ESTIMATION OF PLASMA INSULIN USING HYPOPHYSECTOMIZED-ADRENODEMEDULLATED RATS

KINORI KOSAKA, TAKEHIKO IDE AND NOBUSADA KUZUYA

Okinaka Clinic, School of Medicine, Tokyo University, Tokyo

A reliable and accurate method for the estimation of the insulin concentration in blood should be useful for the elucidation of many problems in the study of metabolism. During the last few years, the technical aspects of this assay procedure have been investigated and two methods became available, one in vivo, the other in vitro.

The in vivo method is based on the hypoglycemic effect of insulin. The test animals used have been prepared in different ways to increase their sensitivity to insulin, i.e. to decrease the threshold value of insulin. Gellhorn et al. (1941) used hypophysectomized-adrenomedullated rats and found that 0.2 milliunit of insulin gave a small but significant lowering of the blood sugar in such preparation. Anderson et al. (1947) prepared hypophysectomized-adrenomedullated rats, previously rendered diabetic with alloxan to prevent the possibility of variation of endogenous insulin secretion. Bornstein (1950), using modification of Anderson's technic, obtained significant effects with insulin doses as low as 0.05 milliunit.

In vitro method, the isolated rat diaphragm is used. This method is based on the fact that small amounts of insulin increase the utilization of glucose by the isolated rat diaphragm (Gemmill, 1939, 1941) and that a quantitative relationship exists between the concentration of insulin in the incubation medium and its effects on the glucose uptake of the diaphragm. Under rigorous control of the experimental conditions, this method can be used for the estimation of plasma-insulin activity (Willebrands et al., 1950; Groen et al., 1952; Perlmutter et al., 1952; Randle, 1954; Vallance-Owen et al., 1954; Takeuchi et al., 1956).

In the present investigation hypophysectomized-adrenomedullated rat have been used for the assay of plasma insulin.

EXPERIMENTALS

Adult male rats weighing from 120 g to 140 g were used. Hypophysectomy was carried out by a modification of Imamichi's technic. After hypophysectomy the rats were kept under temperature of 25 to 29°C and fed constant diet ad libidum to keep in good condition. The adrenal medullas were removed 7 to 10 days after hypophysectomy. These rats were also allowed to drink 1% NaCl and 2% glucose solution, and were used for the experiments 3 to 5 days after the second operation. The rats were kept under fasting condition for 3 to 4 hrs. prior to the insulin assay.

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In the first group, physiological saline solution or plasma kept overnight at room temperature was injected intraperitoneally at a dose level of 1 ml per 100 g of body weight and the blood sugar was determined before and 1 and 2 hrs. after the injection.

In the second group, 1 ml of physiological saline solution containing the various known amounts of insulin (Tronto) per 100 g of body weight was injected.

In the third group, 1 ml of normal human plasma per 100 g of body weight which was kept at room temperature overnight was injected along with known amounts of insulin.

In the fourth group, in order to estimate the insulin content, 1 ml of plasma, which was taken in various experimental conditions, per 100 g of body weight was injected into 5 or 6 test animals as a group. The plasma was separated within 20 minutes after the blood had been withdrawn.

In the last three groups, the blood samples were taken from the tail vein before and sixty minutes after the injection of test materials and the concentrations of glucose were determined by the Hagedorn-Jensen’s method.

RESULTS

The blood sugar level of hypophysectomized-adrenodemedullated rats fasting for 3 hrs. remained stable for 2 hrs. after the injection of physiological saline solution or plasma kept at room temperature overnight (Table I). Therefore fast-

Table 1. Changes of blood sugar levels after injection of saline solution or plasma in fasting hypophysectomized-adrenodemedullated rats

<table>
<thead>
<tr>
<th>No.</th>
<th>Blood sugar value (mg %)</th>
<th>Differences (mg %)</th>
<th>Blood sugar value (mg %)</th>
<th>Differences (mg %)</th>
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<tr>
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<td>0</td>
<td>60min.</td>
<td>120min.</td>
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<tr>
<td>1</td>
<td>82</td>
<td>78</td>
<td>78</td>
<td>4</td>
</tr>
<tr>
<td>2</td>
<td>78</td>
<td>82</td>
<td>80</td>
<td>4</td>
</tr>
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<td>7</td>
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<td>70</td>
<td>68</td>
<td>65</td>
<td>2</td>
</tr>
</tbody>
</table>

ing for 3 hrs. prior to the assay and the following 2 hrs. fasting appears to have no lowering effect of the blood sugar confusing the effect of insulin.

It is found that there is a linear relationship between the logarithm of quantities of injected insulin (in milliunits) per 100 g of body weight and blood sugar differences in tail vein before and 60 minutes after the injection (Fig. 1), and the minimal quantity of insulin which can be determined in the present method is approximately 0.2 milliunit.
When the plasma kept at room temperature overnight is injected with known amounts of insulin, the falls of blood sugar in test animals are within a estimation error from the standard line which is brought out from above experiment (Fig. 2). It seems that this standard line can be applied to the estimation of plasma insulin concentration.

The insulin activity of peripheral blood plasma of human subjects and dogs in fasting state can not be estimated with this method, because the blood sugar of rats does not fall significantly after the injection (Fig. 3).

In normal human subjects, the plasma insulin concentration 90 to 120 minutes after the meal, is about 0.3 milliunit per ml, and in dogs, 105 minutes after intravenous injection of 20 g glucose, 0.3 to 0.4 milliunit per ml (Fig. 3).

The plasma insulin concentrations in superior pancreaticoduodenal vein of fasting dogs are 0.2 to 0.3 milliunit per ml, and in pancreatic branch of splenic vein are about 0.3 milliunit per ml (Fig. 4).

DISCUSSION

Gellhorn et al. (1941) reported that 0.3 milliunit insulin per 100 g of body weight let to a distinct fall in blood sugar of hypophysectomized-adrenodemedulated rats, and an approximately linear relation between the insulin concentration up to 1 milliunit per 100 g of body weight which represented the convulsive threshold and blood sugar 60 minutes after the injection of insulin. With our method, it was found that there was a linear relationship between the logarithm of the quantities of injected insulin per 100 g of body weight and blood sugar differences in tail vein before and 60 minutes after the injection of insulin. The quantities of insulin which could be determined with the present method were approximately 0.2 to 3.0 milliunits.

Bornstein (1950), using alloxan diabetic hypophysectomized-adrenalectomized rats, found that there was a direct relationship between the logarithm of the dose of injected insulin and the depression in blood sugar, and 0.05 milliunit represented the lower limit of insulin which could be estimated by his method. In this point, his method is more sensitive than present one. Bornstein described that it was decided to use to alloxan diabetic hypophysectomized-adrenalectomized rats because hypophysectomized-adrenalectomized rats had a very short life for they were extremely liable to spontaneous hypoglycemia, and it was considered desirable to remove possible interference from endogenous insulin secretion. However, it must be remembered that the blood sugar of diabetic subjects is most unstable, and diabetic animal is not usually more sensitive to insulin. The variation of blood sugar difference used in insulin bioassay by Bornstein is larger than that of present method.

The insulin concentration in peripheral blood plasma of fasting human subjects and dogs is lower than 0.2 milliunit per ml, i.e. minimal dose of insulin which can be estimated in present method. Many investigators estimated the insulin concentration in peripheral blood in fasting state by vivo or vitro method, but their values were reported extremely varied (Gellhorn et al., 1941; Anderson et al., 1947; Bornstein, 1950; Bornstein et al., 1951; Perlmutter et al., 1952; Groen et
Fig. 1. Relationship between the logarithm of quantities of injected insulin and blood sugar differences in tail vein.
Fig. 2. Relationship between the logarithm of quantities of injected insulin with plasma and blood sugar differences in tail vein.
Fig. 3. Plasma insulin concentration in the peripheral blood of human subjects and dogs under various conditions.
Fig. 4. Plasma insulin concentration in pancreatic venous blood of fasting dogs
Therefore, further investigations must be carried out to obtain the actual concentration of insulin in peripheral blood.

After meal or intravenous injection of glucose, the insulin concentration in blood was higher than the fasting level. This results confirmed the reports of Bornstein et al. (1950) and Vallance-Owen et al. (1954). However, whether the hyperglycemia stimulates the insulin secretion from pancreas directly, will be discussed elsewhere.

The insulin concentration of pancreatic vein has not been reported so far. In the present study, the insulin concentrations in blood obtained from the pancreaticoduodenal vein appeared to be 0.2 to 0.3 milliunit per ml and in blood from the pancreatic branch of splenic vein were about 0.3 milliunit per ml in fasting dogs. The course of insulin secretion from pancreas can not be decided yet. From above results, it is considered that, at least insulin is secreted into the pancreatic vein from the islets of Langerhans.

**SUMMARY**

A method using hypophysectomized-adrenomedullated rats for the assay of small quantities of insulin is described.

If known quantities of insulin are added to plasma kept at room temperature overnight, they produce quantitatively similar effects on the rat as in the absence of plasma. The method allows determination of alteration in the insulin concentration of plasma.

The insulin concentration in peripheral blood plasma of fasting human subjects and dogs is lower than the minimal quantities of insulin which could be estimated by the present method.

The insulin concentrations in peripheral human plasma 90 to 120 minutes after meal are 0.2 to 0.3 milliunit per ml, and in normal dogs after intravenous injection of 20 g of glucose are 0.3 to 0.4 milliunit per ml.

The insulin concentrations in blood obtained from the superior pancreaticoduodenal vein in fasting dogs are 0.2 to 0.3 milliunit per ml, and in the blood from the pancreatic branch of splenic vein are 0.3 to 0.4 milliunit per ml.

**REFERENCES**