THE PANCREATIC ALPHA AND BETA CELLS RESPONSES TO L-ARGININE AND INSULIN-INDUCED HYPOGLYCAEMIA IN HYPERTHYROIDISM

By

K. Shima, N. Sawazaki, R. Tanaka, S. Morishita, S. Tarui and M. Nishikawa

ABSTRACT

In order to assess the secretory capacity of the pancreatic alpha and beta cells in patients with hyperthyroidism, the plasma glucagon and insulin responses to l-arginine and insulin-induced hypoglycaemia in 12 patients were compared with those in 6 normal subjects. The response of beta cell to hypoglycaemia was evaluated by measuring the decrease in plasma C-peptide immunoreactivity (CPR) level.

There was a negligible rise in blood glucose and plasma insulin levels in the patients, whereas a significant increase occurred in normal subjects during the arginine infusion. Although no difference in the fasting plasma glucagon concentration between the two groups was found, 30 min after the beginning of the arginine infusion, the plasma glucagon levels rose to a peak of 252 ± 35 pg/ml in the patients, a value significantly lower than 387 ± 53 pg/ml in the normal subjects. The insulin-induced hypoglycaemia caused no significant difference in the peak values of plasma glucagon between the two groups. There was a significant fall in plasma CPR after the insulin injection in both groups but the per cent decrement was rather greater in the patients than in the normal subjects.

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These results suggest that the pancreatic alpha and beta cells in hyperthyroidism have a functional defect in response to l-arginine but not to insulin-induced hypoglycaemia. The mechanism involved in these disorders is discussed.

Thyroid dysfunction is often accompanied by disturbances of several endocrine functions (review: Pittman 1971). An impaired responsiveness of pancreatic alpha and beta cells to l-arginine is one of them (Tayama et al. 1972; Seino et al. 1974; Andreani et al. 1974). However, it is not clear whether the abnormality in the plasma immunoreactive glucagon (IRG) response represents an intrinsic defect in alpha cells or is merely the result of unresponsiveness to l-arginine only since no findings dealing with plasma IRG response to other stimuli in this disorder have been reported.

Furthermore, it is not known whether beta cells in hyperthyroid patients react normally to hypoglycaemia, though they have been reported to respond poorly to l-arginine (Tayama et al. 1972), while responding normally or even excessively to glucose (Doar et al. 1969).

In order to clarify these points, we have undertaken a parallel study of glucagon and insulin or C-peptide levels in blood after provocative tests, l-arginine infusion and insulin-induced hypoglycaemia. To our knowledge, no data have been reported on the suppression of beta cell function by insulin-induced hypoglycaemia by measuring plasma C-peptide immunoreactivity (CPR) in patients with hyperthyroidism, though a rapid fall in plasma CPR level in response to hypoglycaemia was observed in normal subjects (Horwitz & Rubenstein 1974).

**MATERIALS AND METHODS**

The study was conducted in a group of subjects with hyperthyroidism and in a normal control group consisting of 6 healthy medical students and staff of our hospital (Table 1). The diagnosis of hyperthyroidism was established on the basis of clinical symptoms and from the results of certain laboratory examinations as shown in Table 1. None of the subjects studied was grossly overweight. Patients with clinical diabetes were excluded.

The following tests were performed after an overnight fast.

1. Arginine test: 30 g arginine hydrochloride diluted in 300 ml of water was infused at a constant rate for 30 min in the cubital vein in 12 hyperthyroid patients and in 6 normal subjects. Blood was taken from the contralateral cubital vein 15 and 0 min before and 10, 20, 30, 45, 60 and 90 min after the start of the infusion.

2. Insulin tolerance test, ITT: Monocomponent insulin\(^2\), 0.1 unit/kg body weight was injected intravenously as a bolus in 10 hyperthyroid patients (excluding T. J. and M. T. in Table 1) and in 6 normal subjects. Blood was taken from the contralateral

\(^2\) Gift from NOVO Research Institute, Denmark.
A portion of all blood samples was transferred into tubes containing EDTA and lyophilized Trasylol, an inhibitor of proteolysis, in amounts sufficient to provide 500 Kallikrein inactivator units per ml of blood. The samples were centrifuged as soon as possible and the plasma was separated and preserved at −20°C until analyzed. Blood glucose was assayed by the Hoffman method (Hoffman 1937) as applied to the Autoanalyzer. Insulin was determined by a radioimmunoassay using Phadebas insulin kit (Pharmacia, Sweden). The plasma pancreatic glucagon was measured radioimmunologically according to a method described previously (Shima et al. 1975) using antiglucagon serum3) specific for pancreatic glucagon. Plasma C-peptide was determined using C-peptide radioimmunoassay kit (Horwitz & Rubenstein 1974; Kaneko et al. 1974) (Daiichi Radioisotope Labs., LTD. Tokyo).

The plasma alpha amino nitrogen level was determined by the method of Danielson (1939).
Statistical analysis was performed by Student's t-test.

Table 1.
Clinical data in patients with hyperthyroidism and in normal subjects.

<table>
<thead>
<tr>
<th>Case</th>
<th>Age</th>
<th>Sex</th>
<th>Weight (kg)</th>
<th>Height (cm)</th>
<th>T4 (µg/100 ml)</th>
<th>Cholesterol (mg/100 ml)</th>
<th>131I 9% uptake at 24 h</th>
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<td>M</td>
<td>58</td>
<td>167</td>
<td>13.7</td>
<td>134</td>
<td>29.5</td>
</tr>
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<td>19.0</td>
<td>149</td>
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<td>F</td>
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<td>143</td>
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<td>154</td>
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<tr>
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<td>163</td>
<td>16.0</td>
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<tr>
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<td>18.4</td>
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<td>125</td>
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<td>158</td>
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<td>150</td>
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3) Gift from Dr. Piero P. Foá, Dept. of Research, Sinai Hospital of Detroit, 6767 W. Outer Dr. Detroit, Mich 48235.

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Fig. 1.

Blood glucose, plasma alpha amino nitrogen, plasma insulin (IRI), and plasma pancreatic glucagon (IRG) during arginine infusion in 6 controls (broken lines) and 12 patients with hyperthyroidism (solid lines). 300 ml of 10% l-arginine solution was infused from 0 to 30 min. P values calculated according to Student’s t-test. The asterisks represent statistically significant differences between the control and the hyperthyroid groups at a given time. x: P < 0.05  xx: P < 0.025  xxx: P < 0.01  xxxx: P < 0.005.

RESULTS

Fig. 1 shows the blood glucose, alpha amino nitrogen, immunoreactive insulin (IRI) and immunoreactive glucagon (IRG) responses to l-arginine infusion in normal subjects and patients with hyperthyroidism.

In normal subjects the blood glucose level rose significantly from 89.5 ± 1.5 (SEM) mg/100 ml at 0 min to 107.5 ± 2.8 mg/100 ml at 10 min after the commencement of the infusion and decreased with termination of the infusion,
reaching to below basal values at the end of the experiment. On the contrary, no significant change in the blood glucose level was observed during the arginine test in patients with hyperthyroidism (94.3 ± 3.6 mg/100 ml and 96.5 ± 3.8 mg/100 ml at 0 min and 10 min, respectively).

The plasma alpha amino nitrogen level rose in both groups during arginine infusion. The mean peak value of the patient, 14.7 ± 1.16 mg/100 ml, was higher though not by so, than the corresponding value in the normal subjects 11.1 ± 0.87 mg/100 ml. After discontinuation of the arginine infusion, the mean plasma level of alpha amino nitrogen remained higher in the patients compared with that in the normals. The patterns of plasma IRI responses to arginine in

![Graph showing blood glucose, plasma C-peptide immunoreactivity (CPR), and plasma pancreatic glucagon (IRG) during insulin tolerance test in 6 controls (broken lines) and 10 patients with hyperthyroidism (solid lines). 0.1 U/kg of monocomponent insulin was injected at 0 min. P values calculated according to Student's t-test. The asterisks represent statistically significant differences between the control and the hyperthyroid groups at a given time. *: P < 0.025  **: P < 0.01  ***: P < 0.005.](image)

**Fig. 2.**

Blood glucose, plasma C-peptide immunoreactivity (CPR), and plasma pancreatic glucagon (IRG) during insulin tolerance test in 6 controls (broken lines) and 10 patients with hyperthyroidism (solid lines). 0.1 U/kg of monocomponent insulin was injected at 0 min. P values calculated according to Student's t-test. The asterisks represent statistically significant differences between the control and the hyperthyroid groups at a given time. *: P < 0.025  **: P < 0.01  ***: P < 0.005.
normals and hyperthyroid patients were similar to those of the blood glucose responses. The mean fasting IRI levels of the patients, $15.6 \pm 1.5 \mu U/ml$ and $14.9 \pm 1.7 \mu U/ml$ at $-15$ min and $0$ min respectively, were significantly higher than the corresponding values in healthy volunteers, $8.5 \pm 0.8 \mu U/ml$ and $7.8 \pm 0.4 \mu U/ml$. No increase in plasma IRI was noted during the infusion of arginine in the former group, while it rose quickly in the latter, reaching a peak of $48.3 \pm 5.3 \mu U/ml$ at $20$ min. Accordingly, the mean IRI concentrations at $10$, $20$, $30$ and $45$ min in patients with hyperthyroidism were significantly lower compared to the corresponding ones in the normal controls. The mean fasting IRG levels of normal subjects and of patients with hyperthyroidism were about the same ($139 \pm 24 \text{ pg/ml}$ in normals; $121 \pm 23 \text{ pg/ml}$ in patients with hyperthyroidism). Thirty minutes after the beginning of the arginine infusion, the mean IRG level rose to a peak of $252 \pm 35 \text{ pg/ml}$ in the hyperthyroid group, a value significantly lower than $387 \pm 53 \text{ pg/ml}$, observed in the normal subjects.

The mean IRG levels of the patients at $10$ min and $20$ min were also significantly lower than those of the normal subjects.

Fig. 2 shows that, in contrast to the arginine infusion, the insulin-induced hypoglycaemia caused no significant difference between peak values of plasma IRG observed in the two groups ($256 \pm 36 \text{ pg/ml}$) in the patients vs $268 \pm 25 \text{ pg/ml}$ in normal subjects, even though the blood glucose minimum of $37.3 \pm 3.9 \text{ mg/100 ml}$ at $20$ min in patients with hyperthyroidism was significantly higher.

**Fig. 3.**

Per cent decrement of plasma C-peptide immunoreactivity (CPR) during insulin tolerance test in 6 controls (broken lines) and 10 patients with hyperthyroidism (solid lines). $0.1 \text{ U/kg}$ of monocomponent insulin was injected at $0$ min. $P$ values calculated according to Student's $t$-test. The asterisks represent statistically significant differences between the control and the hyperthyroid groups at a given time. $\times\times: P < 0.025$ $\times\times\times: P < 0.005$. 

\begin{figure}[h]
\centering
\includegraphics[width=0.8\textwidth]{fig3.png}
\caption{Percent of fasting value (%)}
\end{figure}
than that of $22.5 \pm 2.5$ mg/100 ml in normal subjects. The rise in plasma glucagon level was significantly greater at 10 min and 20 min following arginine than following insulin in normal subjects, whereas the magnitude of glucagon response to both stimuli was not different in hyperthyroid patients (Figs. 1 and 2). The mean basal levels of plasma CPR were $1.22 \pm 0.06$ ng/ml and $1.17 \pm 0.06$ ng/ml at $-15$ min and 0 min respectively in the normal subjects and $2.03 \pm 0.91$ ng/ml and $1.98 \pm 0.19$ ng/ml in patients with hyperthyroidism. The difference in basal CPR level between both groups was significant. There was a significant fall in plasma CPR after the intravenous injection of insulin in both groups. The mean plasma CPR level during ITT in hyperthyroid patients tended to be higher than those in normal subjects (Fig. 2). However, as shown in Fig. 3, the per cent decrement in plasma CPR level was greater in patients with hyperthyroidism than in normal subjects.

**DISCUSSION**

The failure of blood glucose and plasma IRI levels to rise during the infusion of l-arginine in hyperthyroid patients was demonstrated in the present study. This is in good agreement with the results reported by others (Tayama et al. 1972; Seino et al. 1974). In addition, the current findings revealed that these patients also showed a diminished glucagon response to the arginine infusion. Seino et al. (1974) have already reported that the plasma glucagon response to l-arginine in patients with hyperthyroidism tends to be lower, though not significantly different as compared with that in normal subjects. The reason for the difference in the degree of the hyporesponsiveness to l-arginine between our patients and Seino’s is not clear, but it might be partly due to the difference in the period of the infusion; 300 ml of 10% l-arginine solution was infused over a period of 30 min in our experiment, while it was 45 min in Seino’s. Plasma alpha amino nitrogen level following arginine infusion was not lower but rather higher in patient with hyperthyroidism than in normal subjects, thus the reduced plasma glucagon response to arginine observed in the patients could not reflect an inadequate aminogenic stimulus.

It is now established that hypoglycaemia is a potent stimulus of glucagon release (Unger et al. 1962; Ohmeda et al. 1969; Buchanan et al. 1969). Thus, insulin-induced hypoglycaemia has been suggested as a new test for evaluating pancreatic alpha cell function in man (Gerich et al. 1974). Comparison of the effects of insulin-induced hypoglycaemia and arginine on the release of glucagon in normal subjects shows that arginine is the more potent stimulus. However, the mechanism involved in both tests for glucagon release might be different. Hence, it does not necessarily mean that insulin-induced hypoglycaemia is inferior to arginine for assessing the alpha cell function under various condi-
tions. The present study demonstrates that there is no difference in the magnitude of glucagon response to hypoglycaemia between the two groups, despite the fact that a low glucagon response to arginine occurs in patients with hyperthyroidism, indicating that the pancreatic alpha cells of the patients are relatively insensitive to L-arginine, but not to a fall in plasma glucose concentration. The discrepancy in plasma glucagon response to arginine and to hypoglycaemia has also been observed in juvenile diabetics (Gerich et al. 1973) who, however, respond to arginine but not to hypoglycaemia, just the reverse of that encountered in our patients.

The fact that the pancreatic alpha cell of patients with hyperthyroidism which responds poorly to L-arginine, responds normally to hypoglycaemia, excludes the possibility of a common defect in the secretory apparatus of the cell but supports the assumption that the reactivity to L-arginine of the alpha cell of the patients is impaired selectively. A similar behaviour of the beta cell was observed in the patients, i.e. a failure of the plasma IRI level to rise during the arginine test and a normal decrease in plasma CPR after the injection of insulin.

These findings suggest a functional defect common to both the alpha and beta cell in the response to L-arginine in the patients. The site of such a lesion is speculative but may involve either a loss of arginine recognition because of a defective arginine receptor, if any (Fajans et al. 1971), or aberrant transmission of the information due to a defective intracellular messenger system, possibly connected with a metabolic disturbance of the cells (Seino et al. 1974). Furthermore, the different response of these cells to arginine and to hypoglycaemia may be explained by the existence of different pathways for arginine-induced and hypoglycaemia-induced secretion. Since plasma GH responses to arginine and insulin-induced hypoglycaemia were reduced to the similar extent in our patients (data not shown), the selective impairment of the response to L-arginine is likely to be confined to the pancreatic alpha and beta cells.

The possibility cannot be excluded that the excessive metabolism of IRI and IRG resulted in the lower concentrations of these hormones during the arginine infusion in hyperthyroidism as compared with those in normal subjects. However, if it exists at all, it is probably only of minor importance, because the concentrations of plasma IRG during ITT were similar in the patients to and in the controls.

The failure of blood glucose response to the arginine infusion observed in the patients is difficult to explain, but it may be partly due to the blunted rise in plasma glucagon level during the arginine infusion. In addition, it may be connected with the finding that exogenous glucagon caused only a slight and transient rise in blood glucose in hyperthyroidism (Andreani et al. 1970), probably resulting from the diminished glycogen content of the liver observed in this condition (Hoch 1962).
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