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Thyroxine Treatment and Insulin Secretion in the Rat

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Summary. Thyroxine treatment increases blood glucose and plasma insulin levels in the rat. The hypoglycemic effect of tolbutamide is more pronounced in treated animals. The immediate insulin secretory response of the isolated perfused pancreas to maximal, but not to submaximal, glucose stimuli was increased after thyroxine treatment, especially in the lower dose range. However, as thyroxine treatment reduces insulin release during the prolonged late phase, the total amount of insulin released from the pancreas is reduced. Both the early response to tolbutamide and the subsequent basal secretion were increased after thyroxine treatment. When the pancreas of treated rats was exposed to glucose plus pyruvate the inhibition of the late phase was reversed. Isoprenaline did not overcome the inhibitory effect of thyroxine treatment on the late phase of glucose-induced insulin release. Thyroxine induces a selective inhibition of glucose induced insulin release which is reversed by pyruvate; this indicates that thyroxine interferes with the glycolysis in the beta cell.

Key words: Rat, blood glucose, plasma insulin, pancreas perfusion, insulin secretion, thyroxine, glucose, tolbutamide, pyruvate, isoprenaline.

Hyperthyroidism is known to reduce glucose tolerance in animals and in man [1]. Investigations of the effect of thyroxine treatment on plasma insulin levels, or on insulin release from incubated pieces of pancreatic tissue in response to glucose have yielded apparently controversial results [2, 3, 4, 5, 6]. It is therefore difficult to predict how thyroxine treatment might affect glucose-induced insulin release.

Tolbutamide, another stimulating agent, is markedly more potent in lowering blood glucose in thyroxine treated mice [7] or rats treated with TSH [8]. As tolbutamide-induced insulin release has not perviously been measured in experimental hyperthyroidism we examined the possible effects of thyroxine treatment on glucose- and tolbutamide-induced insulin release.

Using the isolated perfused rat pancreas preparation, we have shown that thyroxine treatment does not only affect the total amount of insulin released, but, in addition, induces selective changes in both the early and late phases of insulin secretion [9].

Materials and Methods

1. Chemicals

The following substances were supplied as gifts: pure rat insulin from Novo GmbH, Mainz, isoprenaline from C. H. Boehringer Sohn, Ingelheim/Rhein, tolbutamide from Farbwerke Hoechst AG, Frankfurt. ^{125}I-labelled insulin was from Farbwerke Hoechst AG, Frankfurt, (−)-thyroxine (sodium salt) and bovine albumin (fraction V) were from Serva, Heidelberg. All other chemicals of analytical grade were from Merck AG, Darmstadt.

2. Animals and Experimental Design

Male albino Wistar rats (Winkelmann, Kirchborchen) were used throughout. They were fed a standard laboratory diet (Altromin®, Altromin GmbH, Lage/Lippe). *In vivo* experiments were performed on animals which weighed approximately 180 g; animals for perfusion experiments weighed approximately 200–220 g. On five subsequent days before animals were taken into an experiment they received a daily intraperitoneal injection of thyroxine (200 μg/kg b. wt. or 600 μg/kg b. wt.) or an equivalent volume of saline. This is sufficient to induce an increased metabolic rate in the rat [10]. Thyroxine treatment caused a weight loss of about 10–20 g during this period.

3. In Vivo Experiments

After five days thyroxine treated animals and control animals were fasted overnight (16 hr). Rats were sacrificed 15 min after the intraperitoneal administration of tolbutamide (50 mg/kg b. wt.) or of an equivalent volume of saline. Arterial blood was collected in heparinized test tubes for determination of plasma insulin and blood glucose.

4. Perfusion Experiments

After five days of treatment the animals were fasted for 24 hr and than anaesthetized by an intraperitoneal injection of pentobarbital (45 mg/kg b. wt.). The pan-

creas and the adjacent part of the duodenum, the spleen and the stomach were removed according to the method of Grodsky *et al.*, with slight modifications [11]. The abdominal aorta and the portal vein were connected to the arterial and venous cannulae, respectively. The perfusion medium consisted of Krebs-Ringer bicarbonate solution containing 0.2% of bovine albumin. It was gassed with 95% oxygen and 5% carbon dioxide, which resulted in a pH of 7.4. The temperature was maintained at 37° C. The flow rate was adjusted to 4 ml/min which resulted in a pressure between 50—70 mm Hg. We confirmed the finding of Bosboom *et al.* [12] that flow rates of 10—12 ml/min, as indicated by Curry *et al.*, often caused considerable leakage of perfusion medium from the preparation [13]. The arterial oxygen tension was 450—550 mm Hg and the venous oxygen tension was 250—350 mm Hg. At the designated zero time, 10 min after the beginning of the perfusion, the perfusate was switched to a medium containing the respective stimulating agent. The venous effluent was collected at various timed intervals (either 10 s, 1 min, or 5 min), as described in the text, and assayed for immunoreactive insulin. The actual glucose concentration in the medium was re-examined as well.

5. Analytical Methods

The immunoreactive insulin was determined by the method of Zaharko and Beck [14]. Pure rat insulin was used as the reference. Blood glucose and glucose in the perfusion medium were estimated by a GOD-Perid method (Boehringer-Test combination).

6. Calculations

Results were tested for statistical significance with the Wilcoxon test or Student's t-test. Results are presented as the mean ± S.E.M.

Results

1. In Vivo Experiments

Blood glucose was increased after thyroxine treatment (200 and 600 μg/kg b. wt./day, for five days). The blood glucose lowering effect of tolbutamide (50 mg/kg b. wt.) was more evident in thyroxine treated rats than in control animals (Table 1). After treatment with the lower thyroxine dose, the increased blood glucose level was associated with an increased insulin secretion, as indicated by the elevated plasma insulin level. Such an increase in plasma insulin cannot be demonstrated after treatment with the higher thyroxine dose (Table 1). Though the blood glucose lowering effect of tolbutamide was more pronounced in animals treated with the higher thyroxine dose, this effect of tolbutamide was not paralleled by a higher plasma insulin level (Table 1).

Table 1. *The effect of tolbutamide (50 mg/kg b. wt.) or an equivalent amount of saline on blood glucose and plasma insulin levels of thyroxine treated (200 or 600 μg/kg b. wt./day, for five days) and of control rats 15 min after administration and after fasting for 16 hr.*

Statistical evaluation: effect of thyroxine: [a] $2p < 0{,}05$, [b] $2p < 0{,}01$;
effect of tolbutamide: [aa] $2p < 0{,}05$, [bb] $2p < 0{,}01$.

treatment (for five days)	controls blood glucose mg/100 ml ± S.E.M.	(n)		tolbutamide	(n)	controls plasma insulin μU/ml ± S.E.M.	(n)		tolbutamide	(n)
saline	78,5 ± 7,4 a	(8)	bb	59,9 ± 2,8 —	(8)	10,8 ± 0,7 b	(8)	bb	22,1 ± 1,3 a	(8)
thyroxine (200 μg/kg)	87,5 ± 6,8 b	(8)	bb	62,5 ± 9,5 a	(8)	21,3 ± 5,5 —	(8)	aa	33,1 ± 3,3 —	(8)
thyroxine (600 μg/kg)	101,3 ± 3,0	(8)	bb	50,9 ± 3,2	(8)	14,6 ± 2,4	(8)	aa	24,1 ± 3,0	(7)

2. Perfusion Experiments

a) Effect of Glucose. Glucose (300 mg/100 ml) produced the well known biphasic insulin secretory pattern in the isolated perfused rat pancreas. After thyroxine treatment (200 or 600 μg/kg b. wt./day, for five days), however, glucose produced a distinctly different pattern in both the early and late phases of insulin secretion (Figs. 1 and 2). Thyroxine treatment with the lower dose resulted in a significantly higher first peak followed by a slightly, but not significantly, reduced second phase. Thyroxine treatment with the higher dose resulted only in a marginally increased immediate response, while there was hardly any second phase of insulin release (Figs. 1 and 2).

b) Effect of Tolbutamide. In response to tolbutamide (20 mg/100 ml), the typical monophasic insulin secretory pattern was obtained (Figs. 1 and 2). After thyroxine treatment (200 or 600 μg/kg b. wt./day, for

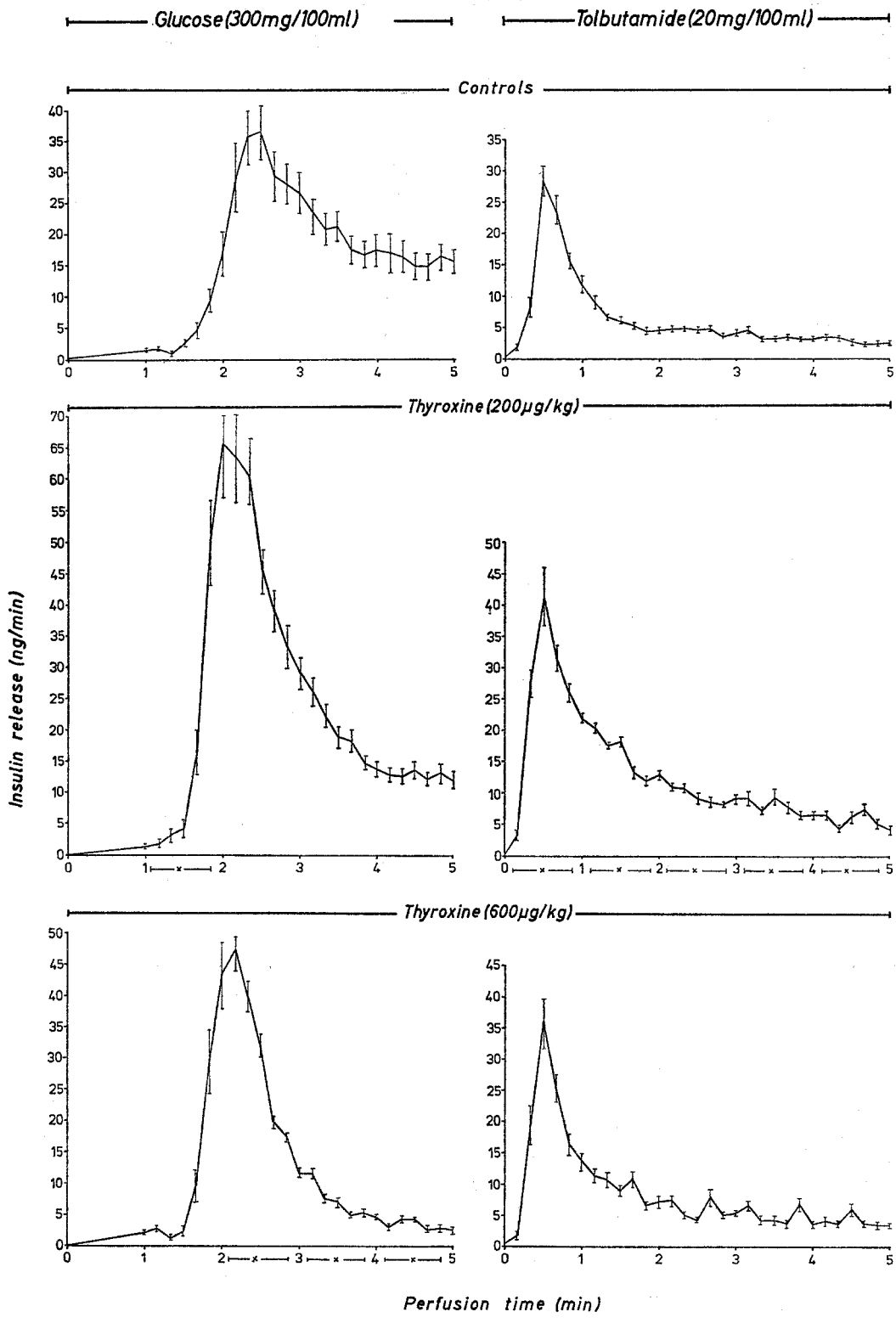

Fig. 1. Comparison between the effect of glucose and tolbutamide on the immediate insulin secretory response (1–5 min) of the perfused pancreas from thyroxine treated rats and controls. Mean ± S.E.M. Number of experiments: N = 7. × 2 $p < 0{,}05$ as compared to controls

five days) the same monophasic pattern could be found, differing from control experiments only in an increased first peak and a slightly increased basal insulin secretion.

c) Effect of a Submaximal Glucose Stimulus. There was no difference in the immediate insulin secretory response between thyroxine treated animals and controls to a submaximal glucose stimulus (150 mg/100 ml) (Fig. 3).

d) Effect of Glucose Plus Pyruvate. In response to glucose (300 mg/100 ml) plus pyruvate (15 mM), however, the late phase of insulin release of the pancreas from thyroxine treated animals (600 μg/kg b. wt./day, for five days), could be restored (Fig. 5). Nevertheless, in comparison with controls, there were distinct differences in the insulin secretory pattern. The first peak was higher and more pronounced, as was the nadir which followed the immediate response (Fig. 4). On the other hand the late phase of insulin release was somewhat higher in controls (Fig. 5).

e) Effect of Isoprenaline on the Late Phase of Insulin Release. Isoprenaline (0.1 mM) added to the perfusion medium 10 min after glucose (300 mg/100 ml), i.e. at the beginning of the second phase of insulin secretion, did not influence insulin release from the isolated perfused pancreas from controls as well as from thyroxine (600 μg/kg b. wt./day, for five days) treated rats. Thus, isoprenaline (0.1 mM) did not overcome the thyroxine-induced inhibition of insulin release.

Discussion

In mice treated with thyroxine an extremely low dose of tolbutamide induced hypoglycaemic convulsions [7]. The present data on plasma insulin levels in rats give little evidence in favour of a more pronounced pancreatic response to tolbutamide in rats following thyroxine treatment (Table 1). As plasma insulin levels determined at a given time do not necessarily reflect either the time course of insulin secretion or the total amount of insulin released, experiments were performed on the isolated perfused rat pancreas.

The results revealed that thyroxine treatment modifies the insulin secretory pattern in response to glucose. Both the early and late phase were changed in a specific way (Figs. 1 and 2). The immediate insulin secretory response to glucose was increased, especially following the lower dose of thyroxine (Fig. 1). In principle such an increased response to glucose could originate from either an elevated capacity of the islet cell to release insulin rapidly or from a more sensitive response to glucose. The latter appears un-

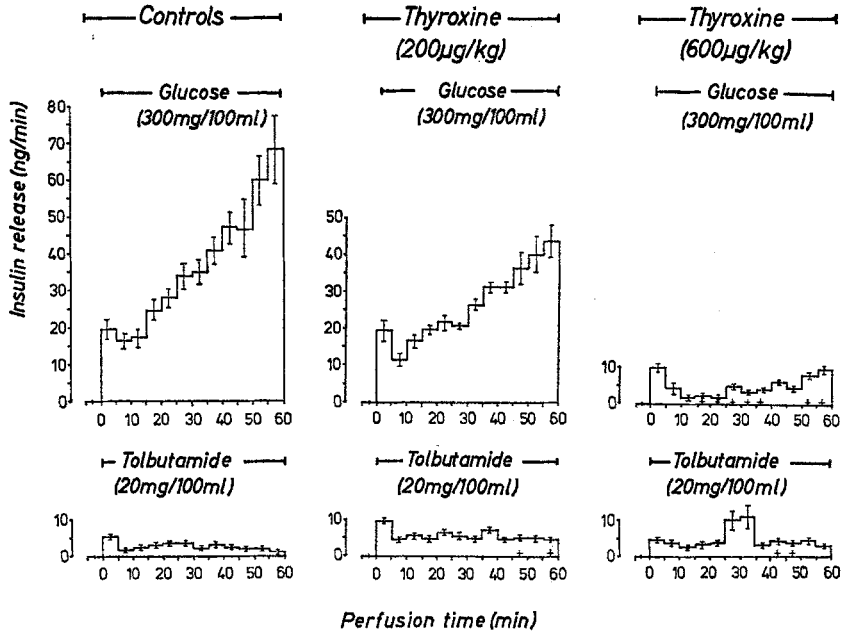

Fig. 2. Comparison between the effect of glucose and tolbutamide on insulin release from the perfused pancreas from thyroxine treated rats and controls. Mean ± S.E.M. Number of experiments: N = 4. + $2p < 0{,}05$ as compared to controls.

likely, however, as the recognition of the glucose stimulus by the beta cell does not seem to be improved, for a submaximal glucose stimulus (150 mg/100 ml) did not result in an increased, immediate insulin secretory response after thyroxine treatment (Fig. 3).

Against these findings, which could be interpreted as indicating that thyroxine is acting as an insulinotropic agent, is the well known diabetogenic effect of hyperthyroidism [1]. In addition, the amount of insulin released from incubated pieces of rat pancreatic tissue in response to glucose was markedly reduced after thyroxine treatment [6]. A resolution of this apparent contradiction is given by the finding that the late phase of insulin release was suppressed or even nearly abolished after thyroxine treatment (Fig. 2). It is this long lasting phase that contributes

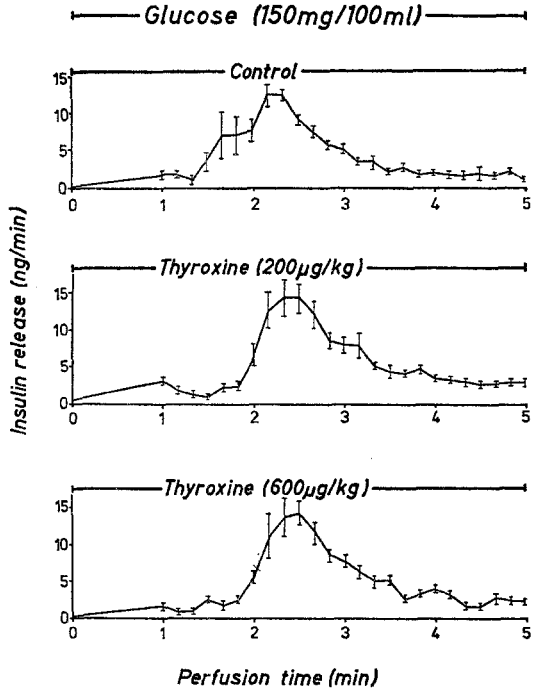

Fig. 3. Effect of a submaximal glucose stimulus on the immediate insulin secretory response (1–5 min) of the perfused pancreas from thyroxine treated rats and controls. Mean ± S.E.M. Number of experiments: N = 6. × 2 $p < 0{,}05$ as compared to control

the major part of the total amount of insulin released from the pancreas in response to glucose stimulation. Any interference with insulin secretion during the late phase should markedly reduce the capacity of the endocrine pancreas to respond adequately to elevated blood glucose levels. This conclusion does not necessarily contradict the results in intact rats treated with thyroxine (Table 1). Basal plasma insulin levels were increased after administration of the lower thyroxine dose and not reduced in response to the higher thyroxine dose. The capacity of the islet cells to respond adequately to a glucose load has not been tested in these experiments.

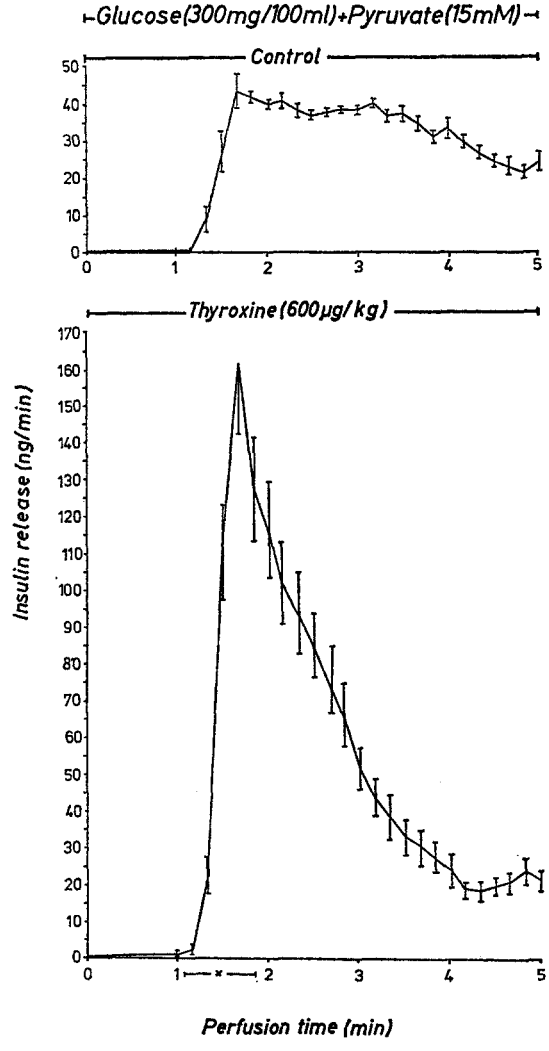

Fig. 4. Effect of a combination of glucose and pyruvate on the immediate insulin secretory response (1–5 min) of the perfused pancreas from thyroxine treated rats and controls. Mean ± S.E.M. Number of experiments: N = 4. × 2 $p < 0{,}05$ as compared to control

Following an initially increased insulin release the secretory response of the perfused pancreas to glucose faded, resulting in a failure to supply insulin for a prolonged period. Among other factors such a failure may reflect an exhaustion of the beta cell. In order to supply a source of metabolic energy which is utilized independently from glycolysis pyruvate was added, in

addition to glucose, to the perfusion medium. In this combination pyruvate was able to restore the late phase of glucose induced insulin release (Fig. 5). Thus insulin was apparently still available for secretion, although not made accessible by glucose alone. Pyruvate has been shown not to significantly affect glucose-induced insulin release from the perfused pancreas of untreated animals (Fig. 5). But some inhibitory effect of thyroxine treatment remained even in the presence of pyruvate (Fig. 5). The ability of pyruvate to induce insulin release during the late

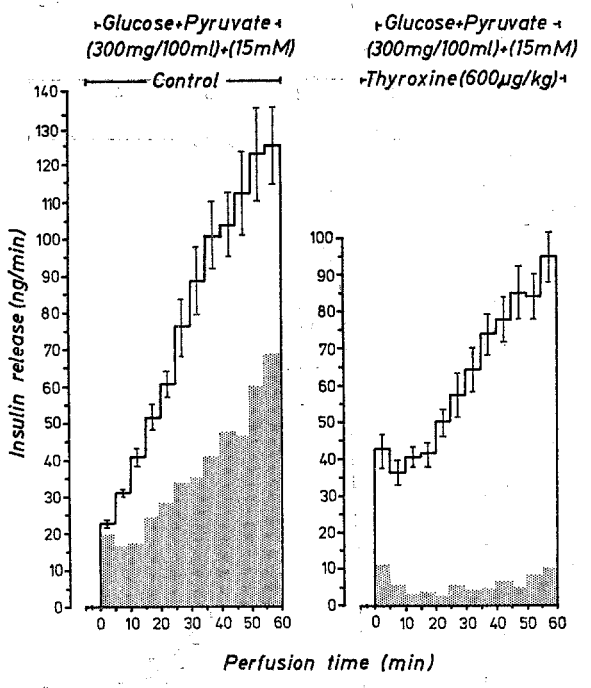

Fig. 5. Effect of a combination of glucose and pyruvate on insulin release from the perfused pancreas from thyroxine treated rats and controls. Shadowed area represents the amount of insulin secreted under identical conditions in response to glucose alone (as shown in Fig. 2). Mean ± S.E.M. Number of experiments: N = 4. × 2 $p < 0{,}05$ as compared to control

phase after thyroxine treatment implies that exposure to thyroxine may possibly interfere with glycolysis in the beta cell. There is at least some similarity to iodoacetate, which has been employed to block glycolysis in islet cells [15], as inhibition of insulin release induced by iodoacetate is reversed by pyruvate [16, 17].

Isoprenaline is known to induce insulin release from the isolated perfused rat pancreas [18] and to increase the intracellular level of cyclic AMP [19]. The failure of isoprenaline to overcome thyroxine-induced inhibition of insulin release may indicate that low cyclic AMP levels are not responsible for the impaired secretion. This conclusion is based on the unproved assumption that sufficient ATP is available in islet cells of thyroxine treated rats to secure an adequate synthesis of cyclic AMP.

Unlike glucose, tolbutamide induced a monophasic insulin secretory pattern. Following thyroxine treatment the immediate secretory response to tolbutamide was similarly increased. This suggests that thyroxine affects the primary response of the beta cell in a way not specific for glucose-induced insulin release.

As the exposure of the islet cell to thyroxine characteristically inhibits the late phase of insulin release, which is typical for glucose and not elicited by sulfonylureas, this inhibitory effect seems to be specifically linked to glucose induced insulin release. Therefore this may reflect a specific interference with glucose metabolism in the beta cell.

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