Comparison of the Changes in Plasma Human Growth Hormone (HGH) and Immuno-Reactive Glucagon (IRG) after Intravenous and Subcutaneous Injection of Glucagon

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Synopsis

Ten healthy male volunteers were studied to compare the effectiveness of intravenous and subcutaneous injections of 1mg of glucagon on HGH secretion. Plasma HGH level rose to a peak of 6ng/ml or greater 120 minutes after the subcutaneous injection of glucagon (sc glucagon) in all subjects, whereas the intravenous injection of glucagon (iv glucagon) caused comparable increments in plasma HGH in only six out of ten subjects. Furthermore, in comparison to those in sc glucagon the periods required to show maximum responses were less consistent in iv glucagon. Plasma IRG levels reached a peak of 102.4±22.6ng/ml at two minutes following iv glucagon, and a peak of 3.33±1.08ng/ml at 15 minutes following sc glucagon. These fell to initial levels at 60 minutes and at 180 minutes, respectively. There was no definite correlation either between the magnitudes of changes in plasma IRG and HGH levels or between the velocities of decrement in blood sugar and HGH responsiveness. Judging from its simplicity and reproducibility it may be concluded that sc glucagon is more suitable for a clinical provocative test of HGH release than is iv glucagon. In regards to the mechanism of glucagon-induced HGH release, neither glucagon per se nor the fall of blood sugar after hyperglycemia was assumed to play any major role. The sustained elevation of plasma IRG for a certain period might be responsible for the glucagon-induced HGH release.

Glucagon has been shown to stimulate various polypeptide hormones including human growth hormone (HGH) and to be applicable for estimation of HGH-secretory capacity. We have, however, as yet very little information as to the serum immuno-reactive glucagon (IRG) levels achieved by intravenous and subcutaneous injection of glucagon and the mechanism of glucagon-induced HGH secretion.

The present study was performed to compare the suitability of different types of glucagon injection for clinical use in a routine test of HGH secretion and to investigate whether the stimulatory effect of glucagon on HGH secretion is related to plasma IRG level per se, or to changes in other substances in plasma.

Materials and Methods

Ten healthy, non-obese male volunteers between the ages 19 and 24 were studied. After an overnight fast the subjects were kept recumbent. Tests were started after 30-minute bed rest. A 19-gauge needle was inserted into an antecubital vein and was kept patent by a slow saline-infusion. After two control samples were obtained, 1 mg of glucagon* was in-
jected either intravenously over a period of two minutes or subcutaneously as a bolus. Venous blood samples were collected 2, 5, 10, 15, 30, 45, 60, 90, 120, 180, and 240 minutes after iv glucagon and 15, 30, 45, 60, 90, 120, 180, and 240 minutes after sc glucagon. When administered intravenously the midpoint of the injection was regarded as zero time for sampling thereafter. Each volunteer was subjected to both iv and sc glucagon tests in random order with one-week intervals.

Blood glucose was determined by an Auto-Analyser with modification by the Hoffman ferri cyanide technique (Hoffman, 1937). Plasma insulin (IRI) was measured by a solid-phase antibody technique using materials purchased from Pharmacia, Sweden (Wide and Porath, 1966). HGH was assayed by the double-antibody radioimmunoassay (Schalch and Parker, 1964) using Wilhelmi HGH as the standard, the lower limit of sensitivity was 1 ng/ml. Glucagon (IRG) was measured by the radioimmunoassay method described previously (Shima et al., 1975) using non-specific antiglucagon serum. Plasma FFA was measured spectrometrically (Duncombe, 1964) and plasma lactate was determined by the method reported by Scholz et al. (1959). Calcium and potassium were determined by flame photometry and inorganic phosphate was determined by the method of Taussky and Schorr (1953).

All values are reported as means±SD unless otherwise specified. Statistical calculations were performed by Student’s t test.

Results

Plasma HGH responses to glucagon

Fig. 1 illustrates individual HGH responses to two types of glucagon injection. In iv glucagon, four of the ten subjects showed no significant responses, two had peaks of HGH responses at 30 and 45 minutes and the other four subjects had peaks after 120 minutes; a significant rise in plasma HGH was defined as a rise of greater than 6 ng/ml above the base line value. The mean maximum HGH increment of these six subjects was 13.4±3.45 ng/ml (p<0.005). On the contrary, sc glucagon caused an increase in all ten subjects with their peaks of HGH responses at after 120 minutes.

Mean plasma HGH responses to iv and sc glucagon are shown in Table 1. With iv glucagon the mean plasma HGH levels rose from the basal level of 3.05±3.82 ng/ml to a peak of 6.80±7.38 ng/ml at 180 minutes, although the difference between these values was not statistically significant. On the other hand, the mean plasma HGH levels rose significantly (p<0.005) from a base line of 1.98±2.46 ng/ml to 21.5±12.7 ng/ml at 120 minutes after sc glucagon.

Plasma IRG patterns after glucagon

As shown in Table 1, two minutes after iv glucagon the mean plasma IRG level reached a peak value of 102.4±22.6 ng/ml then fell rapidly thereafter returning to the base line level at 60 minutes after the injection, while in sc glucagon the mean plasma IRG level had a peak value of 3.33±1.08 ng/ml at 15 minutes, then decreased gradually returning to its basal level after 180 minutes.
Table 1. Mean plasma HGH, IRI, IRG and glucose responses to glucagon (1 mg) injected intravenously (iv) or subcutaneously (sc) in 10 normal subjects

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>-15</th>
<th>0</th>
<th>2</th>
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<th>120</th>
<th>180</th>
<th>240</th>
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<tbody>
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<td><strong>HGH</strong></td>
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<tr>
<td>iv</td>
<td>3.40</td>
<td>3.05</td>
<td>3.29</td>
<td>2.92</td>
<td>3.24</td>
<td>3.54</td>
<td>5.21</td>
<td>4.56</td>
<td>2.82</td>
<td>2.27</td>
<td>4.76</td>
<td>6.80</td>
<td>3.38</td>
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<td>sc</td>
<td>2.02</td>
<td>1.98</td>
<td>1.75</td>
<td>1.49</td>
<td>1.17</td>
<td>1.21</td>
<td>2.01</td>
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<td>± 3.33*</td>
<td>± 3.82</td>
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<td>± 3.13</td>
<td>± 5.82</td>
<td>± 5.43</td>
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<td>± 7.39</td>
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<td>± 2.27</td>
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| **Glucose** |     |     |     |     |     |     |     |     |     |     |     |     |     |
| iv          | 80.5| 79.6| 83.7 | 94.7 | 103.9 | 111.8 | 110.8 | 95.0 | 82.0 | 71.0 | 66.2 | 73.9 | 76.4 |
| sc          | 79.1| 79.8|     |     |     |     |     |     |     |     |     |     |     |
| ± 6.55     | ± 5.74| ± 4.08| ± 7.94| ± 9.57| ± 11.1| ± 20.9 | ± 25.7| ± 19.4| ± 10.9| ± 6.32| ± 6.56| ± 5.83|
| ± 10.5     | ± 10.6|     |     |     |     |     |     |     |     |     |     |     |     |

| **IRI**    |     |     |     |     |     |     |     |     |     |     |     |     |     |
| iv         | 3.89| 3.40| 31.9 | 28.8 | 29.9 | 21.9 | 16.1 | 10.2 | 6.42 | 4.06 | 3.27 | 3.06 | 3.33|
| sc         | 4.94| 4.00|     |     |     |     |     |     |     |     |     |     |     |
| ± 4.43     | ± 3.19| ± 25.4| ± 17.0| ± 15.2| ± 9.85| ± 9.48| ± 5.38| ± 3.04| ± 2.97| ± 2.31| ± 2.60| ± 2.23|
| ± 3.54     | ± 3.44|     |     |     |     |     |     |     |     |     |     |     |     |

| **IRG**    |     |     |     |     |     |     |     |     |     |     |     |     |     |
| iv         | 0.47| 0.46| 102.4| 60.1 | 19.9 | 7.16 | 1.32 | 0.76 | 0.69 | 0.53 | 0.59 | 0.49 | 0.46|
| sc         | 0.50| 0.47|     |     |     |     |     |     |     |     |     |     |     |
| ± 0.28     | ± 0.17| ± 22.6| ± 12.7| ± 6.65| ± 3.49| ± 0.48| ± 0.28| ± 0.31| ± 0.30| ± 0.26| ± 0.15| ± 0.14|
| ± 0.11     | ± 0.15|     |     |     |     |     |     |     |     |     |     |     |     |

*Mean ± S.D.  Significance of differences from control means a; p<0.025  b; p<0.01  c; p<0.005
Blood glucose and IRI responses to glucagon

Blood glucose and IRI responses to glucagon injections are illustrated in Table 1. In iv glucagon the mean blood glucose level rose to a peak of 111.8 mg/dl at 15 minutes, then declined below mean fasting value to 66.2 mg/dl at 120 minutes. After sc glucagon, the mean blood glucose level rose to a peak of 131.4 mg/dl at 30 minutes and fell to a nadir of 64.4 mg/dl at 120 minutes.

Mean falls of blood glucose in intravenous and subcutaneous injections of glucagon were 52.7±14.6 mg/dl and 66.9±22.7 mg/dl, respectively; the difference being significant (p<0.005). But the mean velocities

\[
\frac{\text{BSmax} - \text{BSmin}}{t_{\text{BSmin}} - t_{\text{BSmax}}} 
\]

of the fall of blood glucose in both tests were 0.69±0.35 and 0.75±0.30, there being no significant difference between them. There was no significant correlation between the velocities and the HGH increments (r=0.292, p>0.05).

Mean plasma IRI in iv glucagon rose rapidly from a base line of 3.40 µU/ml to a peak value of 31.9 µU/ml at two minutes, then returned to the basal level at 90 minutes. In sc glucagon mean plasma IRI rose to a peak of 41.9 µU/ml at 30 minutes and returned to the base line at 180 minutes.

Plasma FFA and lactate responses to glucagon injection

The mean plasma FFA fell from a basal level of 573.6 µEq/l to a nadir of 333.3 µEq/l at 60 minutes in iv glucagon and from a base-line level of 486.5 µEq/l to 277.4 µEq/l at 90 minutes following sc glucagon (Fig. 2).

Plasma lactate rose to a peak at 30 minutes in iv glucagon and at 60 minutes in sc glucagon (Fig. 2).

Fig. 2. Mean plasma FFA and lactate responses to glucagon (1 mg) injected intravenously (iv) or subcutaneously (sc) in 10 normal subjects. Vertical bars signify mean±S.D. or mean−S.D.

\( \times \ p<0.05 \) (different from control means)

\( \times \times \ p<0.025 \quad \times \times \times \ p<0.01 \quad \times \times \times \times \ p<0.005 \)

Fig. 3. Mean plasma electrolytes responses to glucagon (1 mg) injected intravenously (iv) or subcutaneously (sc) in 10 normal subjects.
Changes in plasma electrolytes after glucagon

As shown in Fig. 3, plasma potassium and inorganic phosphate were decreased significantly, but calcium did not show any significant change following the injection of glucagon.

Discussion

Mitchell and co-workers (1969) reported increased HGH levels after intramuscular or subcutaneous injection of glucagon. Since then, Cain et al. (1970, 1972), AvRuskin et al. (1971) and Spathis et al. (1974) have confirmed their results. However, Eddy et al. (1970) and Podolsky and Sivaprasad (1972) failed to show a consistent rise in HGH release after glucagon injection. As shown in Fig. 1, in sc glucagon all subjects showed a rise of more than 6 ng/ml in plasma HGH after 120 minutes, whereas in iv glucagon six of ten subjects showed a smaller but significant rise in plasma HGH. These responses appeared at various intervals after the injection; the other four subjects showed no responses. Our results agree with those of Cain et al. (1970, 1972) that either intramuscular or sc injection of glucagon is a potent stimulus for HGH release but 1 mg bolus iv glucagon is not a consistent stimulus for HGH release.

There are various methods as provocative tests for HGH secretion, including the use of insulin (Roth et al., 1963), arginine (Merimee et al., 1965), glucagon (Mitchell et al., 1969), levodopa (Boyd et al., 1970), ACTH (Zahnd et al., 1969) and metyrapone (Kunita et al., 1970). Among them, insulin-induced hypoglycemia has been considered as the most reliable, but in cases suspected of hypopituitarism, this test always necessitates cautious care because of the risk of prolongation of the hypoglycemia. Arginine tests need an intravenous infusion. Contrarily sc glucagon has no risk of hypoglycemia, is simple, and the response can be recognized by taking a few samples between 120 and 180 minutes. Therefore, this is one of the most suitable stimulation tests of HGH secretion for routine clinical use.

The mechanism of glucagon stimulated HGH secretion is still unclear. In order to investigate whether the stimulatory effect of glucagon on HGH release may be associated with plasma IRG level itself, plasma IRG levels were measured during glucagon injection in the present experiment. Glucagon administered intravenously disappeared rapidly from circulation, while it was retained for a longer period when administered subcutaneously as shown in Table 1. In both iv and sc glucagon, most subjects showed peak HGH responses after plasma IRG concentration returned to basal levels. Judging from these findings, together with the fact that there is no definite correlation between concentrations of plasma IRG and HGH responses, it is unlikely that glucagon per se has a direct effect on HGH secretion. The sustained elevation of plasma glucagon for a certain period, however, seems to be necessary for the stimulation of HGH secretion. This assumption is also supported by the results of Cain et al. (1970), who showed that intravenous infusion of 1 mg glucagon for 30 minutes caused HGH responses comparable to those of 1 mg intramuscular glucagon.

There are conflicting reports in relation to whether the fall of blood glucose from its peak may stimulate HGH secretion after glucagon. Mitchell et al. (1970) suggested that falling blood glucose is not the sole mechanism responsible for HGH release by glucagon. Cain et al. (1970 and 1972) pointed out that the rate of fall of blood sugar was similar in iv glucagon but that the HGH response was less consistent in iv glucagon. Podolsky and Sivaprasad (1972) showed that sc glucagon administration did not cause any significant rise in HGH after prolonged fasting. Blood glucose response
to iv glucagon had a peak at 15 minutes and then decreased to a nadir at 120 minutes. On the contrary, following the sc injection of glucagon blood glucose rose to a peak at 30 minutes and fell to a nadir at 120 minutes. The mean fall of blood glucose was significantly ($p<0.05$) greater in sc glucagon than in iv glucagon. However there was no significant difference between the mean velocities of the fall of blood glucose in either test, and no significant correlation between the velocities and the HGH responses. Thus it seems unlikely that the fall of blood glucose after hyperglycemia plays a major role in glucagon-stimulated HGH release.

Irie et al. (1967) reported the fall of FFA induced HGH secretion but that it does not seem to play an important role in the mechanism of glucagon-stimulated HGH secretion because FFA decreased more markedly in iv glucagon than in sc glucagon.

Although the sustained elevation of plasma IRG for a certain period was assumed to be one of the possible factors related to the glucagon-stimulated HGH release, the exact mechanism is still unclear and further studies on this problem are necessary.

It has been well known that glucagon stimulates insulin secretion independently of the hyperglycemia which it causes. The rapid elevation of plasma IRI level prior to an increase in blood sugar observed two minutes after iv glucagon was due to the insulinogenic action of glucagon per se. However, the increase in plasma IRI levels following sc glucagon seems to be caused by both hyperglucagonemia and hyperglycemia.

Plasma lactate levels increased significantly after glucagon injection, but no significant difference was observed between iv and sc glucagon. Plasma potassium and inorganic phosphate levels decreased significantly after glucagon injection but no significant changes were observed in the plasma levels of calcium although the infusion of exogenous glucagon has been shown in most reports (Birge and Avioli, 1969) to lower the serum calcium. This discrepancy is not clear.

References


