Impaired Plasma Insulin Response to Arginine in Hyperthyroidism

Important Role of the Rise of Blood Glucose in the Second Phase of Insulin Release Induced by Arginine

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SUMMARY

Intravenous infusion of 30 gm. of L-arginine over a period of 45 minutes elicited a biphasic insulin response and a moderate blood glucose rise in normal subjects. In patients with hyperthyroidism, both insulin peaks, especially the second one, were low, with virtual absence of the blood glucose response. A single intravenous injection of 4 gm. of arginine provoked similar uniphasic plasma insulin responses in both normal subjects and hyperthyroid patients. Pretreatment with either glucose or xylitol almost completely restored the biphasic insulin response to arginine in patients with hyperthyroidism, whereas pretreatment with aminophylline only partially improved the insulin response. Combined administration of a small amount of glucose (2.25 mg./kg./min.) with arginine also restored the normal second-phase insulin release, with blood glucose rises similar to those in normal subjects given arginine alone. It is concluded that the plasma insulin response to arginine is impaired, especially in its second phase, in patients with hyperthyroidism due to the absence of a blood glucose rise. The second phase of arginine-induced insulin release seems more dependent on glucose than the first phase. DIABETES *25*:961-68, October, 1976.

Since Floyd et al.[1] first reported an increase in plasma insulin during the infusion of amino acids, L-arginine and other amino acids have been extensively used as insulin secretagogues. In our previous studies,[2] we observed that the plasma insulin response to arginine was markedly impaired in patients with hyperthyroidism whereas it was exaggerated in patients with hypothyroidism. This is in contrast to observations that the glucose-induced insulin release is either within normal limits or slightly exaggerated in hyperthyroid patients.[3-5] The present studies were undertaken to elucidate the mechanism responsible for this impaired insulin response to arginine in hyperthyroidism.

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METHODS

Thirty-two patients with unequivocal hyperthyroidism, 19 males and 13 females, aged 19 to 58, were studied. The diagnosis was established by routine thyroid examinations including Triosorb resin sponge uptake (41.7-58.5 per cent) and plasma thyroxine levels (15.4-28 μg./dl.). All were diagnosed as having Graves' disease on the basis of clinical findings and thyroid scintigram. None of them weighed more than 10 per cent over the ideal body weight or had a family history of diabetes. Basal blood glucose levels were within normal limits in all patients.

As controls, 27 normal subjects—22 males and 5

females—aged 19 to 54, were also studied. They were nonobese (less than 15 per cent above the ideal body weight) and apparently normal in endocrine function. None of them had a family history of diabetes or elevated fasting blood glucose levels.

The subjects were not allowed to smoke or to take anything by mouth after 10 p.m. the night before the study. They came to the laboratory at 8:00 to 8:30 a.m. and lay down for at least 30 minutes before the study.

An indwelling needle was placed in the antecubital vein to obtain serial blood samples, and various solutions were infused into the opposite antecubital vein. Some subjects were used for several experiments with an interval of at least three days between experiments, whereas others were used for only one experiment. All were encouraged to eat a weight-maintaining diet with at least 40 per cent of the calories as carbohydrate during the experimental period. The following experiments were performed:

1. *Infusion of arginine.* L-arginine hydrochloride, 30 gm. dissolved in 300 ml. of saline, was infused intravenously over a period of 45 minutes in 15 normal subjects and 21 patients with hyperthyroidism. Blood was withdrawn before and 5, 10, 15, 20, 30, 45, 60, 90, and 120 minutes after the start of the infusion.

2. *Intravenous injection of arginine.* Four grams of L-arginine in 20 ml. saline was injected intravenously within two minutes into 13 normal subjects and 10 patients with hyperthyroidism. Blood was withdrawn before and 3, 5, 10, 20, and 30 minutes after the start of injection.

3. *Glucose and arginine infusions.* Four normal subjects and six hyperthyroid patients received intravenous infusions of arginine, as described above. Three to seven days later, they were challenged with arginine again following an intravenous bolus of glucose (0.25 gm./kg. body weight in a 50 per cent solution) and subsequent infusion of 5 per cent glucose at a rate of 10 mg./kg./min. over a period of 90 minutes. Infusion of arginine was started 90 minutes after the start of glucose infusion. Blood was withdrawn 45, 40, 30, and 15 minutes and immediately before and 5, 10, 15, 20, 30, 45, 60, and 90 minutes after the start of arginine infusion.

4. *Combined intravenous infusion of a small amount of glucose with arginine.* A mixture of 2.25 mg./kg./min. of glucose and 30 gm. of arginine dissolved in 300 ml. of saline was infused intravenously over a period of 45 minutes in six patients with hyperthyroidism. Blood was withdrawn as in experiment 1.

5. *Xylitol and arginine infusions.* Eight normal subjects and six hyperthyroid patients received arginine infusions, once without any pretreatment and once following the intravenous infusion of xylitol, a bolus of 0.25 gm./kg. body weight followed by intravenous infusion at a rate of 15 mg./kg./min. over a period of 90 minutes. Blood was withdrawn at intervals as in experiment 3.

6. *Infusions of aminophylline and arginine.* Five normal subjects and five hyperthyroid patients received arginine infusions, once without any pretreatment and once again after an intravenous infusion of 500 mg. of aminophylline dissolved in 500 ml. of saline over a period of 90 minutes. Blood was withdrawn at intervals as in experiment 3.

Blood was taken into heparinized syringes from the indwelling needle, and an aliquot of blood was used for the determination of blood glucose. The remaining portion was centrifuged as soon as possible. Plasma was separated, frozen, and stored until assayed. Blood glucose was measured by a ferricyanide reduction method with a Technicon AutoAnalyzer. Plasma insulin was determined by the immunoassay kit of the Radiochemical Centre (Amersham, England), which is based on the double-antibody immunoprecipitation technique (method c) of Hales and Randle.[6] Highly purified human insulin (Novo) was used as the standard. The minimal detectable quantity of insulin for the assay was 5 μU./ml. The insulin assay was run simultaneously for corresponding samples with or without pretreatment. Statistical analysis was performed by either Student's t test or paired t test.

RESULTS

Effect of intravenous infusion of arginine on plasma insulin and blood glucose in normal subjects and hyperthyroid patients. The intravenous infusion of arginine caused a biphasic increase in plasma insulin levels in normal subjects, with peak levels occurring at five minutes and 45 minutes; the latter coincided with the end of the arginine infusion (figure 1, table 1). The mean ($\pm$S.E.) first and second peaks of plasma insulin were 35.7 $\pm$ 1.6 μU./ml. and 46.1 $\pm$ 2.7 μU./ml., respectively ($P<0.01$ vs. basal level). Blood glucose rose transiently, with the mean ($\pm$S.E.) peak value of 107.9 $\pm$ 2.1 mg./dl. ($P<0.01$ vs. basal level) occurring 30 minutes after the start of the arginine infusion, as shown in figure 1.

In 21 hyperthyroid patients, arginine infusion elicited only a slight increase in plasma insulin as shown

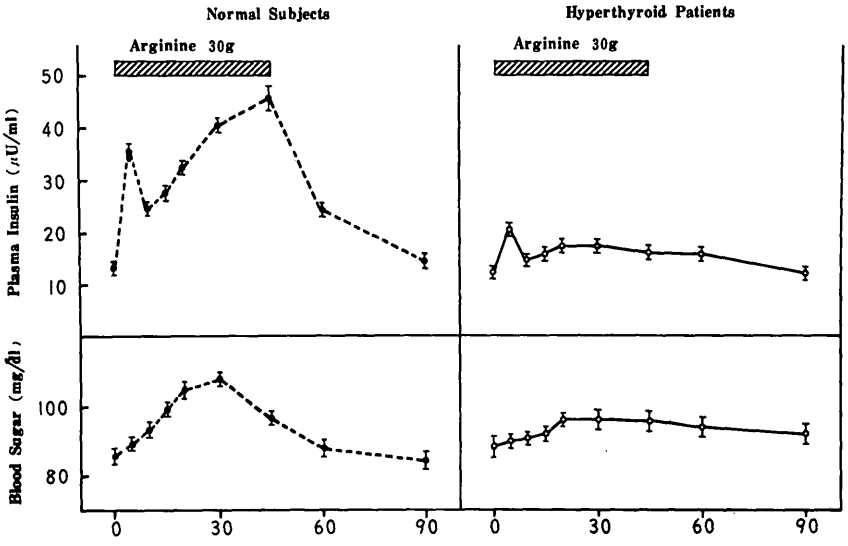

FIGURE 1

Effect of intravenous infusion of 30 gm. of arginine over a period of 45 minutes on plasma insulin and blood glucose levels in 15 normal subjects and 21 patients with hyperthyroidism. Means ± S.E. are shown.

in figure 1 and table 1. Although both insulin peaks were low in hyperthyroidism, the second peak seemed more impaired than the first one (table 1). Plasma insulin levels at five minutes tended to be higher than those at 45 minutes in hyperthyroid patients, whereas the latter levels were more elevated in normal subjects. Blood glucose levels rose only slightly following arginine infusion in hyperthyroid patients (figure 1).

Effect of a single intravenous injection of arginine on plasma insulin and blood glucose in normal subjects and hyperthyroid patients. The intravenous injection of arginine as a bolus caused an abrupt increase of plasma insulin, with the peak occurring three minutes after the start of injection in normal subjects ($P<0.01$ vs. basal level), as shown in figure 2. Blood glucose also rose significantly from a basal level of 89.8 ± 1.2 mg./dl. (mean ± S.E.) to 103.6 ± 2.3 mg./dl. ($P<0.01$). In patients with hyperthyroidism, plasma insulin increased abruptly following the bolus injection of arginine, reaching its peak at three minutes, which was comparable to that in normal subjects (figure 2). Unlike normal subjects, however, these patients showed only a minimal increase in blood glucose levels following the injection of arginine, with the peak blood glucose level of 95.5 ± 3.1 mg./dl.

Effect of pretreatment with glucose on arginine-induced insulin secretion. Intravenous infusion of glucose over a period of 45 minutes raised the mean (±S.E.) blood glucose from 85.0 ± 3.1 to 221 ± 19.7 mg./dl. in normal subjects ($P<0.01$). Subsequent infusion of arginine did not raise blood glucose further but gradually lowered it, as shown in figure 3. Plasma insulin levels rose significantly following glucose infusion from the pretreatment levels of 9.5 ± 2.1 μU./ml.

TABLE 1

First and second peaks of plasma insulin during arginine infusion with or without glucose, xylitol, or aminophylline pretreatment in normal subjects and patients with hyperthyroidism

Treatment	Normal subjects		Hyperthyroid patients		Normal vs. hyperthyroid	
	1st Peak	2nd Peak	1st Peak	2nd Peak	1st Peak	2nd Peak
Arginine	35.7 ± 1.6*	46.1 ± 2.7	20.8 ± 1.6	17.6 ± 1.6	P < 0.001	P < 0.001
Arginine	33.5 ± 2.9	45.2 ± 2.2	17.6 ± 3.3	16.6 ± 3.3	P < 0.05	P < 0.001
Glucose + arginine	275.0 ± 60.2	183.0 ± 59.4	194.6 ± 12.4	102.8 ± 25.2	n.s.	n.s.
Control vs. glucose	P < 0.05	P < 0.05	P < 0.01	P < 0.02		
Arginine	34.2 ± 1.8	43.6 ± 4.5	19.3 ± 4.5	17.6 ± 4.7	P < 0.02	P < 0.001
Xylitol + arginine	65.6 ± 6.3	69.0 ± 10.6	45.0 ± 5.9	47.8 ± 8.0	n.s.	n.s.
Control vs. xylitol	P < 0.01	P < 0.05	P < 0.01	P < 0.01		
Arginine	36.0 ± 2.7	46.8 ± 2.8	18.4 ± 2.5	18.2 ± 2.3	P < 0.01	P < 0.001
Aminophylline + arginine	41.2 ± 3.5	58.2 ± 4.7	26.4 ± 3.9	26.8 ± 2.2	P < 0.05	P < 0.01
Control vs. aminophylline	n.s.	P < 0.02	n.s.	n.s.		

*μU./ml., means ± S.E.M. are shown.

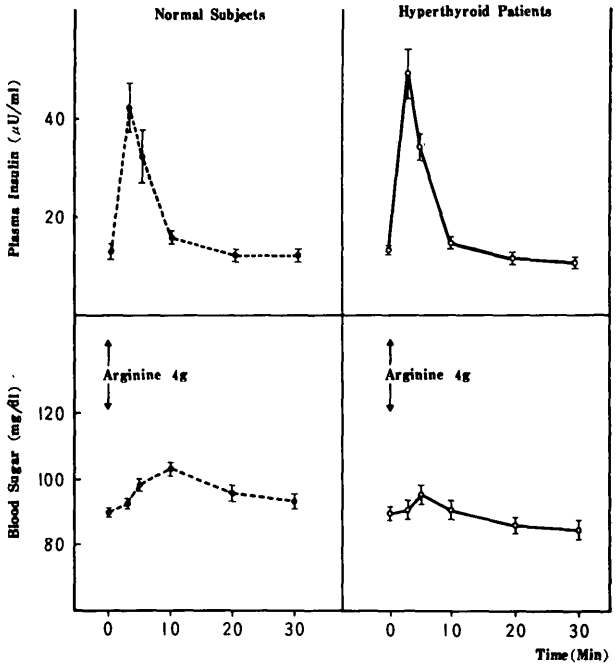

FIG. 2. Effect of a single intravenous injection of 4 gm. of arginine on plasma insulin and blood glucose levels in 13 normal subjects and 10 patients with hyperthyroidism. Means ± S.E. are shown.

(mean ± S.E.) to 48.0 ± 6.2 μU./ml. ($P<0.01$). Subsequent infusion of arginine elicited a sharp increase in plasma insulin, with the peak at five minutes, followed by a gradual decrease in spite of continuing arginine infusion (figure 3). The mean peak insulin level at five minutes was significantly higher than that induced by arginine alone ($P<0.05$) (table 1).

In patients with hyperthyroidism, pretreatment with glucose raised blood glucose levels from 93.6 ± 3.9 mg./dl. (mean ± S.E.) to 254.8 ± 14.0 mg./dl., both of which were slightly but not significantly higher than those in normal subjects. The intravenous infusion of arginine, begun 45 minutes after the start of the glucose infusion, gradually lowered blood glucose levels as in normal subjects (figure 3). Plasma insulin levels increased gradually from basal levels of 11.2 ± 2.2 μU./ml. (mean ± S.E.) to 38.2 ± 2.7 μU./ml. following glucose infusion and then rose abruptly after the start of the arginine infusion, as shown in figure 3. The peak insulin level at five minutes was significantly higher than that induced by arginine alone ($P<0.01$) (table 1). Plasma insulin then declined gradually, without the second peak that usually appears at the end of the arginine infusion. Peak insulin levels following glucose-arginine infusion in hyperthyroidism were slightly but not significantly lower than those in normal subjects (194.6 ± 12.4 μU./ml. vs. 275 ± 60.0 μU./ml.).

Effect of the concomitant administration of a small amount of glucose on arginine-induced insulin secretion in hyperthyroid patients. In order to simulate the slight increase of blood glucose during the infusion of arginine in normal subjects, 2.25 mg./kg./min. of glucose mixed with arginine was infused into patients with hyperthyroidism. As shown in figure 4, the blood glucose rise in this experiment was almost the

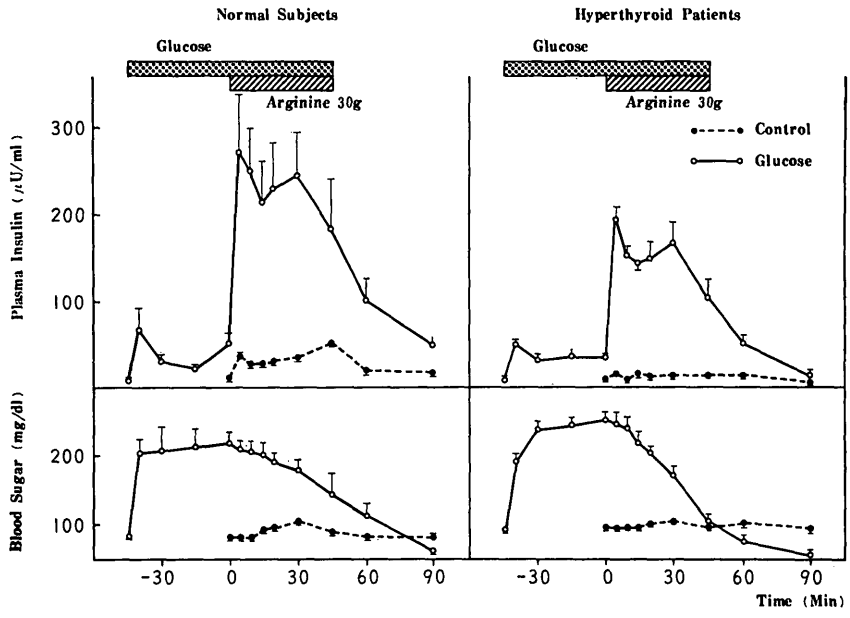

FIGURE 3

Effect of pretreatment with glucose (0.25 gm./kg. body weight as a bolus plus 10 mg./kg./min. as intravenous infusion for 90 minutes) on plasma insulin and blood glucose responses to arginine in four normal subjects and six patients with hyperthyroidism. Means ± S.E. are shown.

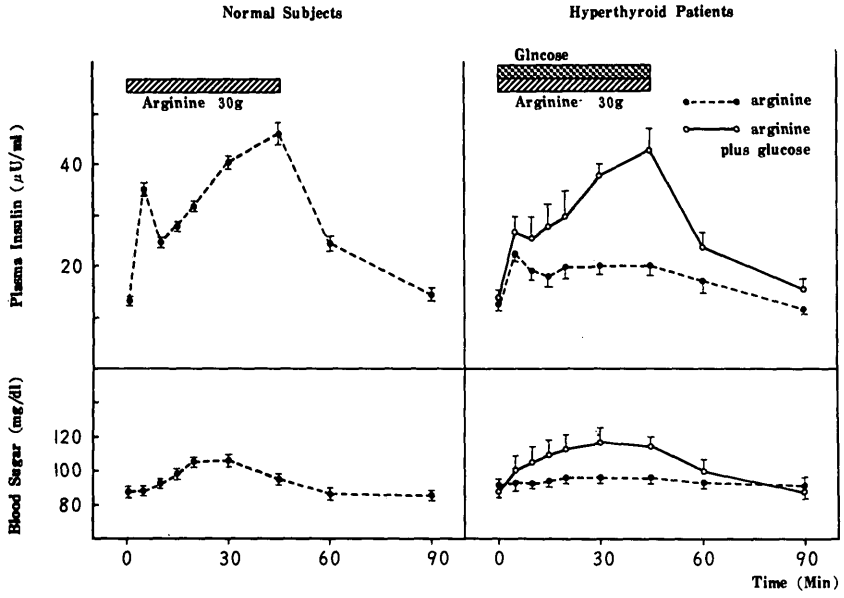

FIGURE 4

Effect of a combined intravenous administration of glucose (2.25 mg./kg./min.) and arginine (30 gm.) on plasma insulin and blood glucose levels in six patients with hyperthyroidism (right panel). For comparison, plasma insulin and blood glucose levels during the arginine infusion in normal subjects are shown in the left panel. Means ± S.E. are shown.

same as in normal subjects receiving arginine alone. Plasma insulin in hyperthyroid patients rose significantly, showing a biphasic pattern similar to that in normal subjects (figure 4). Plasma insulin 45 minutes after the start of the combined administration was significantly higher than during arginine infusion (42.3 ± 5.1 μU./ml. vs. 20.0 ± 2.4 μU./ml., $P<0.01$).

Effect of pretreatment with xylitol on arginine-induced insulin secretion. The intravenous infusion of xylitol over a period of 45 minutes raised blood glucose levels only slightly but abolished the blood glucose rise following arginine infusion in normal subjects (figure 5). Plasma insulin levels were virtually unchanged during xylitol infusion but their responses to arginine were significantly exaggerated with the mean (±S.E.) levels at five and 15 minutes of 65.6 ± 6.3 μU./ml. and 69.0 ± 10.6 μU./ml., respectively (figure 5). These levels were significantly higher than those seen during the infusion of arginine alone ($P<0.05$).

In hyperthyroid patients, xylitol infusion did not alter blood glucose levels and their responses to arginine infusion, as shown in figure 5. Plasma insulin levels were slightly elevated during the infusion of xylitol alone and their responses to arginine were significantly enhanced; the mean plasma insulin level at

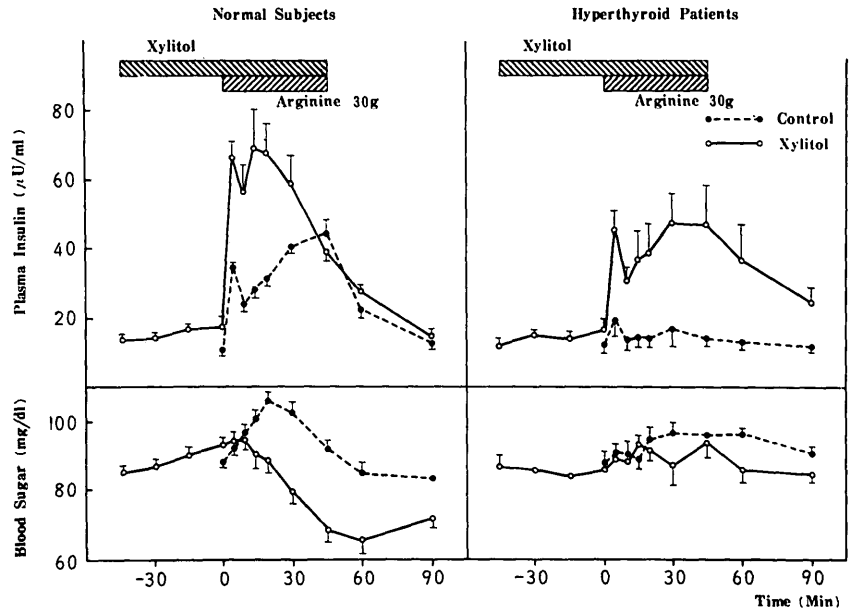

FIGURE 5

Effect of pretreatment with xylitol (0.25 gm./kg. body weight as a bolus plus 15 mg./kg./min. as intravenous infusion for 90 minutes) on plasma insulin and blood glucose responses to arginine in eight normal subjects and six patients with hyperthyroidism. Means ± S.E. are shown.

five minutes was 45.0 ± 5.9 μU./ml., which was significantly higher than the mean level of 19.3 ± 4.5 μU./ml. following the infusion of arginine alone ($P<0.05$). The second insulin peak usually observed at the end of arginine infusion was 46.5 ± 12.6 μU./ml. following the xylitol-arginine infusion, which was also significantly higher than the value, 14.5 ± 2.7 μU./ml., following the infusion of arginine alone ($P<0.01$). The plasma insulin level five minutes after the start of xylitol-arginine infusion was still lower in hyperthyroid patients than in normal subjects (45.0 ± 5.9 μU./ml. vs. 65.6 ± 6.3 μU./ml., $P<0.05$).

Effect of pretreatment with aminophylline on arginine-induced insulin secretion. As shown in figure 6, intravenous infusion of aminophylline over a period of 45 minutes affected neither blood glucose levels nor their response to subsequent arginine infusion in normal subjects. Aminophylline infusion did not alter basal insulin levels but slightly enhanced their response to arginine infusion, as shown in figure 6. Plasma insulin levels at five and 45 minutes, 41.2 ± 3.5 μU./ml. and 57.2 ± 5.7 μU./ml., respectively, were slightly higher than corresponding control (arginine alone) levels of 36.0 ± 2.7 μU./ml. and 46.8 ± 2.8 μU./ml., respectively.

In hyperthyroidism, aminophylline infusion did not affect basal blood glucose levels but slightly raised blood glucose responses to arginine infusion. Arginine infusion following pretreatment with aminophylline elicited a slightly greater rise in plasma insulin, the first and second peaks being higher than those induced by arginine alone (figure 6).

DISCUSSION

The present study confirms our previous observation[2] that plasma insulin response to arginine infusion is markedly impaired in patients with hyperthyroidism. As already known,[7] arginine infusion elicits a biphasic increase in plasma insulin, with the first peak within five minutes and the second peak at the end of the infusion. In hyperthyroidism, both insulin peaks were lower than those in normal subjects; the second peak, which was higher than the first in normal subjects, was affected much more than the first.

In order to elucidate whether the first phase of arginine-induced insulin release is really impaired in hyperthyroidism, we compared plasma insulin response with a bolus injection of arginine in normal subjects and hyperthyroid patients. Both groups showed similar insulin peaks, appearing at three minutes. Although we have not studied dose-response relationship, our results suggest that only the second phase of arginine-induced insulin release is impaired in hyperthyroidism. The apparent lower first insulin peaks in hyperthyroid patients given arginine infusion can be explained by the timing of blood sampling; i.e., the first blood sampling at five minutes in the arginine infusion experiment might miss the real first insulin peak, which occurs within three minutes, and overlapping of the second-phase insulin release could not be eliminated.

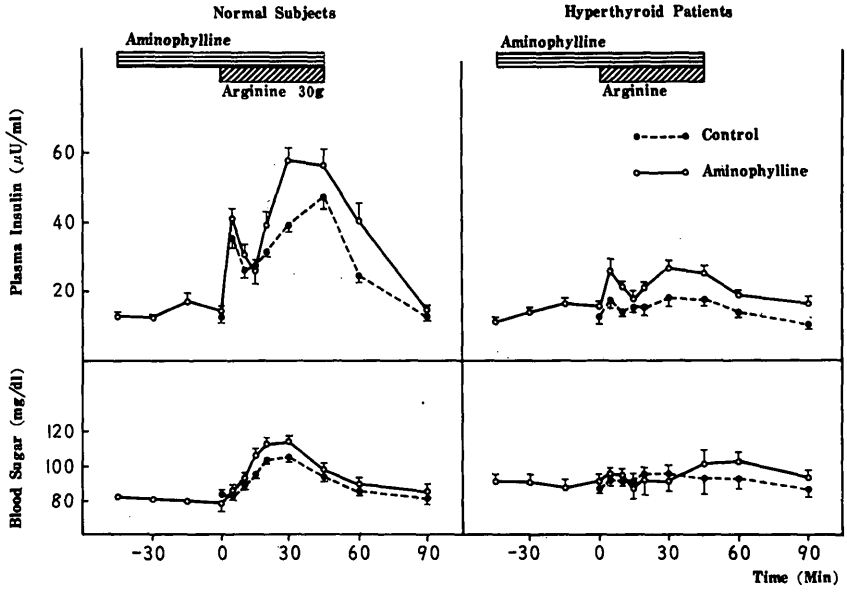

FIGURE 6

Effect of pretreatment with aminophylline (500 mg. as intravenous infusion for 90 minutes) on plasma insulin and blood glucose responses to arginine in five normal subjects and five patients with hyperthyroidism. Means ± S.E. are shown.

The reason for the impaired second-phase insulin release induced by arginine infusion in hyperthyroidism was not clear.

The possibility that accelerated metabolism of L-arginine in hyperthyroidism might lessen the stimulatory effect on the pancreatic islet is unlikely, because we found normal plasma glucagon response to arginine in hyperthyroidism.[2] Sakurai et al.[8] reported that plasma arginine levels during the infusion of arginine were only slightly, but not significantly, lower in hyperthyroid patients than in normal subjects. These results suggest that the impaired insulin response to arginine in hyperthyroidism can not be explained solely by the accelerated arginine clearance.

Thyroid hormones are known to enhance the beta-adrenergic-stimulating effect of catecholamines in adipose tissue, heart, and certain other tissues, although their effects on the pancreatic beta cells remain unclear. Bowers[9] reported that the increased tissue cyclic-AMP concentrations of the pituitary gland in thyroidectomized rats is lowered by the administration of triiodothyronine. It is possible, therefore, that thyroid hormones lower the cyclic AMP concentration in the pancreatic beta cell, causing decreased insulin secretion in response to arginine. This is probably not the case, however, because aminophylline, which is known to raise tissue cyclic AMP concentration by inhibiting the action of phosphodiesterase, only partially restored the arginine-induced insulin secretion in hyperthyroid patients.

Our present studies demonstrated that elevated blood glucose levels induced by pretreatment with glucose infusion restored the plasma insulin response to arginine infusion in hyperthyroid patients. That is, the first and second insulin peaks during the infusion of arginine after pretreatment with glucose were significantly higher than those in control experiments given arginine alone and only slightly, but not significantly, lower than those in normal subjects receiving glucose and arginine. This points to the importance of glucose in arginine-induced insulin secretion.

In the standard arginine test, blood glucose rose moderately following the start of arginine infusion, reaching a peak at 30 minutes in normal subjects, while it was virtually unchanged in hyperthyroid patients. Since plasma glucagon response to arginine infusion in hyperthyroid patients is almost within normal limits,[2] the impaired blood glucose response to arginine may be ascribed to a decreased glycogen store[10] in the liver. In order to simulate the normal blood glucose rise during the infusion of arginine, a small amount of glucose mixed with arginine was infused into patients with hyperthyroidism. In this experiment, a blood glucose rise almost comparable to that in normal subjects given arginine alone was achieved, as well as an almost-normal insulin response to arginine. These results suggest that the impaired arginine-induced insulin release in hyperthyroidism is caused by the lack of blood glucose rise during the infusion of arginine. The first insulin peak, observed five minutes after the start of the arginine infusion, preceded the blood glucose rise in normal subjects and appears to be less dependent on blood glucose. This is in accordance with the fact that the response of the first insulin pool was not impaired in hyperthyroidism.

Controversial results have been reported on arginine-induced insulin secretion in the absence of glucose. Some[11-15] have observed that arginine in the absence of glucose is capable of stimulating insulin secretion, while others[16-18] failed to find this effect. They agree, however, that the intrinsic ability of arginine to stimulate insulin release is, if any, very small and that glucose significantly augments arginine-induced insulin secretion. The present observations of humans agree with the results of animal experiments, suggesting that there is a close interaction between glucose and arginine in insulin secretion.

The exact mechanism by which glucose augments arginine-induced insulin release remains to be clarified. It has been suggested, especially on the basis of recent experiments using glucose anomers, that glucose may stimulate insulin release directly via a glucose receptor.[19-23] It is possible, therefore, that glucose receptors interact with amino acid receptors, possibly on the surface of pancreatic beta cells, as suggested by Pagliara et al.[15] However, other data suggest that some metabolite(s) of glucose may be the actual stimulant.[24-26] In the present experiment, xylitol, which has little effect on insulin release by itself, significantly augmented arginine-induced insulin release not only in normal subjects but also in hyperthyroidism. Since xylitol is considered to be metabolized through the glucuronic acid-xylulose pathway and subsequently the pentose phosphate shunt, the resulting metabolite(s) may enhance insulin secretion induced by arginine. These results seem to favor the metabolism hypothesis as far as the second-phase insulin release is concerned. However, Montague and Taylor[27] reported that isolated rat islets incubated with xylitol released insulin without any increase in intracellular glucose-6-phosphate concentration. Although xylitol alone has little, if any,

effect on insulin release in man, the receptor hypothesis cannot be ruled out completely.

Another interesting feature of the present experiments is the fact that the second-phase insulin release induced by arginine seems more dependent on blood glucose than the first-phase insulin release. Efendić et al.[28] reported that insulin-induced hypoglycemia lowered the first peak of plasma insulin in response to arginine infusion whereas glucose infusion augmented it. The latter observation agrees with ours. These results indicate that the first phase of arginine-induced insulin release may also be influenced by blood glucose levels, although the first blood sampling at five minutes in these experiments may not have completely excluded the overlapping of the second-phase insulin release. Efendić et al.[28] observed an absence of the second peak during arginine infusion in a normal subject who showed a decline in blood glucose levels. Using the isolated perfused rat pancreas, Gerich et al.[14] studied the effect of various concentrations of glucose on insulin response to 3.2 mM arginine and found only the first peak of insulin release at low concentrations of glucose. Our present experiments suggest that the second phase of arginine-induced insulin release becomes apparent when blood glucose exceeds certain levels in man also.

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