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EFFECTS OF α-KETOCARBOXYLIC ACIDS AND 4-PENTENOIC ACID ON INSULIN SECRETION FROM THE PERFUSED RAT PANCREAS

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Abstract—Pyruvic acid, α-ketobutyric acid, α-ketovaleric acid, α-ketooctanoic acid, α-ketononanoic acid, α-ketoisovaleric acid, α-ketoglutaric acid, α-ketoadipic acid, hydroxypyruvic acid, and 4-pentenoic acid did not induce significant insulin secretion from the perfused rat pancreas. β-phenylpyruvic acid and α-keto-β-methylvaleric acid induced only minimal insulin secretion from the perfused rat pancreas which was characterized by a monophasic secretory pattern. Both α-ketocaproic acid and α-ketoisocaproic acid were potent insulin secretagogues which induced a biphasic insulin secretory pattern comparable to the secretory pattern in response to glucose. The insulin secretory potency of this group of chemically related α-ketoacids is discussed.

The effect of α-ketocarboxylic acids on insulin secretion from the perfused rat pancreas was investigated with special reference to the relation between chemical structure of α-ketocarboxylic acids and its effect on insulin secretion. The insulinotropic potency of α-ketoisocaproic acid has attracted special attention earlier because the transamination of α-ketoisocaproic acid results in the formation of the aminoacid L-leucine, which is a well known insulin secretagogue [1]. However, several other α-ketocarboxylic acids seem to be only weak insulin secretagogues [1–7]. A number of different α-ketocarboxylic acids is available. They differ in the number of C-atoms and carboxygroups as well as in chain length. This is a favourable prerequisite to define exact structural requirements for α-ketocarboxylic acids to induce insulin secretion. It is intended to characterize structural requirements for the insulin secretory potency of α-ketocarboxylic acids and to discuss these in comparison with the effects of α-hydroxycarboxylic acids, fatty acids, amino acids, sugars, and sulfonureas on insulin secretion [1–10].

MATERIALS AND METHODS

Chemicals. Pure rat insulin was kindly supplied by Novo GmbH, Mainz, Germany, bovine albumin (fraction 5) was obtained from Serva, Heidelberg, Germany, ^{125}I-labelled bovine insulin from Behringwerke AG, Frankfurt, Germany. The following α-ketoacids were obtained as sodium salts from Sigma, St. Louis, MO., U.S.A.: pyruvic acid, α-ketobutyric acid, α-ketovaleric acid, α-ketoisovaleric acid, DL-α-keto-β-methyl-*n*-valeric acid, α-ketocaproic acid, α-ketoisocaproic acid, α-ketooctanoic acid, α-ketononanoic acid, α-ketoglutaric acid, α-ketoadipic acid, β-phenylpyruvic acid, and hydroxypyruvic acid. 4-pentenoic acid was from ICN K and K Labs, Plainview, NY, U.S.A. All other chemicals of analytical grade including D-glucose were from Merck AG, Darmstadt, Germany.

Experimental design and analytical methods. The pancreas plus the adjacent part of the duodenum, the spleen and the stomach were removed from 24 hr fasted male Wistar rats (190–250 g body wt) according to the method of Grodsky *et al.* [11] with slight modifications [12].

At the designed zero time, 10 min after the beginning of the perfusion, the perfusate was switched to a medium containing 5 mM of the respective α-ketoacid or 4-pentenoic acid. The venous effluent of all perfusions was collected at various timed intervals (10 sec, 1 min or 5 min), as described in the Figures and assayed for immunoreactive insulin by the method of Zaharko and Beck [13].

Calculations. Results were tested for statistical significance with Student's '*t*' test.

RESULTS

There was insignificant basal insulin secretion without any special kinetic pattern during a 60 min perfusion from the perfused rat pancreas in response to pyruvic acid (5 mM), α-ketobutyric acid (5 mM), α-ketovaleric acid (5 mM), α-ketooctanoic acid (5 mM), α-ketononanoic acid (5 mM), α-ketoisovaleric acid (5 mM), α-ketoglutaric acid (5 mM), α-ketoadipic acid (5 mM), and hydroxypyruvic acid (5 mM) (Table 1).

Though the total amount of insulin released during 60 min from the perfused pancreas induced by β-phenylpyruvic acid (5 mM) and α-keto-β-methylvaleric acid (5 mM) was also minimal (Table 1) it was characterized by a monophasic insulin secretory pattern (Figs 1 and 2). β-phenylpyruvic acid and α-keto-β-methylvaleric acid (5 mM) induced an immediate insulin secretory response with a latency of 1–2 min (Fig. 1) followed by

Table 1. The effect of various α-ketocarboxylic acids and 4-pentenoic acid on insulin secretion from the perfused rat pancreas. N = Number of experiments. * $P < 0.01$ compared with perfusions without any substrate

Test substance	N	Insulin secretion μg/60 min Mean ± S.E.M.
No substrate	4	0.16 ± 0.03
 Pyruvic acid (5 mM)	4	0.01 ± 0.01
 α-Ketobutyric acid (5 mM)	4	0.08 ± 0.03
 α-Ketovaleric acid (5 mM)	4	0.18 ± 0.04
 α-Ketocaproic acid (5 mM)	5	3.63 ± 0.72*
 α-Ketooctanoic acid (5 mM)	4	0.24 ± 0.04
 α-Ketononanoic acid (5 mM)	4	0.34 ± 0.07
 α-Ketoisovaleric acid (5 mM)	4	0.29 ± 0.01
 α-Ketoisocaproic acid (5 mM)	5	2.82 ± 0.60*
 α-Keto-β-methylvaleric acid (5 mM)	3	0.27 ± 0.07
α-Ketoglutaric acid (5 mM)	4	0.19 ± 0.07
 α-Ketoadipic acid (5 mM)	3	0.15 ± 0.07
 Hydroxypyruvic acid (5 mM)	4	0.35 ± 0.05
 β-Phenylpyruvic acid (5 mM)	4	0.40 ± 0.11
$CH_2=CH(CH_2)_2COOH$ 4-Pentenoic acid (5 mM)	4	0.17 ± 0.01
$CH_2OH(CHOH)_4COH$ D-Glucose (16.7 mM)	4	3.86 ± 0.41*

basal insulin secretion only during the rest of the 60 min perfusion time (Fig. 2). Only α-ketocaproic acid (5 mM) and α-ketoisocaproic acid (5 mM) were potent insulin secretagogues (Table 1). The total amount of insulin released from the perfused rat pancreas during a 60 min perfusion (Table 1), as well as the biphasic insulin secretory pattern (Figs 1 and 2) were comparable with that induced by D-glucose (16.7 mM) (Table 1, Figs 1 and 2), the most important physiological insulin secretagogue. The immediate insulin secretory respone to α-ketocaproic acid (5 mM) as well as to α-ketoisocaproic acid (5 mM) appeared after a latency of 1–2 min (Fig. 1) and was followed by a sustained late phase of insulin secretion from the perfused pancreas (Fig. 2). α-ketoisocaproic acid (5 mM) was not significantly less potent than α-ketocaproic acid (5 mM) (Table 1). 4-pentenoic acid (5 mM), though hypoglycemic *in vivo* [8–10], induced only insignificant basal insulin secretion without any special kinetic pattern from the perfused pancreas (Table 1).

DISCUSSION

Out of a great number of α-ketocarboxylic acids tested only α-ketocaproic acid and α-ketoisocaproic acid were potent insulin secretagogues and induced a biphasic insulin secretory pattern (Table 1 and Figs 1 and 2). α-keto-β-methylvaleric acid and β-phenylpyruvic acid were weak insulin secretagogues and induced only a monophasic secretory pattern (Table 1 and Figs 1 and 2). Some of the ketoacids tested in this communication were also tested by Matschinsky *et al.* [6]. In contrast to their results an

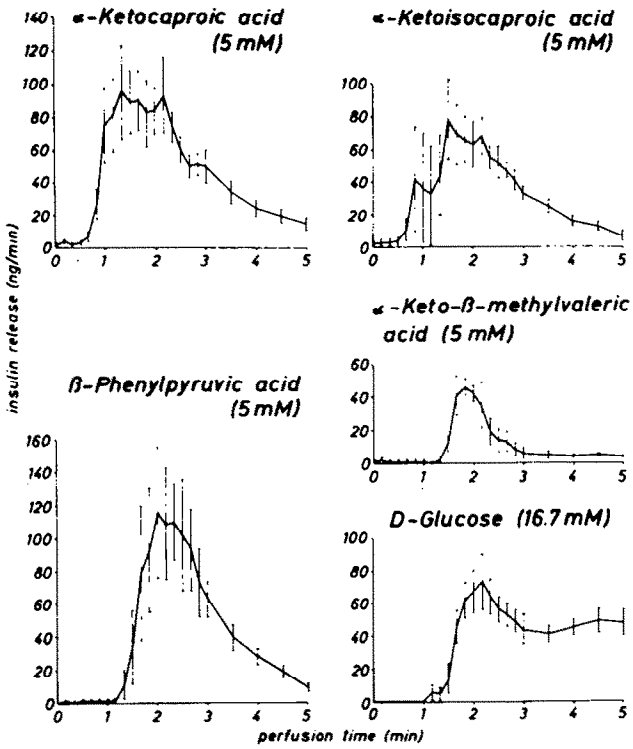

Fig. 1. The effect of α-ketocaproic acid (5 mM), α-ketoisocaproic acid (5 mM), β-phenylpyruvic acid (5 mM), α-keto-β-methylvaleric acid (5 mM), and D-glucose (16.7 mM) on the immediate insulin secretory response (1–5 min) of the perfused rat pancreas. Mean ± S.E.M. Number of experiments as shown in Table 1.

insulin secretory response of the perfused rat pancreas to α-ketooctanoic acid and α-ketononanoic acid was not observed while there was a small insulin secretory response to α-keto-β-methylvaleric acid (Fig. 1). These discrepancies may be due to overinterpretation of original data [2, 3]. This can sometimes occur when weak insulin secretagogues are tested and the perfusion medium is collected during large intervals only. Fatty acids, including caproate and isocaproate as well as α-hydroxycarboxylic acids have no stimulating effects on insulin secretion indicating that the α-ketogroup

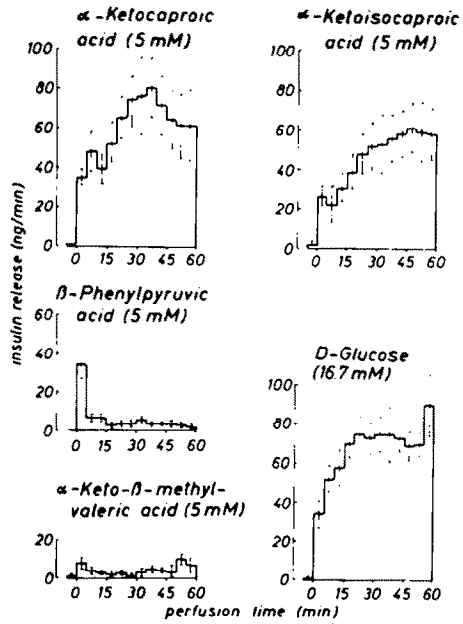

Fig. 2. The effect of α-ketocaproic acid (5 mM), α-ketoisocaproic acid (5 mM), β-phenylpyruvic acid (5 mM), α-keto-β-methylvaleric acid (5 mM), and D-glucose (16.7 mM) on insulin secretion from the perfused rat pancreas. Mean ± S. E. M. Number of experiments as shown in Table 1.

is essential for the insulin secretory potency [6]. This view is also supported by the finding that 4-pentenoic acid, a carboxylic acid, had only a very small effect on insulin secretion. 4-pentenoic acid is the simplest compound to fulfill the structural requirements (a carbon double bond separated by two carbon atoms from a carboxylic group) which have been postulated to be essential for the hypoglycemic action of hypoglycin [8–10]. Hypoglycin, 4-pentenoic acid and related carboxylic acids can induce severe hypoglycemias *in vivo* in contrast to other carboxylic acids but even these carboxylic acids are apparently only weak insulin secretagogues [8–10].

The results presented here argue against the conclusion drawn by Matschinsky *et al.* [6] that the potency of α-keto-monocarboxylic acids increases with the length of the aliphatic chain. Both α-ketooctanoic acid and α-ketononanoic acid though they have a longer aliphatic chain than α-ketocaproic acid were ineffective as well as all α-ketoacids with less than six C-atoms. On the other side the number of C-atoms does not seem to determine the insulin secretory potency. α-ketocaproic acid, α-ketoisocaproic acid, α-keto-β-methylvaleric acid, and α-ketoadipic acid all have six C-atoms but had a decreasing insulin secretory potency in this order (Table 1). The α-ketodicarboxylic acid α-ketoadipic acid was completely ineffective (Table 1).

α-ketocaproic acid, α-ketoisocaproic acid, and α-keto-β-methylvaleric acid are isomers but differed greatly in their insulin secretory potency (Table 1).

α-ketocaproic acid and α-ketoisocaproic acid both induced an immediate insulin secretory response (Fig. 1), followed by a sustained late phase of insulin release (Fig. 2) and exhibited a biphasic insulin secretory pattern which is surprisingly similar to that one induced by glucose (Figs 1 and 2). This is an interesting phenomenon in view of the fact that ketoacid and glucose metabolism are not related to each other. In contrast the isomer α-keto-β-methylvaleric acid induced only a small immediate insulin secretory response (Fig. 1), and no late phase of insulin release (Fig. 2). This secretory pattern is similar to the monophasic insulin secretory pattern which is typical for tolbutamide [7, 12]. α-ketovaleric acid induced no insulin secretory response (Table 1).

A simple explanation for the ability of β-phenylpyruvic acid to induce an immediate insulin secretory response of the pancreas (Fig. 1) is not available at present. It may be possible that pyruvic acid in connection with the phenol ring, α-ketocaproic acid, α-ketoisocaproic acid, and α-keto-β-methylvaleric acid have in common a chemical conformation or physicochemical properties which are prerequisites for the ability of these α-ketoacids to induce an insulin secretory response.

It can be concluded that the straight chain α-ketocaproic acid with its six C-atoms is as potent as α-ketoisocaproic acid (Table 1). L-Leucine, an amino acid with insulin secretory potency, is a transamination product of α-ketoisocaproic acid. α-ketocaproic acid, however, is unrelated to leucine metabolism. Therefore it is unlikely that the chemical relationship to leucine is important for the insulin secretory potency of α-ketoisocaproic acid. Moreover, it is interesting that α-ketoacids which are unrelated to glycolysis have an insulin secretory potency similar to that of glucose and induce a comparable secretory pattern. The insulin secretory potency of α-ketoisocaproic acid, α-keto-β-methylvaleric acid, and α-ketovaleric acid decreases in this order. The methylgroup is apparently important for the stimulatory capacity of these compounds and the secretory potency seems also to depend on the position of the methylgroup (Table 1). Therefore the ketoacids present a tool for further investigations into the structural requirements for insulin secretagogues, their physicochemical properties and metabolic characteristics.

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