Influence of Age on Responsiveness to Diabetogenic Action of Growth Hormone

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SUMMARY

Responsiveness to the growth-promoting action of growth hormone (GH) develops gradually during postnatal life, and as the organism ages, sensitivity to the hormone declines. Our study was undertaken to determine whether there is a similar age-related pattern of sensitivity to the diabetogenic action of purified native human GH (hGH) in the obese (*ob*/*ob*) mouse. The *ob*/*ob* mouse was used because adults of this strain respond to chronic GH treatment with increases in fasting plasma insulin and blood glucose concentrations and with glucose intolerance. When hGH was given subcutaneously to adult (4-mo-old) female mice at doses of 5, 10, or 25 μg/day for 3 days, a significant increase in fasting blood glucose concentration and an impairment in glucose tolerance were produced by the 10-μg/day dose. Larger effects were obtained with the 25-μg/day dose. A threefold increase in fasting plasma insulin concentration was also produced with this dose of the hormone. By contrast, 25 μg/day of hGH had no effect on fasting plasma insulin, blood glucose, or glucose tolerance in 1-mo-old *ob*/*ob* mice. When a dose of 100 μg/day of hGH was given to 1-mo-old animals, a significant increase in fasting plasma insulin concentration occurred, but again there were no effects on fasting blood glucose or glucose tolerance. Mice 1.5 mo old showed marginal changes in fasting blood glucose and glucose tolerance when given hGH at doses of 25 or 100 μg/day. Mice 9 or 12 mo old exhibited responsiveness similar to that of 4-mo-old animals. Thus, responsiveness to the diabetogenic action of GH develops gradually in the *ob*/*ob* mouse during postnatal growth. However, there does not appear to be a significant decline in sensitivity to the diabetogenic action of GH as the adult animal ages. *Diabetes* 36:88–92, 1987

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Animals as well as humans develop responsiveness to the growth-promoting action of pituitary growth hormone (GH) during postnatal life; the fetus and neonate respond poorly, if at all, to this biological property of the hormone (1). In the rat (2) and mouse (3) the onset of responsiveness to the growth-promoting action of GH has been reported to occur at ~1 wk of age, and by 28 days of age, further somatic growth depends on the hormone (4). The cellular basis for this gradual development of sensitivity to the hormone is not known, although it has been shown that the onset of responsiveness is correlated with an increase in binding sites for GH, at least in the liver (5,6). As the organism ages, sensitivity to the growth-promoting action of GH gradually declines (7,8), and again the basis for this loss of responsiveness is not understood.

Whether there is a similar age-related pattern of responsiveness to the diabetogenic action of GH has not been established. Many years ago, Young reported (9,10) that large doses of alkaline extracts of the anterior pituitary, which stimulated growth in puppies and kittens, did not have diabetogenic effects in these animals, whereas the extracts did produce glucosuria in adult dogs and cats. These early observations suggested that young animals are not sensitive to the diabetogenic action of GH and that responsiveness to this property of the hormone develops during postnatal life, as does sensitivity to the growth-promoting action of GH. However, to our knowledge, this has not been documented in a systematic way with GH itself. Therefore, our study was undertaken to determine whether there is an age-related pattern of sensitivity to the diabetogenic action of purified native human GH (hGH) in the hereditarily obese (*ob*/*ob*) mouse. The *ob*/*ob* mouse was chosen for this work because adults of this strain respond predictably to chronic treatment with either native or biosynthetic hGH with an increase in fasting blood glucose concentration and glucose intolerance (11,12).

TABLE 1
Body weights of *ob/ob* mice of various ages

Age (mo)	*N*	Body weight (g)*	Range (g)
1	20	18 ± 0.6	14–22
1.5	27	34 ± 0.6	27–40
4	27	59 ± 1.0	49–70
9	26	80 ± 1.3	69–92
12	5	84 ± 3.0	78–92

*Values are means ± SE.

MATERIALS AND METHODS

Native hGH (type A) was isolated and purified by ion-exchange chromatography on DEAE-cellulose as previously described (13).

Female *ob/ob* mice (C57BL/6J *ob/ob*) were obtained from the Jackson Memorial Laboratory, Bar Harbor, ME. The animals were maintained at 22°C on a 12-h light/dark cycle and given access to Purina rat chow and tap water ad libitum. Individual groups of animals were used for experiments when they were 1, 1.5, 4, 9, or 12 mo old. The body weights of the mice at these ages are given in Table 1.

The diabetogenic activity of native hGH in *ob/ob* mice at the above ages was assessed by a method previously described in detail (11). In brief, groups of animals were injected subcutaneously once daily for 3 consecutive days with saline and on the 4th day were given 2 μg dexamethasone phosphate subcutaneously, fasted for 6 h, and then given an intraperitoneal glucose tolerance test. An initial blood sample (20 μl) was taken from the tip of the tail to determine fasting blood glucose concentration, and then the animals were given an intraperitoneal injection of glucose (1 mg/g body wt). Blood samples (20 μl) were then taken at 30, 60, and 90 min after the injection of glucose. Seven days after the start of saline treatment, the same mice were injected subcutaneously for 3 days with various doses of hGH, and the tolerance test was then repeated as described above. Glucose-tolerance curves obtained after hGH treatment were compared with those obtained after the course of saline treatment to determine if the hormone had caused a rise in fasting blood glucose concentration and a decrease in glucose tolerance. Statistical comparisons were made with the paired *t* test, and effects were considered to be significant at $P < .05$. In comparing the effects of various doses of hGH on animals of various ages, note that the indicated dose represents the actual amount of hormone injected per animal per day. No attempt was made to correct for the body-weight differences among the various age groups (see Table 1). The validity of such a correction can be questioned in view of the obvious increase in the ratio of fat to lean body mass as the animals age and the lack of knowledge concerning the relative distribution of hGH between the fat and lean compartments of the mouse. In any event, it seems reasonable to assume that a given amount of hormone injected into 1-mo-old mice represents a substantially larger dose for the animal than the same amount given to the much larger 9-mo-old mice.

To determine the effect of hGH on plasma insulin concentration, groups of mice were injected subcutaneously with either saline or hGH for 3 consecutive days and on the 4th day were given 2 μg dexamethasone phosphate, fasted for 6 h, and then an 80-μl sample of blood was taken from the tip of the tail for the measurement of blood glucose and plasma insulin. Plasma insulin concentration was measured by the method of Hayashi et al. (14).

RESULTS

When native hGH was administered to 4-mo-old *ob/ob* mice at doses of 5, 10, or 25 μg/day for 3 days, diabetogenic responses typical for young adult animals were obtained. The results are shown in Fig. 1. At a dose of 5 μg/day, hGH had no effect on either fasting blood glucose concentration or glucose tolerance. The fact that the two tolerance curves obtained in this experiment are superimposable illustrates that in adult mice, the second course of treatment with a subthreshold diabetogenic dose of GH (or saline, not shown) and the subsequent glucose tolerance test have no influence themselves on the outcome of the tolerance test, as has been demonstrated repeatedly (12,15). A dose of 10 μg/day of hGH produced a significant increase in fasting blood glucose concentration and a modest impairment in glucose tolerance. A pronounced increase in fasting blood glucose level and impairment in glucose tolerance were obtained with a dose of 25 μg/day of hGH (Fig. 1, Table 2). Table 2 shows that a dose of 25 μg/day of hGH also produced a marked increase in plasma insulin concentration in the 4-mo-old *ob/ob* mouse, in accord with previous observations on the hyperinsulinemic effects of GH in humans and certain animals, including the *ob/ob* mouse (16). However, this increase in circulating insulin was obviously insufficient to overcome peripheral insulin resistance resulting from the action of the hGH because the animals exhibited an increase

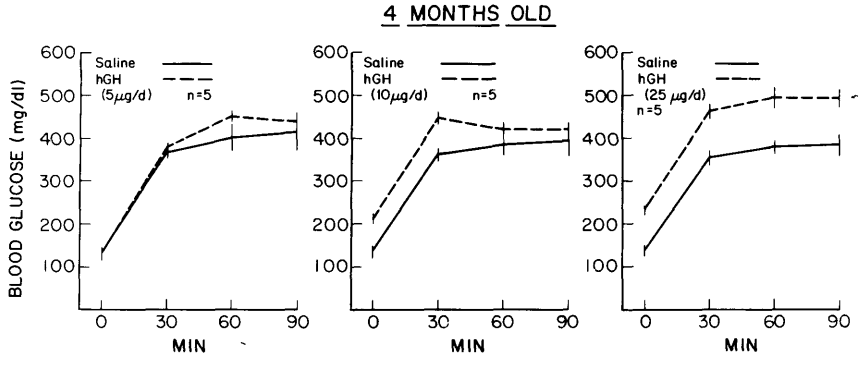

FIG. 1. Effects of treatment with 3 doses (5, 10, or 25 μg/day for 3 days) of hGH on glucose tolerance of fasted 4-mo-old *ob/ob* mice. Each *point* represents mean of *N* observations. *Vertical lines* through *points* indicate 2 SE. Effects of 10-μg/day dose were statistically significant ($P < .05$) at 0 and 30 min. Effects of 25-μg/day dose were statistically significant ($P < .05$) at all time points.

TABLE 2
Effects of hGH on fasting concentrations of blood glucose and plasma insulin in *ob/ob* mice

Treatment	Age (mo)	*N*	Blood glucose (mg/dl)*	Plasma insulin (μU/ml)*
Saline	4	6	169 ± 14	217 ± 85
hGH (25 μg/day)	4	6	214 ± 17†	728 ± 122†
Saline	1	12	133 ± 8	31 ± 10
hGH (25 μg/day)	1	4	124 ± 11	21 ± 13
hGH (100 μg/day)	1	10	126 ± 4	93 ± 24†

*Values are means ± SE.
†Significantly different from saline-treated controls, $P < .05$.

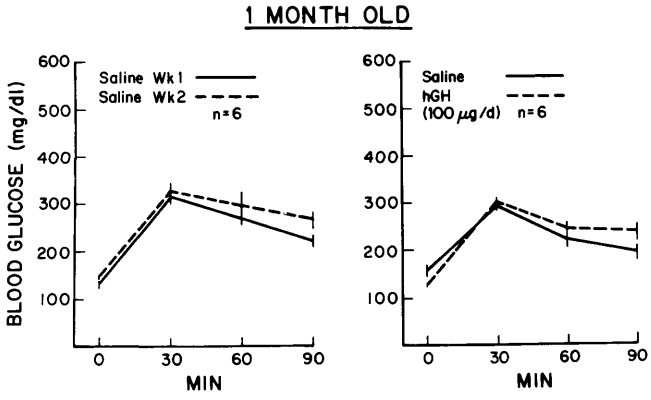

FIG. 2. Effects of treatment with saline or hGH (100 μg/day for 3 days) on glucose tolerance of fasted 1-mo-old *ob/ob* mice. Each *point* represents mean of *N* observations. *Vertical lines* through *points* indicate 2 SE. Effects of saline and 100-μg/day dose of hGH were statistically significant ($P < .05$) at 0 and 90 min.

in fasting blood glucose level. Table 2 also shows that the fasting concentration of plasma insulin in the saline-treated 4-mo-old control mice was also quite high. The hyperinsulinemia of the adult *ob/ob* mouse is well documented (17) and is presumably related to the gradual development of peripheral insulin resistance in this animal.

By contrast, large doses (25 or 100 μg/day) of hGH had no effect on fasting blood glucose concentration in 1-mo-old *ob/ob* mice (Table 2) and little effect, if any, on glucose tolerance (Fig. 2, *right panel*). The slight decrease in glucose tolerance after the course of hGH treatment probably cannot be attributed to the diabetogenic action of the hormone because a similar marginal change in glucose tolerance was observed when 1-mo-old mice were given a second course of saline injections rather than the hGH treatment (Fig. 2, *left panel*). The reason for this slight change in the glucose tolerance of these young animals during the 2nd wk of the experimental protocol is not clear, but as noted above, this is not seen when adult animals are used (e.g., Fig. 1). The 25-μg/day dose of hGH also had no effect on fasting plasma insulin concentration in the 1-mo-old mice, although this dose was sufficient to produce a threefold increase in plasma insulin in 4-mo-old animals (Table 2). However, when the dose of hGH was increased to 100 μg/day, a significant increase in fasting plasma insulin concentration occurred in the 1-mo-old animals. Presumably, this increase in circulating insulin was sufficient to overcome any peripheral insulin resistance produced in these animals by the 100-μg/day dose of hGH because these animals did not exhibit elevated fasting blood glucose concentrations or decreased glucose tolerance compared with the saline-treated controls. Considering that 4-mo-old mice, which weigh more than three times as much, exhibited elevated fasting blood glucose and decreased glucose tolerance to only 10 μg/day of the hormone, the 1-mo-old animals are quite insensitive to the diabetogenic action of the hormone. Doses of hGH > 100 μg/day were not given to the 1-mo-old mice.

Figure 3 illustrates experiments performed on 1.5-mo-old mice. When hGH was administered at a dose of 12.5 μg/day, it had no effect on either fasting blood glucose concentration or glucose tolerance. However, when the dose of hormone was increased to 25 μg/day, small but significant changes were produced in fasting blood glucose concentration and in glucose tolerance. A somewhat greater effect on glucose tolerance was obtained with a dose of 100 μg/day. Thus, the 1.5-mo-old mouse responds to the diabetogenic property of GH, but like the 1-mo-old animal, its responsiveness appears to be considerably less than that of 4-mo-old animals when body weight differences and effective doses are compared.

Experiments were also conducted to determine whether there is any change in the responsiveness of adult *ob/ob* mice to the diabetogenic action of GH as the animals age. Figure 4 summarizes experiments conducted on 9-mo-old mice. These animals did not respond to a dose of 10 μg/day of hGH but had a marked increase in fasting blood glucose concentration and impairment in glucose tolerance when the dose of hormone was raised to 25 μg/day (Fig. 4*A*). Much larger effects were obtained when hGH was given at doses of 50 and 100 μg/day (Fig. 4*B*). The very large

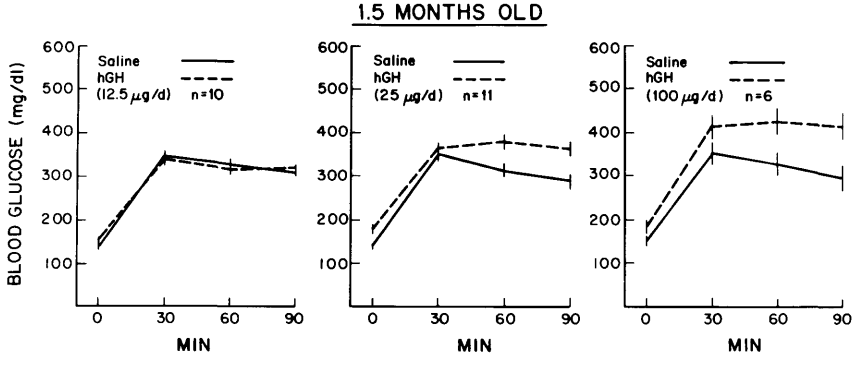

FIG. 3. Effects of treatment with 3 doses (12.5, 25, or 100 μg/day for 3 days) of hGH on glucose tolerance of fasted 1.5-mo-old *ob/ob* mice. Each *point* represents mean of *N* observations. *Vertical lines* through *points* indicate 2 SE. Effects of 25-μg/day dose were statistically significant ($P < .05$) at 0, 60, and 90 min. Effects of the 100-μg/day dose were statistically significant ($P < .05$) at all time points.

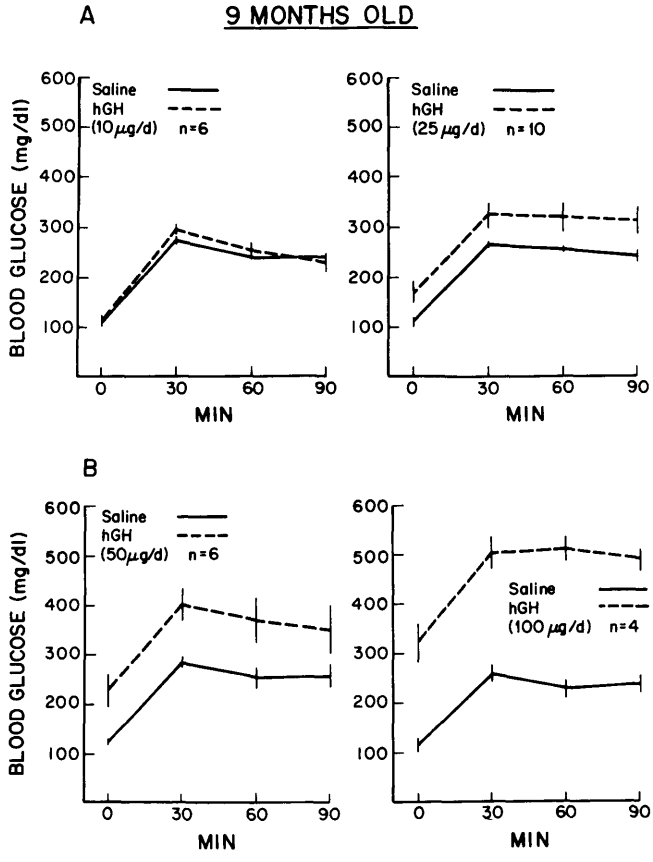

FIG. 4. Effects of treatment with 4 doses (*A*: 10 or 25 μg/day for 3 days; *B*: 50 or 100 μg/day for 3 days) of hGH on glucose tolerance of fasted 9-mo-old *ob/ob* mice. Each *point* represents mean of *N* observations. *Vertical lines* through *points* indicate 2 SE. Effects of 25-, 50-, and 100-μg/day doses were statistically significant ($P < .05$) at all time points.

response obtained with a dose of 100 μg/day of hGH again underscores the lack of sensitivity of the 1-mo-old animals to the diabetogenic effect of GH, given their considerably smaller body weight and lack of responsiveness to this dose of the hormone. When these dose-response studies on 9-mo-old animals are compared with those performed on 4-mo-old animals (Fig. 1), there is some suggestion that the 9-mo-old mouse may be somewhat less sensitive to the diabetogenic effect of GH because it did not give a significant response to an hGH dose of 10 μg/day. If this apparent difference is real, it is insignificant. Finally, a limited series of experiments were conducted on 12-mo-old *ob/ob* mice, which, when given hGH at a dose of 25 μg/day, exhibited a significant increase in fasting blood glucose concentration and an impairment in glucose tolerance essentially equivalent to those of the 9-mo-old animals (results not shown). Thus, a significant change in responsiveness of the adult *ob/ob* mouse to the diabetogenic effect of GH as it ages does not seem to occur, at least up to the age of 1 yr.

DISCUSSION

The results of our study indicate that responsiveness to the diabetogenic action of GH develops gradually during postnatal life, as does sensitivity to the growth-promoting property of the hormone. Compared with adult animals, which exhibited elevated fasting blood glucose and plasma insulin levels and impaired glucose tolerance in response to moderate doses of GH, 1-mo-old *ob/ob* mice responded to only a very high dose of hGH (100 μg/day for 3 days) with an elevation in fasting plasma insulin concentration. Even this dose of hGH did not appear to be very diabetogenic in the 1-mo-old animals because fasting blood glucose and glucose tolerance were not affected. Definite effects of hGH were obtained on fasting blood glucose concentration and glucose tolerance in 1.5-mo-old mice, presumably because hGH-induced hyperinsulinemia was not sufficient to overcome peripheral insulin resistance resulting from the action of the hormone. However, these effects were only seen in 1.5-mo-old animals when doses of hGH were used that were substantially greater than those producing comparable effects in adult animals. Therefore, our experiments suggest that the early observations of Young (9,10), indicating an age-related change in the ability of crude alkaline extracts of the anterior pituitary to cause glucosuria in dogs and cats, were in fact reflecting age-related changes in the sensitivity of these animals to the GH present in the extracts. In this study there was no indication of a significant change in responsiveness of the adult *ob/ob* mouse to the diabetogenic action of GH with advancing age. This is interesting in view of the well-documented decline in sensitivity with age in humans and various animals to the growth-promoting action of GH.

There are numerous possible explanations for the gradual development of postnatal responsiveness to the diabetogenic action of GH. For example, receptors for this activity of the hormone may be only gradually expressed as the animal ages. On the other hand, the maturation of a postreceptor effector mechanism may be required for the development of full sensitivity to this property of the hormone. Whatever the reason, the transition of the *ob/ob* mouse from a poorly responsive to a fully responsive state should provide a useful tool for the study of the mechanism of the diabetogenic action of GH.

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