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COMPARISON OF THE EFFECTS OF LEUCINES, NON-METABOLIZABLE LEUCINE ANALOGUES AND OTHER INSULIN SECRETAGOGUES ON THE ACTIVITY OF GLUTAMATE DEHYDROGENASE

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Both L-leucine and its non-metabolizable analogue b(−)-2-aminobicyclo-(2,2,1)heptane-2-carboxylic acid [b(−)-BCH] stimulate insulin release from the pancreatic B-cells whereas their stereoisomers D-leucine and b(+)-BCH do not [1, 12]. So far no molecular mechanism has been found in the pancreatic B-cells which exhibits the same stereospecificity for these amino acids as does insulin release. CHRISTENSEN et al. [1] suggested the L-system for amino acid transport as trigger site for insulin release since it is shared by L-leucine and BCH. This hypothesis was weakened by the finding that D-leucine also appeared to be transported into the B-cells by the L-system [10]. Pyridine nucleotides have been suggested to be of great importance in stimulating insulin release [9] and both L-leucine and b(−)-BCH increase the fluorescence from reduced pyridine nucleotides in the pancreatic B-cells [14, 17]. Enzymes affecting the state of reduction of pyridine nucleotides, *e.g.* glutamate dehydrogenase (GLDH), have been considered as possible sites of action for regulators of insulin release [6, 7]. Since YIELDING and TOMKINS [22] demonstrated stereospecific L-leucine stimulation of bovine liver GLDH, it is of great interest to compare the effects of leucine and BCH isomers on the GLDH activity of B-cells. Isolation of any substantial amount of pure B-cell GLDH is almost impossible in view of the limited mass of tissue. I therefore used bovine liver GLDH as a model of the B-cell enzyme to study how its activity is affected by leucine and BCH isomers and other insulin secretagogues at concentrations normally used for *in vitro* studies of insulin release.

MATERIAL AND METHODS

Bovine liver GLDH, NADH and α-ketoglutarate were purchased from Boehringer Mannheim GmbH, Mannheim, Germany. Sigma Chemical Co., St. Louis/Mo., U.S.A., supplied L-leucine, D-leucine, L-arginine, α-ketoisocaproic acid, diethylstilbestrol and bovine serum albumin (fraction V). The resolved isomers of 2-aminobicyclo(2,2,1)heptane-2-carboxylic acid (BCH) were gifts from Professor H. N. Christensen, Department of

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Biochemistry, the University of Michigan, Ann Arbor/Mich., U.S.A. 1-Butyl-3-sulfanilylcarbamide (carbutamide), 1-(p-toluenesulfonyl)-3-butylurea (tolbutamide) and 1-([p-/2-(5-chloro-2-methoxybenzamido)-ethyl/-phenyl]sulfonyl)-3-cyclohexylurea (glibenclamide) were donated by Farbwerke Hoechst AG, Frankfurt/Main, Germany, and N-(4-[β-/5-methylpyrazine-2-carboxamido/-ethyl]-benzene-sulfonyl)-N'-cyclohexylurea (glipizide) was given by Pfizer Inc., New York/N.Y., U.S.A. All other chemicals were commercially available and of analytical grade. Distilled and deionized water was used throughout.

Bovine liver GLDH was dissolved in a solution of 1 mg/ml bovine serum albumin and 0.9% NaCl. In most cases 20 μM diethylstilbestrol and the substance to be tested were added to this solution. GLDH activity was measured essentially as described by SCHMIDT [18]. The assay was carried out at 37 °C (instead of at 25 °C) using a Beckman DB spectrophotometer connected to a logarithmic recorder. The rate of reductive amination of α-ketoglutarate was measured by recording the initial rates of decrease in absorbance of the reduced pyridine nucleotides at 340 nm.

Results are expressed as changes in extinction per min (ΔE_{340}/min). The statistical significance was estimated from the difference between paired test and control data using the two-tailed *t*-test.

RESULTS

In the absence of enzyme inhibitors or activators the concentration of GLDH used had an activity of 0.398 ± 0.010 ΔE_{340}/min (mean of 20 experiments ± SEM). Addition of 20 μM diethylstilbestrol depressed this activity to 0.079 ± 0.002 ΔE_{340}/min ($p < 0.001$). The most effective stimulators of diethylstilbestrol-inhibited GLDH activity were b(−)-BCH and L-leucine (tab. 1). Both of these insulin-releasing amino acids completely reversed the diethylstilbestrol inhibition and caused stimulation beyond the activity observed with the pure enzyme alone ($p < 0.001$). The non-secreting stereoisomers, b(+)-BCH and D-leucine, were less effective and only partially counteracted the diethylstilbestrol inhibition. L-arginine and the leucine metabolite, α-ketoisocaproic acid, lacked significant effects on GLDH activity. Small and diverging effects were obtained with the sulfonylurea compounds: carbutamide slightly stimulated GLDH activity, tolbutamide or glipizide had no effect, and glibenclamide actually further inhibited the diethylstilbestrol-inhibited enzyme. Glucose, which is the most important physiological stimulus of insulin secretion, did not influence GLDH activity.

DISCUSSION

The action of different modifiers of GLDH activity is largely dependent on substrate concentrations [11, 21]. A pure system with strict control of all parameters should therefore be preferred for the enzymatic assays. However, isolation of any substantial amount of pure B-cell GLDH is almost impossible in view of the limited mass of tissue. Since it has been shown previously that both purified bovine liver GLDH [2, 21, 22] and GLDH activity in homogenates of B-cell-rich pancreatic mouse islets [6, 7] are stimulated by L-leucine, ADP and carbutamide, it seemed reasonable to assume that GLDH from these sources interacts in a similar manner with regulating molecules. For these reasons the commercially available bovine liver enzyme was employed as a model of the B-cell enzyme. To facilitate detection of stimulation of GLDH activity, diethylstilbestrol was used to inhibit the enzyme [22].

test substance		enzyme activity (ΔE_{340}/min)	difference from control
control	(20)	0.079 ± 0.002	—
20 mM L-leucine	(13)	0.628 ± 0.026	0.550 ± 0.027 **
20 mM D-leucine	(13)	0.107 ± 0.004	0.029 ± 0.004 **
20 mM b(−)-BCH	(6)	0.632 ± 0.038	0.556 ± 0.041 **
20 mM b(+)-BCH	(6)	0.292 ± 0.011	0.217 ± 0.010 **
20 mM L-arginine	(7)	0.089 ± 0.003	0.010 ± 0.004
20 mM α-KIC	(7)	0.073 ± 0.005	− 0.006 ± 0.006
20 mM D-glucose	(6)	0.083 ± 0.008	0.007 ± 0.006
0.1 mM carbutamide	(6)	0.084 ± 0.003	0.004 ± 0.001 *
0.1 mM glibenclamide	(7)	0.034 ± 0.003	− 0.047 ± 0.003 **
0.1 mM glipizide	(7)	0.078 ± 0.005	− 0.002 ± 0.006
0.1 mM tolbutamide	(7)	0.076 ± 0.004	− 0.004 ± 0.004

* $p < 0.02$; ** $p < 0.001$

Table 1 - The effects of insulin secretagogues and related substances on diethylstilbestrol-inhibited glutamate dehydrogenase activity. The results are expressed as change of extinction at 340 nm/min. The number of experiments included in the analyses is stated within parentheses. Results are given as mean values ± SEM. (BCH = 2-amino-bicyclo(2,2,1)heptane-2-carboxylic acid; α-KIC = α-ketoisocaproic acid).

The best stimulators of GLDH activity were L-leucine and b(−)-BCH, which initiate insulin secretion. Their stereoisomers, D-leucine and b(+)-BCH, which do not initiate insulin release, were much less effective as stimulators of the enzyme. Since the pancreatic B-cells can decarboxylate glutamic acid to GABA [4], activation of GLDH could result in increased CO_2 production from substrates entering the citric acid cycle. It is therefore notable that b(−)-BCH [3], L-leucine and D-leucine [5] stimulate glucose oxidation in the B-cell. However, GLDH stimulation may not explain the increased glucose oxidation, since b(+)-BCH, despite being a better activator of GLDH than D-leucine, had no effect on glucose oxidation.

Both L-leucine and b(−)-BCH have been reported to increase the fluorescence from reduced pyridine nucleotides in mouse pancreas islets [14, 17]. Metabolic degradation of L-leucine is probably not the cause of reduction of pyridine nucleotides since an almost identical fluorescence pattern was observed with b(−)-BCH [16]. Stimulation of GLDH activity, on the other hand, may account for an increased state of pyridine nucleotide reduction. The insulin-releasing leucine metabolite, α-ketoisocaproic acid, also increased the fluorescence from reduced pyridine nucleotides in mouse pancreatic islets [17] but did not stimulate GLDH activity. The latter observation does not necessarily imply that exposure of B-cells to α-ketoisocaproic acid does not result in increased GLDH activity since this compound could first be converted to L-leucine [17]. Glucose, the most important physiological stimulator of insulin release, had no effect on GLDH activity. The prompt increase of fluorescence from reduced pyridine nucleotides seen after exposing B-cells to glucose might be explained mainly as an increased supply of substrate for the phosphoglyceraldehyde-dehydrogenase reaction [15].

Neither sulfonylurea compounds nor arginine affect the fluorescence from reduced pyridine nucleotides in mouse pancreatic islets [13, 14]. Arginine had no effect on GLDH activity. Although two of the sulfonylureas tested had some small effects on GLDH activity, one of these effects was in fact an

inhibition. Therefore, it seems unlikely that arginine or sulfonylureas regulate insulin release by affecting GLDH activity, particularly since sulfonylureas may not even enter the B-cells [8, 19, 20].

The specificity of the insulin-releasing amino acids L-leucine and b(−)-BCH in stimulating GLDH activity makes it tempting to speculate about a connection between allosteric regulation of pyridine nucleotide-dependent enzymes and insulin release. However, since different modifiers of GLDH activity depend on substrate concentrations [11, 21], and since the bovine liver enzyme was studied, the assay system may not be representative of the pancreatic B-cell.

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SUMMARY

Glutamate dehydrogenase (GLDH) from bovine liver was employed in a model system for testing a possible role of GLDH in insulin release. The ability of different insulin secretagogues to stimulate the activity of the diethylstilbestrol-inhibited enzyme was tested. The two insulin-releasing amino acids, L-leucine and its non-metabolizable analogue 2-aminobicyclo(2,2,1)heptane-2-carboxylic acid [b(−)-BCH], were the best stimulators of GLDH activity. The non-secreting stereoisomers, D-leucine and b(+)-BCH, were less effective. Glucose, L-arginine and the leucine metabolite α-ketoisocaproic acid lacked significant effects on GLDH activity. Small and diverging effects were obtained with sulfonylurea compounds: whereas carbutamide caused slight stimulation, tolbutamide and glipizide had no effect, and glibenclamide was an inhibitor. The specificity of the insulin-releasing amino acids L-leucine and b(−)-BCH in stimulating GLDH activity makes it tempting to speculate about a connection between allosteric regulation of pyridine nucleotide-dependent enzymes and insulin release.

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