Transamination of 3-Phenylpyruvate in Pancreatic B-cell Mitochondria*

(Received for publication, June 20, 1983)

Sigurd Lenzenz, Ingo Rustenbeck, and Uwe Panten
From the Institute for Pharmacology and Toxicology, University of Göttingen, Göttingen, Federal Republic of Germany

High aminotransferase activities catalyzing the reaction between L-glutamate and the aromatic ketomocarboxylic acid, 3-phenylpyruvate, were observed in the mitochondria from pancreatic B-cells. At very low concentrations of 3-phenylpyruvate, L-glutamine was an effective amino group donor. The aminotransferase activities for the aliphatic ketomocarboxylic acids, pyruvate and 2-ketoisovalerate, were lower in B-cell mitochondria. High rates of transamination of 2-ketoisocaproate with L-glutamine were observed and may be an important prerequisite for the insulin secretory potency of this 2-keto acid.

Since B-cell mitochondria are well supplied with 1-glutamine and L-glutamate, 3-phenylpyruvate-induced 2-ketoglutarate production may explain the insulin secretory potency of 3-phenylpyruvate which is not a fuel for pancreatic islet cells.

3-Phenylpyruvate is a potent initiator of insulin secretion (1–3) but not a fuel for pancreatic islets (3–6). Sener et al. (5) and Malaisse et al. (6) attributed the insulin secretory potency of 3-phenylpyruvate to its capacity to increase total 2-ketoisocaproate from endogenous amino acids in pancreatic islet cells. However, in earlier work, these authors reported higher total rates of transamination in pancreatic islet homogenates with the non-insulin releasing transamination partners, pyruvate and 2-ketoisovalerate, than with the insulin secretagogues, 3-phenylpyruvate and 2-ketoisocaproate (7). BCH1-induced insulin secretion from pancreatic B-cells has been proposed to result from activation of glutamate dehydrogenase, thereby enhancing production of NADH and 2-ketoglutarate from endogenous glutamate in mitochondria (8, 9). However, allosteric activation of glutamate dehydrogenase is not the mechanism of 3-phenylpyruvate-induced insulin secretion and there is also no indication for triggering of insulin release by allosteric activation of other enzymes (4). Therefore, we investigated the transamination of 3-phenylpyruvate as a possible alternative source for enhanced mitochondrial production of 2-ketoglutarate.

EXPERIMENTAL PROCEDURES

Animals and Methods—24-h fasted ob/ob mice (40–60 g, body weight) were used for the experiments. Pancreatic islets were isolated from the pancreas following collagenase digestion (10). Islets were selected under a stereomicroscope and maintained in Krebs-Ringer bicarbonate medium containing 10 mM Hepes, 3 mM glucose, and 0.1% bovine albumin. Pancreatic islets were homogenized in ice-cold Tris/sucrose buffer (10 mM Tris, 250 mM sucrose, pH 7.4) using a Potter-Elvehjem homogenizer with a Teflon pestle and maintained on ice. Sediments obtained after centrifugation for 10 min at 100,000 × g and another 10 min at 500 × g at 4 °C were discarded. The mitochondrial pellet was obtained by centrifugation of the supernatant for 10 min at 8,700 × g at 4 °C and resuspended in Tris/sucrose buffer. The cytoplasmic fraction was obtained by centrifugation of the supernatant for another 20 min at 100,000 × g. Protein was determined according to McKeown (11). Monoamine oxidase was determined using the method of Ichihara and Koyama (7). L-[4-14C]Glutamate was determined in these fractions. Oxidation of 2-keto[U-14C]glutamate by isolated pancreatic islets was performed essentially as described for other keto acids elsewhere (14).

Determination of Transamination—Transamination rates were measured as described previously (15) using modifications of the methods of Ichihara and Koyama (16), Cooper and Meister (17, 18), Ichihara et al. (19), and Cooper and Gross (19). Samples (20 μl, 5–10 μg of protein) of the tissue homogenates, the mitochondrial fractions, or the cytoplasmic fractions from pancreatic islets were incubated for 30 min at 37 °C in 40 μl of Tris/sucrose buffer containing varying concentrations of L-[1-14C]glutamate (0.5–42.0 Ci/mmol) or L-[1-14C]glutamine (0.4–4.0 Ci/mmol) and supplemented with 0.1 mM pyridoxal phosphate and 5 mM of the keto acid (2-ketoisovalerate, 2-ketoisocaproate, 3-phenylpyruvate, or pyruvate). L-[1-14C]Glutamate was dried at 56 °C with a constant stream of N2, while L-[1-14C]glutamine was dried at 37 °C only. This was necessary to avoid generation of high blank values in the presence of 3-phenylpyruvate due to nonenzymatic conversion of labeled glutamine to [1-14C]2-pyrrolidine-5-carboxylic acid (19). In control experiments, it was confirmed that transamination rates in 0.1 M phosphate buffer instead of Tris/sucrose buffer yielded similar rates of transamination. Transamination rates in pancreatic islet tissue were not dependent on pH in the range between 7.0 and 8.6 and were linear for incubation times up to 60 min. Freezing and thawing the samples three times in Tris buffer (10 mM, pH 7.4) devoid of sucrose did not affect the transamination rates in tissue homogenates, mitochondrial fractions, or cytoplasmic fractions, indicating that transport of 2-ketoisovalerate, 2-ketoisocaproate, 3-phenylpyruvate, pyruvate, glutamate, or glutamine was not ratelimiting. Blank values were obtained by incubating the medium without homogenate or fractions. At the end of the incubation period, the incubation mixture was passed immediately onto an ion exchange column (10.0 × 0.5 cm; 500 mg Dowex 50-WX8; 200–400 mesh; H+ form; Sigma; prewashed with 2 mI of ice-cold distilled water. The eluted 2-keto[U-14C]glutamate and/or 2-keto[U-14C]glutamate were determined by liquid scintillation counting after the addition of 10 ml of scintillation liquid.

Materials—3-Phenylpyruvate, 2-ketoisovalerate, 2-ketoisocaproate, L-glutamate, 2-ketoglutarate, and pyridoxal phosphate were purchased from Sigma; L-glutamine was from Calbiochem, La Jolla, CA; crude collagenase (type IV) from Worthington; fatty acid poor bovine albumin (fraction V; Penfext) from Miles Laboratories, Elkhart, IN; pyruvate from Fluka AG, Buchs, Switzerland. All other reagents of analytical grade were from Sigma or Merck AG, Darmstadt, Germany.

* This work was supported by grants from the Deutsche Forschungszemgemeinschaft, Bonn-Bad Godesberg, Germany. Some of the results of this study were obtained during medical thesis work by I. R. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

† To whom correspondence and reprint requests should be addressed, at the Institute for Pharmacology and Toxicology, Robert-Koch-Strasse 40, D-3400 Göttingen, Federal Republic of Germany.

The abbreviations used are: BCH, 2-endoaminonorbornane-2-carboxylic acid; Hepes, 4-(2-hydroxyethyl)-l-piperazineethanesulfonic acid.
Transamination of 3-Phenylpyruvate in B-cell Mitochondria

2-keto[14C]glutamate were purchased from the Radiochemical Centre, Amersham, Bucks, Great Britain.

RESULTS

The ability of various keto acids to act as a partner in the transamination of L-[14C]glutamate to 2-keto[14C]glutamate and of L-[14C]glutamate to 2-keto[14C]glutamate and/or 2-keto[14C]glutamate was examined in tissue homogenates, mitochondrial fractions, and cytoplasmic fractions of pancreatic islets from ob/ob mice. The rates of transamination are expressed as nmol of 2-keto acid/mg of protein/30 min. In Table I, the results of a direct comparison of the rates of transamination between various keto acids at a 5 mM concentration and both the amino acids, L-[14C]glutamate and L-[14C]glutamine, at a 2 mM concentration are shown. When L-[14C]glutamate was the transamination partner, the rates of transamination of 2-ketoisovalerate and 2-ketoisocaproate in pancreatic islet homogenates were very low compared to the high rates observed with 3-phenylpyruvate and pyruvate; rates obtained with pyruvate were three times higher than rates obtained with 3-phenylpyruvate (Table I). In mitochondrial and cytosolic fractions of pancreatic islets, rates of transamination of 2-ketoisovalerate and 2-ketoisocaproate were also low (Table I). The rates of transamination of 3-phenylpyruvate and pyruvate were significantly higher (Table I). However, in contrast to total homogenates of pancreatic islets, the rates of transamination of 3-phenylpyruvate were 2.5 times higher than those of pyruvate in mitochondrial fractions (Table I). On the other hand, in cytoplasmic fractions, the rates of transamination of pyruvate were 5.5 times higher than those of 3-phenylpyruvate (Table I). Only in the presence of 3-phenylpyruvate were the rates of transamination higher in the mitochondrial fractions than in the cytosolic fractions of pancreatic islets (Table I). When L-[14C]glutamine was the transamination partner, the rates of transamination of 2-ketoisocaproate, 3-phenylpyruvate, and pyruvate were similar both in homogenates and in cytoplasmic fractions of pancreatic islets from ob/ob mice, while rates of transamination of 2-ketoisovalerate were significantly lower (Table I). In mitochondrial fractions, rates of transamination of L-[14C]glutamine were significantly higher in the presence of 2-ketoisocaproate or 3-phenylpyruvate than in the presence of 2-ketoisovalerate or pyruvate (Table I). Comparing the two amino group donors, L-[14C]glutamate and L-[14C]glutamine, the results show that L-[14C]glutamate is the preferred intramitochondrial transamination partner for 3-phenylpyruvate at a concentration of 5 mM (Table I). To further elucidate the importance of transamination of 3-phenylpyruvate for the initiation of insulin secretion, concentration dependencies were studied. Both the concentrations of L-[14C]glutamate and L-[14C]glutamine and of 3-phenylpyruvate and pyruvate were varied. Over the whole range of concentrations of both L-[14C]glutamate and L-[14C]glutamine up to 20 mM, rates of transamination in homogenates, mitochondrial fractions, and cytosolic fractions from pancreatic islets increased steadily in the presence of pyruvate (5 mM) (Fig. 1). When 3-phenylpyruvate (5 mM) was the transamination partner, only the rates of transamination of L-[14C]glutamine increased steadily up to 20 mM (Fig. 1). In the presence of L-[14C]glutamate, rates reached maxima at concentrations of 2-5 mM in homogenates and mitochondrial fractions and cytosolic fractions; at higher concentrations, rates of transamination between L-[14C]glutamate and L-[14C]glutamine decreased again (Fig. 1). Thus, in contrast to transamination with pyruvate, rates of transamination of 3-phenylpyruvate in pancreatic islet homogenates and mitochondrial fractions were higher in the presence of L-[14C]glutamine than in the presence of L-[14C]glutamate when amino acid concentrations were 5 mM or higher (Fig. 1).

Rates of transamination of L-[14C]glutamate (1 mM) steadily increased with increasing concentrations of pyruvate or of 3-phenylpyruvate in homogenates, mitochondrial fractions, and cytoplasmic fractions from pancreatic islets from ob/ob mice, but with one noticeable exception (Fig. 2). At concentrations of 3-phenylpyruvate above 10 mM, rates of transamination of L-[14C]glutamate (1 mM) reached a plateau in the mitochondrial fraction (Fig. 2). Maximal rates of transamination of L-[14C]glutamine were lower than those of L-[14C]glutamate (Fig. 2). Maximal rates of L-[14C]glutamine transamination were obtained with micromolar concentrations of both pyruvate and 3-phenylpyruvate in homogenates as well as in mitochondrial and cytoplasmic fractions from pancreatic islets from ob/ob mice (Fig. 2).

### Table I

| Keto acid (5 mM) | Tissue homogenates | Mitochondrial fractions | Cytoplasmic fractions |
|----------------|--------------------|-------------------------|-----------------------|
|                | L-[14C]Glutamate (2 mM) | L-[14C]Glutamine (2 mM) | L-[14C]Glutamate (2 mM) | L-[14C]Glutamine (2 mM) |
| 2-Ketoisovalerate | 15.2 ± 4.5 | 2.9 ± 1.4 | 6.4 ± 2.8 | 24.3 ± 8.9 | 3.3 ± 1.1 |
| 2-Ketoisocaproate | 15.7 ± 4.6 | 11.2 ± 2.3 | 26.4 ± 1.9 | 216.0 ± 19.4 | 12.8 ± 2.9 |
| 3-Phenylpyruvate | 51.4 ± 4.8 | 9.7 ± 2.9 | 24.2 ± 6.7 | 216.0 ± 19.4 | 12.8 ± 2.9 |
| Pyruvate         | 147.8 ± 12.4 | 13.0 ± 1.8 | 10.0 ± 3.1 | 216.0 ± 19.4 | 12.8 ± 2.9 |

Transamination rates (nmol 2-ketoglutarate and/or 2-ketoisocaproate/mg protein × 30 min)
Transamination of 3-Phenylpyruvate in B-cell Mitochondria

Pyruvate

I

600

3-Phenylpyruvate

2045

FIG. 1. Concentration-dependent transamination of L-[1-14C]glutamate (0.1; 0.2; 0.5; 1; 2; 5; 10 mM) (○—○) and L-[U-14C]glutamine (0.2; 0.5; 1; 5; 20 mM) (□—□) in the presence of 3-phenylpyruvate (5 mM) (left) or pyruvate (5 mM) (right) in homogenates (upper graphs), mitochondrial fractions (middle graphs), and cytoplasmic fractions (lower graphs) of pancreatic islets from ob/ob mice. Values shown are the means ± S.E. for 4–6 experiments.

was especially striking in the mitochondrial fractions, resulting in considerably lower transamination rates of L-[U-14C]glutamate than of L-[U-14C]glutamate with millimolar concentrations of keto acids (Fig. 2).

In a control experiment, the rate of oxidation of 2-keto[U-14C]glutarate (5 mM) by isolated incubated ob/ob mouse pancreatic islets was found to be as low as 0.39 ± 0.27 mmol/kg dry weight (n = 8).

DISCUSSION

The results provide support for the existence of a high aminotransferase activity catalyzing the reaction between L-glutamate and the aromatic ketomonocarboxylic acid, 3-phenylpyruvate, in the mitochondria from pancreatic islets (Table I). The aminotransferase activity for the ketomonocarboxylic acids, pyruvate, 2-ketoisovalerate, and 2-ketoisocaproate was significantly lower in islet cell mitochondria in the presence of L-glutamate (Table I). Therefore, transamination with glutamate cannot be the sole cause of the strong insulin-releasing capacity of 2-ketoglutarate (1, 2, 14, 15, 20). Our mitochondrial fraction also contained insulin secretory granules. But it is very unlikely that these organelles are sites of transamination. We are not aware of reports describing secretory granules as sites of transamination. Since we used ob/ob mouse pancreatic islets in the present study, which contain more than 90% B-cells (21), the results are representative for B-cell mitochondria. In total homogenates of pancreatic islets, we observed, in accordance with results from Malaisse et al. (7), higher rates of transamination of pyruvate which apparently originate from cytosolic transamination (Table I). The present observation of a high rate of transamination between L-glutamate and 3-phenylpyruvate in mitochondria from pancreatic islets is in accordance with the description of an intramitochondrial phenylalanine aminotransferase with high capacity in pig brain and heart (22). An aminotransferase catalyzing the reaction between L-glutamate and 3-phenylpyruvate has been extracted from mitochondria (23) and there is evidence for the existence of a specific aromatic amino acid aminotransferase (23).

There are apparently also other aminotransferases in the mitochondria which can form 2-ketoglutarate in the presence of 3-phenylpyruvate (24, 25). Glutamine is transaminated (19, 24, 25) yielding 2-ketoglutaramate (26) followed by an ω-deamidation reaction in which 2-ketoglutarate is converted to 2-ketoglutarate (25). In our investigation, we found transamination rates of glutamine in the presence of millimolar concentrations of 3-phenylpyruvate (the optimal range for initiation of insulin secretion by this insulin secretagogue (3)), which were significantly lower in homogenates, mitochondrial fractions, and cytoplasmic fractions from pancreatic islets from ob/ob mice when compared to glutamate transamination (Table I). Maximal rates of pancreatic islet mitochondrial glutamine transaminase in the micromolar concentration range of 3-phenylpyruvate (Fig. 2) are in accordance with very low apparent K_m values for 3-phenylpyruvate as reported for rat kidney, brain, and liver mitochondrial glutamine transaminase (19, 24). Our results support the recent conclusion by Cooper and Meister (24) that apparent K_m values for 3-phenylpyruvate exhibited by the glutamine
transaminase K are several orders of magnitude lower than those reported for other 3-phenylpyruvate-utilizing transaminases. Thus, at 5 mM 3-phenylpyruvate, which is a concentration in the optimal range for initiation of insulin secretion, mitochondrial transamination of L-glutamate seems to be more important for initiation of insulin secretion by 3-phenylpyruvate than transamination of L-glutamate. On the other hand, in vivo where 3-phenylpyruvate plasma concentrations are normally around 5 μM and up to 100–200 μM in phenylketonuria (27), a possible potentiating effect of 3-phenylpyruvate on glucose-induced insulin secretion should be mainly dependent on L-glutamine transamination. With 2-ketoisocaprate, the rate of transamination in the mitochondria from pancreatic B-cells was higher with L-glutamine than with L-glutamate (Table I). Glutamine aminotransferase activity was more important for initiation of insulin secretion by 3-phenylpyruvate than transamination of L-glutamine. On the other hand, in vivo where 3-phenylpyruvate plasma concentrations are normally around 5 μM and up to 100–200 μM in phenylketonuria (27), a possible potentiating effect of 3-phenylpyruvate on glucose-induced insulin secretion should be mainly dependent on L-glutamine transamination. With 2-ketoisocaprate, the rate of transamination in the mitochondria from pancreatic B-cells was higher with L-glutamine than with L-glutamate (Table I). Glutamine aminotransferase activity was very low with 2-ketoisovalerate as substrate in pancreatic islet homogenates and subcellular fractions (Table I) which is in agreement with findings in liver tissue reported by Meister (26). Therefore, the high rates of L-glutamine transamination in pancreatic B-cell mitochondria in the presence of 2-ketoisocaprate as compared to virtually negligible rates in the presence of 2-ketoisovalerate (Table I) may help to explain the ability of 2-ketoisocaprate in contrast to 2-ketoisovalerate to induce insulin secretion from pancreatic islets (1, 2, 14, 20).

Since B-cell mitochondria are well supplied with L-glutamine and L-glutamate (7), 3-phenylpyruvate-induced 2-ketoglutarate production may explain the insulin secretory potency of this aromatic ketomonocarboxylic acid. Thus, the two fuel analogues, 3-phenylpyruvate and BCH, may induce insulin secretion by reactions started by enhanced intramitochondrial availability of 2-ketoglutarate. One such reaction may be active metabolism of 2-ketoglutarate through the segment of the Krebs’ cycle leading to the formation of malate and thus providing more reducing equivalents as recently documented in HeLa cells (28) and in several other mammalian cells such as those from the small intestine (29). Exogenous 2-ketoglutarate administered to intact pancreatic B-cells apparently cannot replace 2-ketoglutarate produced intramitochondrially. This keto acid is excluded apparently not only from liver cells (30) but also from pancreatic islet cells as shown by the extremely low rates of oxidation of radioactively labeled 2-ketoglutarate (see “results”).

REFERENCES

1. Matschinsky, F. M., Fertel, R., Koder-Brajtburg, J., Stillings, S., Ellerman, J., Raybaud, F., and Thurston, H. H. (1973) Proceedings of the Midwest Conference on Endocrinology and Metabolism 8th Conference, (Breitenbach, R. P., and Mussachia, X. J., eds) pp. 63–87, University of Missouri, Columbia, MO
2. Lenzen, S. (1978) Biochem. Pharmacol. 27, 1321–1324
3. Lenzen, S., and Panten, U. (1981) Biochem. Med. 25, 366–372
4. Panten, U., and Langer, J. (1981) Biochem. J. 198, 353–356
5. Sener, A., Welsh, M., Lehrn, P., Garcia-Morales, P., Saceda, M., Malaisse-Lagae, F., Herchuelz, A., Valverde, J., Hellerstrom, C., and Malaisse, W. J. (1980) Biochem. J. 210, 913–919
6. Malaisse, W. J., Sener, A., Welsh, M., Malaisse-Lagae, F., Hellerstrom, C., and Christophe, J. (1985) Biochem. J. 210, 921-927
7. Malaisse, W. J., Sener, A., Malaisse-Lagae, F., Hutton, J. C., and Christophe, J. (1981) Biochim. Biophys. Acta 677, 39–49
8. Panten, U., Holze, S., and Lenzen, S. (1980) Horm. Metab. Res. 10, (suppl.) 27–30
9. Malaisse-Lagae, F., Sener, A., Garcia-Morales, P., Valverde, I., and Malaisse, W. J. (1982) J. Biol. Chem. 257, 3754–3758
10. Lernmark, A. (1974) Diabetologia 10, 431–438
11. McKnight, G. S. (1977) Anal. Biochem. 78, 86–92
12. Lenzen, S., Nahrstedt, H., and Panten, U. (1982) Naunyn-Schmiedeberg's Arch. Pharmacol. 324, 190–195
13. Wurtman, R. J., and Axelrod, J. (1983) Biochem. Pharmacol. 12, 1439–1441
14. Lenzen, S., and Panten, U. (1980) Biochem. J. 186, 135–144
15. Lenzen, S., Formanek, H., and Panten, U. (1982) J. Biol. Chem. 257, 6631–6633
16. Ichihara, A., and Koyama, E. (1966) J. Biochem. 59, 169–169
17. Cooper, A. J. L., and Meister, A. (1970) Methods Enzymol. 57, 1016–1023
18. Cooper, A. J. L., and Meister, A. (1972) Biochemistry 11, 661–671
19. Cooper, A. J. L., and Gross, M. (1977) J. Neurochem. 28, 771–778
20. Panten, U., von Kiegstein, E., Poser, W., Schönborn, J., and Hasselblatt, A. (1972) FEBS Lett. 20, 225–228
21. Hellman, B. (1985) Ann. N. Y. Acad. Sci. 131, 541–558
22. Shrawder, E., and Martines-Carrion, M. (1972) J. Biol. Chem. 247, 2486–2492
23. Scandurra, R., Cannella, C., and Ferretti, M. G. (1967) Eur. J. Biochem. 3, 219–223
24. Cooper, A. J. L., and Meister, A. (1981) Comp. Biochem. Physiol. 69B, 137–145
25. Cooper, A. J. L., and Meister, A. (1977) CRC Crit. Rev. Biochem. 4, 281–303
26. Meister, A. (1980) J. Biol. Chem. 187, 173–187
27. Langenbeck, U., Behbehani, A., and Kuthe, H. (1981) J. Inher. Metab. Dis. 4, 69–70
28. Reitzer, L. J., Wice, B. M., and Kenneil, D. (1979) J. Biol. Chem. 254, 2669–2676
29. Windmueller, H. G., and Spaeth, A. E. (1974) J. Biol. Chem. 249, 5070–5079
30. Ross, B. D., Hens, R., and Krebs, H. A. (1967) Biochem. J. 102, 942–951
Transamination of 3-phenylpyruvate in pancreatic B-cell mitochondria.
S Lenzen, I Rustenbeck and U Panten

*J. Biol. Chem.* 1984, 259:2043-2046.

Access the most updated version of this article at [http://www.jbc.org/content/259/4/2043](http://www.jbc.org/content/259/4/2043)

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at [http://www.jbc.org/content/259/4/2043.full.html#ref-list-1](http://www.jbc.org/content/259/4/2043.full.html#ref-list-1)