The fact that rebound of plasma FFA after arginine infusion is impaired (14) and recovery from insulin-induced hypoglycemia is delayed (15) in selective growth hormone-deficient patients also suggests the phsyiologically important role of growth hormone for energy supply.

Summary. Plasma HGH, FFA, glucose, and α-amino nitrogen levels were determined in seven young men after heparin administration. Plasma FFA increased rapidly, followed by a gradual fall. Corresponding to the fall of plasma FFA, a marked rise of plasma HGH was demonstrated. This HGH rise was diminished when plasma FFA was kept elevated, or when glucose was given. From these results it was concluded that the fall of plasma FFA could stimulate HGH secretion as in the case of falling blood glucose values and that the fluctuation of plasma FFA could be one of the factors regulating the secretion of HGH.

The authors express their thanks to Prof. Kiku Nakao and Dr. Kazuo Shizume (Chief, Dept. of Endocrinology, Toranomon Hospital, Akasaka, Tokyo) for their advice and encouragement. Technical assistance of Miss Junko Shibazaki and Miss Miki Komiyama is greatly appreciated.

1. Irie, M., Sakuma, M., Tsushima, T., Shizume, K., and Nakao, K., Proc. Soc. Exp. Biol. Med. **126**, 708 (1967).

2. Schalch, D. S., and Parker, M. L., Nature **203**, 1141 (1964).

3. Hugget, A. St. G., and Nixon, D. A., Lancet **2**, 369 (1957).

4. Dole, V. P., J. Clin. Invest. **35**, 150 (1956).

5. Fisher, L. J., Bunting, S. L., and Rosenberg, L. E., Clin. Chem. **9**, 573 (1963).

6. Roth, J., Glick, S. M., Yalow, R. S., and Berson, S. A., Science **140**, 987 (1963).

7. Hunter, W. M., Clarke, B. F., and Duncan, L. J. P., Metabolism **15**, 596 (1966).

8. Roth, J., Glick, S. M., Yalow, R. S., and Berson, S. A., Metabolism **12**, 577 (1963).

9. Roth, J., Glick, S. M., Yalow, R. S., and Berson, S. A., Recent Progr. Horm. Res. **21**, 241 (1965).

10. Knopf, R. F., Conn. J. W., Fajans, S. S., Floyd, J. C., Guntche, E. M., and Rull, J. A., J. Clin. Endocrinol. **25**, 1140 (1965).

11. Schalch, D. S., and Kipnis, D. M., J. Clin. Invest. **44**, 2010 (1965).

12. Owen, O. E., Morgan, A. P., Kemp, H. G., Sullivan, J. M., Herrera, M. G., and Cahill, G. F., Jr., J. Clin. Invest. **46**, 1589 (1967).

13. Vignais, P. M., Gallagher, C. H., and Zabin, I., J. Neurochem. **2**, 283 (1958).

14. Merimee, T. J., Burgess, J. A., and Rabinowitz, D., Diabetes **7**, 478 (1967).

15. Rimoin, D., Merimee, T. J., and McKusick, V. A., Science **152**, 1635 (1966).

Received Nov. 12, 1969. P.S.E.B.M., 1970, Vol. 133.