INHIBITORY EFFECT OF QUINALDIC ACID ON GLUCOSE-INDUCED INSULIN RELEASE FROM ISOLATED LANGERHANS ISLETS OF THE RAT*

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Received June 4,1974

SUMMARY

Influence of quinaldic acid on glucose-induced insulin release from isolated Langerhans islets of rats was studied. Glucose, in high concentration (15 mM), stimulated insulin release for 30 min after incubation (the first phase), followed by distinctly increasing release during the subsequent 60 min (the second phase). Addition of 12 mM quinaldic acid to an incubation medium containing 15 mM glucose caused almost complete inhibition in the second phase of glucose-induced insulin release, while the first phase was insensitive to quinaldic acid. A possible mechanism concerning the effect of quinaldic acid on β-cells of Langerhans islets is discussed.

We have previously shown that the level of kynurenine 3-hydroxylase in hyperthyroid rats decreased to about 50 % of the control value, while the level of kynurenine aminotransferase increased to about 150 %, resulting in the over production of kynurenic acid (1). There are papers reporting observations of an elevated formation of kynurenic acid and xanthurenic acid in steroid-treated patients and pregnant women (2-5).

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* This work has been supported in part by the Scientific Research Fund of the Ministry of Education of Japan and by a grant from the Tanabe Amino Acid Research Foundation.

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It was reported that kynurenic acid and xanthurenic acid were further metabolized to quinaldic acid and 8-hydroxyquinaldic acid, respectively (6-8), and that normal human subjects excreted quinaldic acid after ingestion of kynurenic acid in quantities sufficient to account for as much as 30 % of the dose (6). However, almost no attention has so far been paid to the physiological or pathological significance of quinaldic acid or 8-hydroxyquinaldic acid. Recently, we have made an observation indicating that quinaldic acid and its relatives have a property which causes insulin release from isolated Langerhans islets of rats (9).

The present work describes the inhibitory effect of quinaldic acid on the glucose-induced insulin release from the islets. Evidence of inhibition by quinaldic acid in the second phase of glucose-induced insulin release is presented.

METHODS AND MATERIALS

Pancreatic islets of Langerhans were isolated from male Wistar rats weighing 400 g by the method of Lacy and Kostianovsky (10). Batches of 6 islets, of comparable size, per flask were incubated in 0.5 ml of Krebs-Ringer bicarbonate solution supplemented with 5 mM pyruvate, 5 mM fumarate, 5 mM glutamate and bovine serum albumin (2 mg/ml) (10). The incubation medium for insulin release studies contained various concentrations of quinaldic acid and glucose, as indicated in Results. Incubation was carried out at 38° in an atmosphere of O_2 : CO_2 (95:5 %, v/v). The insulin content of the incubated media was measured by the double antibody radioimmunoassay technique (11). Collagenase (Code : CLS IV) was purchased from Worthington Biochemical Corporation. Quinaldic acid (quinoline-2-carboxylic acid) was purchased from Wako Pure Chemical Industries, LTD., Osaka, Japan, and added to the incuba-

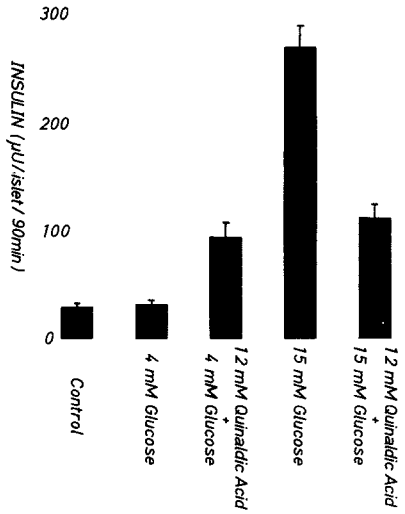

Fig. 1. Effect of quinaldic acid on insulin release from Langerhans islet.
Incubation medium as described in Methods, supplemented with glucose and quinaldic acid at the indicated concentration. A control experiment without test substance was run parallel. Results represent means ± S.E. in μU of insulin released in 4 incubation flasks.

tion medium after adjustment to pH 7 with NaOH. Puromycin was a product of Nutritional Biochemicals Corporation. Crystalline human insulin, pork insulin labeled with ^{125}I, anti-insulin guinea pig serum and anti-γ-G sheep serum were obtained commercially from Dainabot Radioisotope Laboratories, LTD., Tokyo, Japan.

RESULTS

Fig. 1 summarizes those studies which are pertinent to the effect of quinaldic acid on insulin release in the presence of 4 mM and 15 mM glucose. It can be seen that only a small amount of insulin (30 or 32 μU/islet/90 min) was found in the incubated medium, with or without addition of 4 mM glucose. In the presence of 4 mM glucose, as much as 92 μU of insulin release was caused by 12 mM quinaldic acid. Glucose by itself, at high concentration (15 mM), induced a significant release of insulin (270 μU/islet/90

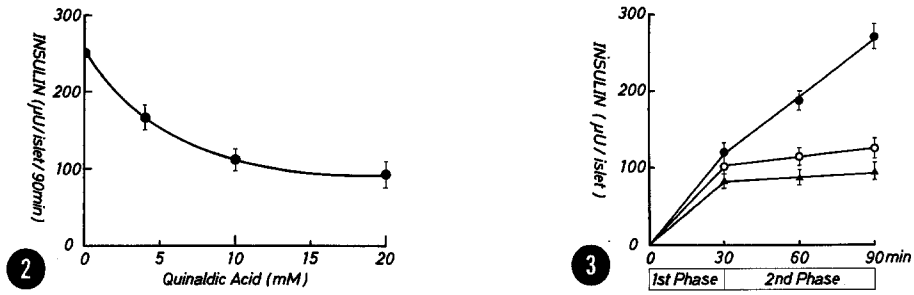

Fig. 2. Effect of different concentrations of quinaldic acid on glucose-induced insulin release.
15 mM glucose-containing incubation medium supplemented with quinaldic acid at the concentration indicated. Results represent means ± S.E. in μU of insulin released in 4 incubation flasks.

Fig. 3. Time course study of insulin release.
Incubation medium supplemented with 15 mM glucose (●) or 12 mM quinaldic acid (▲) or both (○). The points at the different time intervals for each curve are the mean ± S.E. in μU of insulin released in 4 incubation flasks.

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min). In the presence of 12 mM quinaldic acid, a reduction of the glucose-induced insulin release to 112 μU occurred.

Fig. 2 shows the results of experiment, in which the relation between the quinaldic acid concentration and the inhibition of glucose-induced insulin release was studied; the concentration of half maximum inhibition was approx. 4 mM.

Fig. 3 shows the results of time course studies: (a) With 15 mM glucose, 119 μU of insulin was released from an islet during the first 30 min after incubation (the first phase), and during the subsequent 60 min (the second phase) the rate of insulin release was approx. linear with a total production of 272 μU. (b) 12 mM quinaldic acid produced an increase (80 μU) in insulin release during the first 30 min, but there was no significant rise in insulin release during the subsequent 60 min. (c) In the presence of 12 mM quinaldic acid, the second phase of glucose-induced insulin release was completely abolished.

We further observed that addition of puromycin in concentration (0.2 mM) sufficient to block all protein synthesis, affected neither the insulin release by quinaldic acid nor the first phase of glucose-induced insulin release, whereas the inhibitor did suppress the second phase of glucose-induced insulin release.

DISCUSSION

The results¶ described herein as well as the results described in our previous paper (9), which were obtained with isolated Langerhans islets of rats, show that quinaldic acid causes insulin release on the one hand and that it inhibits the second phase of glucose-induced insulin release on the other.

The pattern of glucose-induced insulin release has been shown to be biphasic in isolated perfused preparations of rat pancreas (12) and of its islets (13) maintained in vitro : it has been suggested that the first phase of release, which is insensitive to puromycin, may be due to the release of prestored insulin from β-granules and the second phase, which is sensitive to puromycin, may be due to the synthesis of insulin and the subsequent association of new β-granules. It has been reported that insulin is stored as zinc-complex in β-granules of Langerhans islets (14). In the study of chelate chemistry, it is also well known that quinaldic acid forms an insoluble complex with zinc (15). Therefore, it is suggested that quinaldic acid has dual functions against β-cells of Langerhans islets : 1) quinaldic acid induces insulin release by deprivation of zinc from insulin-zinc complex in preformed β-granules, and 2) quinaldic acid inhibits the for-

¶ Quite recently, similar results were also obtained in in vivo experiments in rats (Okamoto, H., Miyamoto, S., Mabuchi, H., & Takeda, R., Manuscript in preparation).

mation of new β-granules by blocking the association of insulin-zinc complex.

There have been many papers reporting the increased level of kynurenine metabolites, such as kynurenic acid and xanthurenic acid, in urine of steroid-treated patients, women using contraceptive steroids, pregnant women (2-5) and hyperthyroid rats (1). In this connection, it is very significant that in such cases hyperinsulinemia, or diabetic state, was commonly observed (16-20) —— a curious coincidence. However, any reasonable biochemical explanation for the hyperinsulinemia, or diabetic state, has not yet been presented. The present results as well as our previous results (9) indicate that quinaldic acid affects insulin release from Langerhans islets. Thus, it was supposed that excessive formation of quinaldic acid (or its relatives) due to disorder in tryptophan metabolism is involved in the pathogenesis of hyperinsulinemia or diabetic state.

Acknowledgements———The authors would like to express their appreciation to Dr. Y. Yoneyama and Dr. S. Kuno, Department of Biochemistry, Kanazawa University School of Medicine, for their help and encouragement.

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