Effect of Leucine on the Pyridine Nucleotide Contents of Islets and on the Insulin Released—Interactions In Vitro with Methylene Blue, Thiol Oxidants, and p-Chloromercuribenzoate

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SUMMARY

In the presence of glucose (2 mg/ml), leucine (10 mM) noticeably increased islets' NADPH contents as well as the NADPH:NADP ratio; the changes occurred as soon as 1 min after its addition. NADH concentrations were also increased by leucine. The NADPH:NADP ratio as well as insulin release stimulated by glucose plus leucine were markedly decreased by methylene blue. The thiol oxidants diamide and tert-butyl hydroperoxide also inhibited insulin secretion in response to glucose plus leucine. Employing the perfused pancreas technique, the insulin-releasing action of p-chloromercuribenzoate was further enhanced by leucine. The combined effects were inhibited by tert-butyl hydroperoxide, however.

Our data suggest that the insulin-releasing action of leucine depends on the islets' NADPH and reduced glutathione (GSH); in addition, leucine may contribute to insulin secretion by increasing the islet NADPH:NADP ratio and the NADH:NAD ratio. From the data, we assume that the observed increase of NADPH may lead via GSH to an increase in the number of such thiol groups in the β-cell membrane, which are believed to be related to stimulation of insulin release and, thus, to increase the sensitivity of the β-cell to stimulation by glucose and/or leucine. DIABETES *28*:593–599, June 1979.

Although the insulin-releasing effect of the amino acid leucine is well established,[1] its mechanism of action on insulin secretion is still poorly understood. Panten et al.[2] observed that stimulation of insulin release triggered by leucine in the presence of glucose (3.3 mM) was accompanied by an increase of the fluorescence of reduced pyridine nucleotides.

We recently hypothesized that the insular NADPH:NADP ratio is important in the mechanism of insulin secretion, perhaps by keeping glutathione in the reduced state.[3] It is therefore conceivable that, at least in part, the action of leucine in insulin secretion could be to provide NADPH, which, in turn, might be responsible for keeping the glutathione molecule in the reduced form.

To obtain evidence for such a possibility, we examined the effect of leucine on the pyridine nucleotides in the pancreatic islets. Also, the insulin-releasing effect of leucine was studied using isolated islets and perfused pancreas in the absence and presence of some metabolic inhibitors that lower the islet NADPH and/or reduced glutathione (GSH). Moreover, the interaction between leucine and a thiol reagent, p-chloromercuribenzoate, was studied to observe a possible synergism between these substances in releasing insulin. The presumption was based on the suggestion that the insulin-triggering effect of the latter depends on the islets' NADPH and GSH contents.[3]

MATERIALS AND METHODS

Chemicals. Tris(hydroxymethyl)aminomethane, NADP, NADPH, NAD, NADH, G-6-P-dehydrogenase, glutamate dehydrogenase, α-ketoglutaric acid, and adenosine diphosphate were purchased from Boehringer, Mannheim. p-Chloromercuribenzoate and tert-butyl hydroperoxide were obtained from Merck-Schuchardt, Hohenbrunn. Methylene blue was purchased from Fluka. Dextran (mol wt 60,000–90,000), L-leucine, and α-D-glucose were obtained from Serva Feinbiochemica, Heidelberg. Diamide [diazene dicarboxylic acid bis(N,N-dimethylamide)] was prepared and donated by HAG, Bremen, through the courtesy of Dr. O. Vitzthum. Bovine serum albumin was purchased from Behringwerke, Marburg, and collagenase (196 U/mg) from Worthington Biochemicals, Harrison, NJ. Rat insulin was obtained from Novo Laboratories, Copenhagen, Denmark. The insulin radioimmunoassay kit (INSIK-I), a product of CIS-SORIN, Italy, was supplied by Isotopen Dienst

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West, Sprendlingen. All other chemicals and reagents of analytic grade were obtained from E. Merck, Darmstadt.

Animals. Wistar rats of either sex, taken from a local strain reared in our laboratories and weighing between 300 and 350 g, were used for these studies. They were maintained on a standard pellet diet (Altromin) and on tap water ad libitum.

EXPERIMENTAL PROCEDURES

ISOLATED PANCREATIC ISLET STUDIES

Pancreatic islets were isolated by the collagenase incubation procedure.[4] The harvested islets were preincubated for half an hour in 1 ml of Krebs-Ringer-Bicarbonate (KRB) buffer containing 0.3 mg/ml glucose and 20 mg/ml albumin while being gassed with Carbogen (95% O_2 and 5% CO_2). After preincubation, islets were kept in ice for 3 min and washed three times with ice-cold Hanks' solution. For studies on the effect of leucine on insular NADPH, NADP, NADH, and NAD contents, the test incubations were performed with batches of 10 islets in 0.7 ml KRB buffer (pH 7.4) at 37 °C while being gassed with Carbogen supplemented with albumin (20 mg/ml), leucine (10 mM), and glucose (2 mg/ml) for 1, 5, or 60 min, as indicated in detail in the tables. The effect of methylene blue (2 μg/ml) on the above parameters was studied in the absence and presence of leucine. In the experiments performed for 60 min, the insulin released into the medium was also measured.

After incubation, the amount of insulin released into the medium was determined as described later. Immediately after the medium was removed, NaOH (40 mM, 0 °C) was added to disintegrate the islets, and assays of total NADPH, NADP, NADH, and NAD contents were performed by means of enzymatic cycling as described by Lowry et al.[5] The cycling reaction was carried out for 60 min at 38 °C. Since these pyridine nucleotides were determined from standard curves (0.25–3 pmol), which were prepared from authentic compounds taken through the same procedures, no corrections for recovery were needed. The values for pyridine nucleotides in pancreatic islets given in this paper are similar to those reported by others.[6,7]

In separate experiments, the effects of diamide and tert-butyl hydroperoxide on leucine-induced insulin release were studied. Batches of five islets were incubated in 1 ml of the medium described before, containing leucine (10 mM) and/or glucose (2 mg/ml) in Krebs-Ringer-Bicarbonate buffer (pH 7.4) with and without diamide (1 mM) or tert-butyl hydroperoxide (2 mM). Islets were incubated at 37 °C and were continuously gassed with Carbogen. At the end of a 90-min incubation period, samples were collected for the determination of insulin.

PERFUSION STUDIES

The rat pancreas was isolated by the technique described by Curry et al.[10] The isolation and perfusion of pancreas is described in detail elsewhere.[3] The perfusion medium contained Krebs-Ringer Bicarbonate with 4% dextran and 0.25% bovine serum albumin. The pH of final solution was always adjusted to 7.35. All pancreases were first allowed to equilibrate for 15 min; the next 5 min was the basal period, designated in Figure 1 by −5 to 0 min. During this period, effluent samples were collected every minute for determination of insulin. At zero time, test substances, dissolved in the basic medium, were allowed to flow into isolated pancreas by changing the medium reservoir, and the effluent samples were collected every 30 s for the next 7 min. During the next 23 min, samples were again taken every minute. Thus, the period from −5 to 0 min represents the prestimulation period and that from 0 to 30 min the stimulation period.

First, the insulin-stimulatory effects of leucine (10 mM) and p-chloromercuribenzoate (0.1 mM) in the presence of glucose (1 mg/ml) were studied separately by perfusing these substances during the stimulatory phase (Figure 1A and B). Then, to study the interaction of p-chloromercuribenzoate with leucine (10 mM) in the presence of glucose (1 mg/ml), the respective combinations were perfused during the stimulatory phase (Figure 1C). Moreover the effect of tert-butyl hydroperoxide (2 mM) on the insulin secretion induced by p-chloromercuribenzoate (0.1 mM) + leucine (10 mM) in the presence of glucose (1 mg/ml) was studied by adding tert-butyl hydroperoxide both during and before perfusion of the respective drug combination (Figure 1D). The duration of perfusion of these substances is shown with arrows in the figures.

ASSAY OF INSULIN

Insulin concentration in effluent from perfused pancreas and that released into the medium by the isolated islets was measured in duplicate by a two-antibody radioimmunoassay[11] using the insulin reagent kit already described. Samples (10 μl) from incubation or perfusion experiments were added to 90 μl of phosphate-buffer with 0.1% of albumin (equal to buffer of test kit). Rat insulin was used as standard. The range of the standard curve was 1.25 to 20 μU IRI. Results of perfused pancreas were expressed as amounts of immunoreactive insulin (IRI) per milliliter. Insulin released by the isolated islets was expressed as microunits per milliliter per 60- or 90-min incubation period. The values are given as mean ± SEM, and the Student's t test was used for statistical analysis.

RESULTS

ISLETS' NADPH, NADP, AND INSULIN RELEASE

Table 1 shows the islet concentration of NADPH, NADP, and the NADPH:NADP ratio at 0, 1, and 5 min after addition of either glucose (2 mg/ml) alone or in combination with leucine (10 mM). Even 1 min but also 5 min after the addition of glucose or glucose plus leucine there was a noticeable increase in NADPH, a concomitant decrease in NADP, and an increase of the NADPH:NADP ratio in comparison with zero time (removal of ice-cold medium immediately after its addition). The changes in response to glucose plus leucine were more pronounced than with glucose alone.

Since only small quantities of insulin were secreted at between 1 and 5 min by as few as 10 islets, measurement of the insulin released into the medium is not possible in an incubation system for analytic reasons. Therefore, in further studies used for direct comparison, the concentration of pyridine nucleotides and the accumulated secretion of insulin were measured after 60 min of incuba-

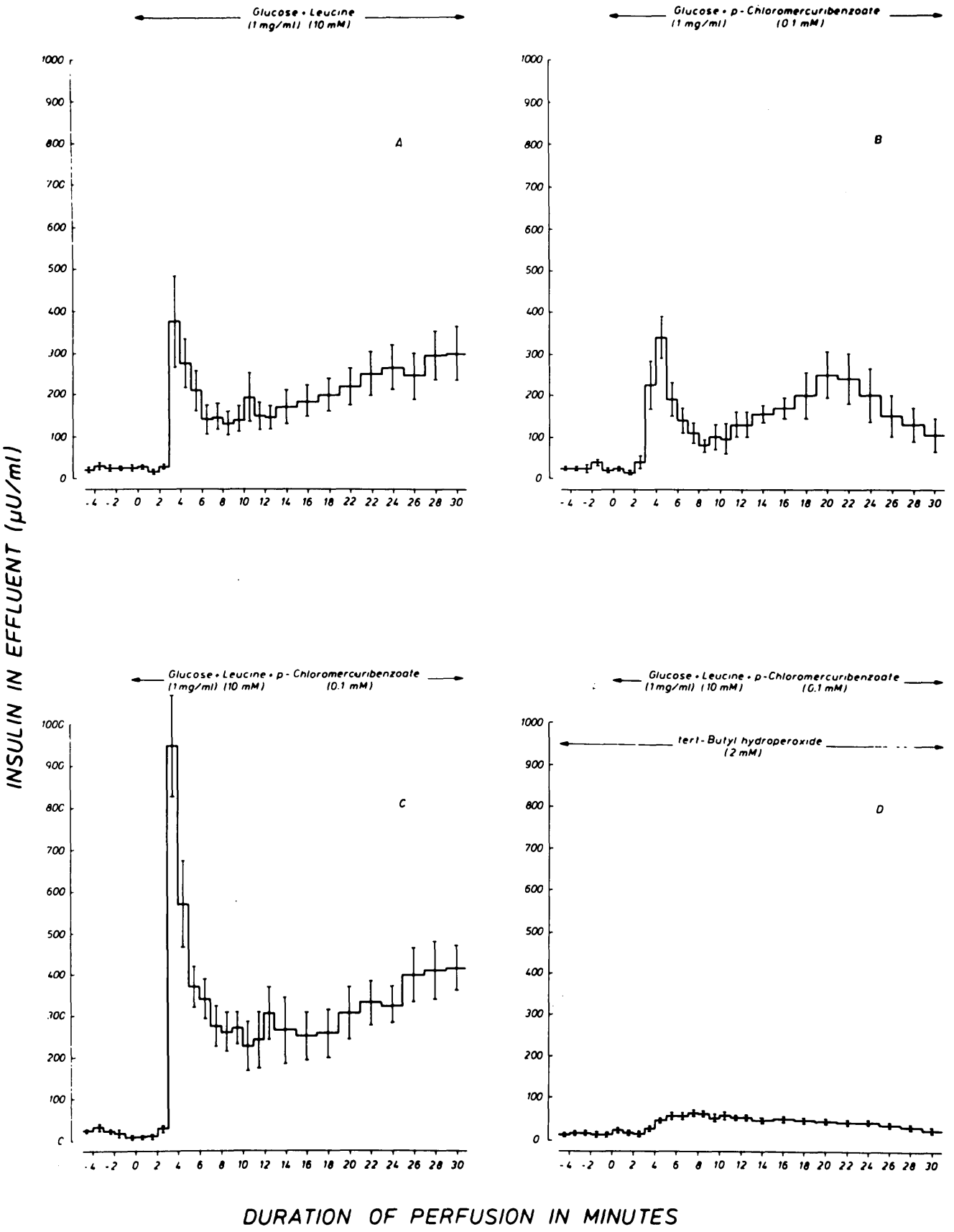

FIGURE 1. Interaction of p-chloromercuribenzoate with glucose- and glucose + leucine–induced insulin release from isolated perfused rat pancreas; effect of tert-butyl hydroperoxide. All perfusions were carried out for 35 min. After the 15-min equilibration period, samples were collected for insulin determination every minute for 5 min (from −5 to 0 min). Then either glucose, leucine, or p-chloromercuribenzoate was added to the perfusion medium for 30 min. Samples were taken every 30 s for the first 7 min and every minute for the remaining 23 min. Inhibitor (D) was added both before and during perfusion with glucose plus leucine plus p-chloromercuribenzoate. Mean ± SEM; n = 6.

TABLE 1
Effect of glucose alone and in combination with leucine on NADPH, NADP, and the NADPH:NADP ratio in isolated rat pancreatic islets 1 and 5 min after addition to the incubation medium

	pmol/10 islets								
	NADPH			NADP			NADPH:NADP		
Time (min)	0	1	5	0	1	5	0	1	5
Glucose (2 mg/ml)	A: 0.51 ± 0.07 (n = 11)	C: 1.09 ± 0.08 (n = 12)	E: 1.12 ± 0.11 (n = 12)	G: 1.23 ± 0.07 (n = 21)	I: 0.81 ± 0.05 (n = 23)	L: 0.79 ± 0.06 (n = 22)	0.42	1.33	1.42
Glucose (2 mg/ml) + Leucine (10 mM)	B: 0.59 ± 0.05 (n = 12)	D: 1.44 ± 0.08 (n = 12)	F: 1.68 ± 0.21 (n = 11)	H: 1.21 ± 0.08 (n = 17)	K: 0.56 ± 0.03 (n = 18)	M: 0.58 ± 0.04 (n = 18)	0.49	2.56	2.90

10 islets were incubated in 0.7 ml of medium for 0, 1, and 5 min. Mean ± SEM; n = number of experiments.
Statistical analysis: A vs. C:, B vs. D:, B vs. F:, I vs. K:, G vs. I:, G vs. L:, H vs. K:, H vs. M: $P < 0.001$; C vs. D:, A vs. D:, L vs. M: $P < 0.01$; E vs. F: $P < 0.05$; A vs. B:, G vs. H: NS

tion. The concentrations of islets' NADPH and NADP and their ratio (NADPH:NADP), as well as the insulin released during 60 min of incubation with glucose or glucose + leucine with and without methylene blue are shown in Table 2. Incubation of islets with glucose (2 mg/ml) led to a noticeable increase in the insulin released and in NADPH, a concomitant decrease of NADP, and an increase of the NADPH:NADP ratio.

In the presence of this concentration of glucose, leucine noticeably raised NADPH, the NADPH:NADP ratio, and insulin secretion further. In the presence of methylene blue (2 μg/ml), NADPH and the NADPH:NADP ratio as well as the insulin released from islets incubated with glucose alone and in combination with leucine were markedly decreased. The decrease of insulin release was lower in the experiments with glucose plus leucine than in those with glucose alone.

ISLETS' NADH AND NAD

Table 3 shows that glucose (2 mg/ml), after 60 min of incubation, noticeably increased the islets' NADH and NAD concentrations; however, the NADH:NAD ratio hardly

TABLE 2
Effect of glucose alone and in combination with leucine with and without methylene blue (2 μg/ml) on NADPH, NADP, the NADPH/NADP ratio, and on insulin released by isolated rat pancreatic islets

	pmol/10 islets			
	NADPH	NADP	NADPH:NADP	μU IRI/ml Insulin
Buffer	A: 0.74 ± 0.04 (n = 10)	F: 1.72 ± 0.05 (n = 37)	0.43	L: 111 ± 28 (n = 13)
Glucose (2 mg/ml)	B: 1.17 ± 0.05 (n = 19)	G: 1.39 ± 0.09 (n = 26)	0.84	M: 392 ± 62 (n = 15)
Glucose (2 mg/ml) + Leucine (10 mM)	C: 1.40 ± 0.07 (n = 17)	H: 1.09 ± 0.06 (n = 29)	1.28	N: 613 ± 88 (n = 14)
Glucose (2 mg/ml) + Methylene blue (2 μg/ml)	D: 0.64 ± 0.05 (n = 20)	I: 1.58 ± 0.1 (n = 23)	0.40	O: 187 ± 49 (n = 14)
Glucose (2 mg/ml) + Leucine (10 mM) + Methylene blue (2 μg/ml)	E: 0.53 ± 0.07 (n = 18)	K: 1.75 ± 0.1 (n = 18)	0.30	P: 292 ± 38 (n = 15)

10 islets were incubated in 0.7 ml of medium for 60 min. Mean ± SEM; n = number of experiments.
Statistical analysis: L vs. M:, A vs. B:, B vs. D:, C vs. E:, F vs. G:, H vs. K: $P < 0.001$; B vs. C:, G vs. H:, N vs. P: $P < 0.01$; M vs. N: $P < 0.05$; M vs. O: $P < 0.02$; G vs. I: NS

TABLE 3
Effect of glucose alone and in combination with leucine on NADH, NAD, and the NADH:NAD ratio in isolated rat pancreatic islets

	pmol/10 islets		
	NADH	NAD	NADH:NAD
Buffer	A: 0.48 ± 0.07 (n = 22)	E: 4.06 ± 0.32 (n = 23)	0.12
Glucose (2 mg/ml)	B: 0.82 ± 0.10 (n = 22)	F: 5.97 ± 0.36 (n = 22)	0.14
Glucose (2 mg/ml) + Leucine (10 mM)	C: 1.18 ± 0.14 (n = 22)	G: 6.17 ± 0.42 (n = 20)	0.19

10 islets were incubated in 0.7 ml of the medium for 60 min. Mean ± SEM; n = number of experiments.
Statistical analysis: A vs. B: $P < 0.01$; E vs. F: $P < 0.001$; B vs. C: $P < 0.05$; F vs. G: NS.

changed. The addition of leucine to the medium containing 2 mg/ml glucose noticeably raised the NADH concentration further. There was no further change of NAD. Methylene blue (2 μg/ml) did not decrease NADH or the NADH:NAD ratio (data not shown).

THIOL OXIDANTS AND THE INSULIN RELEASED BY ISOLATED ISLETS

Immunoreactive insulin (IRI) that accumulated in 1 ml of the incubation medium during 90 min of incubation of five islets in various groups is shown in Table 4. As expected, in the presence of 2 mg/ml of glucose, a significant stimulation of insulin release occurred that was markedly inhibited by diamide (1 mM) and tert-butyl hydroperoxide (2 mM). These inhibitory effects were previously shown to be reversible.[3] Leucine (10 mM) alone did not release insulin noticeably, but, when combined with glucose (2 mg/ml), there was a marked potentiation. When the incubation of islets was carried out in the presence of diamide (1 mM), however, an inhibition of the insulin release stimulated by glucose plus leucine (1037 ± 69 vs. 168 ± 17) was observed. Likewise, tert-butyl hydroperoxide decreased the insulinogenic response of glucose + leucine (1037 ± 69 vs. 143 ± 13). Similar to our findings with methylene blue, the inhibition of insulin release induced by glucose plus leucine was more pronounced than that of glucose alone.

THIOL OXIDANTS, p-CHLOROMERCURIBENZOATE, AND THE INSULIN RELEASED BY ISOLATED PERFUSED PANCREASES

We already showed that perfusion of pancreas with glucose alone at a concentration of 1 mg/ml produces no significant release, but the combination of leucine (10 mM) and glucose (1 mg/ml) produced a biphasic response (Figure 1A). As we showed previously,[3] p-chloromercuribenzoate (0.1 mM) in the presence of 1 mg glucose per milliliter also caused a biphasic insulin-secretory response (Figure 1B). To determine how p-chloromercuribenzoate interacts with the insulin-releasing effect of leucine, a medium containing glucose (1 mg/ml), leucine (10 mM), and p-chloromercuribenzoate (0.1 mM) was perfused. We demonstrated that at least the initial peak of insulin released was higher than that produced by leucine + glucose or p-chloromercuribenzoate + glucose (compare Figure 1C with 1A and 1B). However, the addition of tert-butylhydroperoxide (2 mM) to the medium, both before and during perfusion with glucose + leucine + p-chloromercuribenzoate, greatly depressed their insulin-releasing effect (Figure 1D).

DISCUSSION

LEUCINE-INDUCED INSULIN RELEASE

It is well documented that leucine stimulates insulin release.[1,10] So far, it is unsettled whether the insulin-releasing action of this amino acid requires the presence of glucose. Thus, its insulin-triggering effect occurs even in the absence of glucose.[1,12,13] On the other hand, several workers have shown that its insulin-releasing capacity depends on the presence of glucose.[14,15] Confirming the data of Basabe et al.,[15] we observed that leucine (10 mM) produces a biphasic response of insulin secretion in the presence of a substimulatory glucose concentration.

As regards the possibility of an interaction between leucine and glucose, β-cell metabolism could participate in at least three ways:

TABLE 4
Effect of diamide and tert-butyl hydroperoxide (t-BHP) on glucose plus leucine–induced insulin release by isolated rat pancreatic islets

	Insulin (μU IRI/ml)			
	Buffer	Glucose (2 mg/ml)	Leucine (10 mM)	Glucose (2 mg/ml) + Leucine (10 mM)
Control	A: 92 ± 21 (n = 18)	D: 590 ± 37 (n = 13)	G: 81 ± 17 (n = 6)	J: 1037 ± 69 (n = 22)
Diamide (1 mM)	B: 117 ± 24 (n = 24)	E: 224 ± 20 (n = 14)	H: 105 ± 26 (n = 8)	K: 168 ± 17 (n = 13)
t-BHP (2 mM)	C: 69 ± 10 (n = 10)	F: 117 ± 20 (n = 20)	I: 109 ± 17 (n = 6)	L: 143 ± 13 (n = 15)

Five islets were incubated in 1 ml of medium for 90 min. Mean ± SEM; n = number of experiments.
Statistical analysis: A vs. B: A vs. C: G vs. H: G vs. I: } NS; D vs. E: D vs. F: J vs. K: J vs. L: } $P < 0.001$

1. Since insular glucose metabolism increases after raising the glucose concentration,[16] glucose metabolism might provide increasing amounts of some metabolites or cofactors required for the induction of insulin secretion by leucine.
2. Leucine, from its own metabolism or that of glucose, might provide metabolites or cofactors that themselves could further increase the insulin-secretory effect of glucose.
3. Leucine, from its own metabolism or that of glucose, might provide metabolites or cofactors that could further increase the insulin-triggering action of leucine itself.

The first possibility is difficult to evaluate, as the results are controversial concerning the glucose dependency of leucine in stimulating insulin release.

The facts that leucine is readily metabolized by the β-cell[17] and several leucine metabolites stimulate insulin release[18] are consistent with the idea that leucine metabolism is an important factor in stimulation of insulin release. Studies on the effect of leucine on glucose oxidation in pancreatic islets are not conclusive. Gylfe and Sehlin[19] were unable to demonstrate an increase of ^3H_2O formation from tritiated glucose by leucine, suggesting that this amino acid did not further affect the β-cell glycolysis. On the other hand, these authors observed about a 33% increase of $^{14}CO_2$ production from U-C^{14}-glucose by leucine, and Hellman et al.[17] observed a tendency towards stimulation at glucose concentrations of 3, 10, and 20 mM.

Studies by Panten et al.[2] on the fluorescence of reduced pyridine nucleotides in single, perifused, pancreatic islets showed that leucine increases such fluorescence, but the method used by these workers did not distinguish between NADPH and NADH. We recently suggested that the oxidation-reduction state of the NADPH:NADP ratio is needed for the insulin-releasing mechanism of glucose, p-chloromercuribenzoate, and tolbutamide.[3,20] Therefore, we found it of interest to study the insular NADPH:NADP ratio and the insulin released after incubation with leucine. Prerequisite for a function of the NADPH:NADP ratio in glucose- and/or leucine-stimulated insulin secretion is its rapid rise in response to the addition of glucose and leucine to the incubation medium. This is, in fact, the case, since this ratio is increased as early as 1 min after glucose is added, and a further increase is evident when, in addition to glucose, leucine is supplied. As shown in Table 2, these changes could still be observed, although to a lower degree, after 60 min of incubation. This suggests that, at least in part, the interaction between glucose and leucine, which under the above mentioned conditions leads to a potentiation of insulin release (buffer, 111; glucose alone, 392; and leucine plus glucose, 613 μU IRI/ml), may be due to an increase of the islets' NADPH:NADP ratio produced by leucine. If this is the case, a decrease of the islets' NADPH:NADP ratio should lead to an inhibition of the glucose plus leucine–induced insulin secretion.

When methylene blue (2 μg/ml), an oxidant of NADPH, was employed, the decrease of the ratio was associated with an inhibition of glucose plus leucine–induced insulin release (Table 2). The properties, specificity, and nontoxicity of methylene blue and the two thiol oxidants also employed later in the study were described in detail elsewhere.[3] From our data, showing that NADPH is needed for the insulin-releasing effect of glucose, leucine, and/or glucose + leucine, no firm statement can be made as to whether the increase of the NADPH:NADP ratio produced by leucine increases the insulinogenic action of glucose, of leucine, or of both.

The reduced pyridine nucleotides include not only NADPH but also NADH. Therefore, it was of interest to check the levels of NADH. Table 3 shows that glucose (2 mg/ml) alone increases the islet NADH. This suggests that NADH, too, may be needed in the mechanism of glucose-induced insulin release. When glucose was combined with leucine, a further increase of NADH was observed. Whereas glucose alone could have increased the islets' NADH contents by increasing the glycolytic flux, the further increase of NADH by leucine, which does not itself influence glycolysis,[19] is not clear. It is possible, however, that NADH is produced by the metabolism of leucine, as in other organs. Leucine was observed to be metabolized by islet tissue.[17] Irrespective of the source of NADH, it appears that it may also contribute in some way to the combined action of leucine and glucose on insulin release.

It is also not clear how leucine increases islet NADPH. A possible source is NADH. Formation of NADPH via stimulation of the pentose-phosphate shunt by leucine, however, is less likely, since leucine does not change oxidation of ^{14}C-1- and ^{14}C-6-glucose nor does it change the 6-phosphogluconate:glucose-6-phosphate ratio (unpublished observations).

For technical reasons, it was not possible until now to differentiate between the different compartments of the pyridine nucleotides in the β-cell and between the free and the bound forms. If the ratio of free pyridine nucleotides in the different compartments behaves differently from that of total pyridine nucleotides, it would limit the interpretation of our data, but this possibility remains to be evaluated.

In insulin secretion, the NADPH is believed to keep the islet GSH in the reduced state, which, in turn, keeps some β-cell membrane thiol groups in the reduced state.[3] Preliminary results of our laboratory showed that leucine (10 mM), when incubated with pancreatic islets, markedly increased GSH and decreased GSSG concentrations (unpublished observation). If leucine stimulates insulin secretion via NADPH → GSH → SH-groups, then its insulin-releasing effect should be inhibited by decreasing insular GSH. Table 4 shows that diamide, an oxidant of GSH and other thiols, and tert-butyl hydroperoxide, an indirect oxidant of GSH, by way of being a substrate of glutathione-peroxidase, which were recently shown to decrease the islets' GSH:GSSG ratio,[21,22] significantly diminished glucose- and glucose + leucine–induced insulin secretion. These results suggest that the insulin-releasing effect of leucine is, at least in part, related to GSH, be it by either providing GSH via NADPH, or by depending on GSH itself, or by both of them. Since NADPH acts on both the cell membrane and the secretion granule membrane,[23,24] it is also possible that NADPH, provided by leucine, could act directly on the β-cell membrane and/or on the secretion granule membrane SH-groups to facilitate insulin release. In order to evaluate whether leucine via GSH produces more thiol groups in the β-cell membrane, an indirect approach was used that employed p-chloromercuribenzoate. The latter compound, which hardly penetrates the β-cell membrane, is supposed to trigger insulin release by reacting with superficial thiol groups in the β-cell membrane.[25] As shown in Figure 1C,

leucine potentiated at least the first peak of the p-chloromercuribenzoate–induced release of insulin by the isolated perfused pancreas system, suggesting that leucine might provide, via NADPH and GSH, more membrane thiols for the insulin-releasing action of p-chloromercuribenzoate. The observation that synergism was less pronounced during the second phase might be explained if we consider the findings that p-chloromercuribenzoate enters the β-cell after about 20 min and then blocks some intracellular SH-groups in the β-cell.[26] The possibility of synergism between leucine and p-chloromercuribenzoate by some other mechanism, however, cannot be excluded.

In conclusion, our data indicate that the insulin-releasing action of leucine depends on islet NADPH and GSH and, perhaps, on membrane thiols. Leucine, by increasing the islets' NADPH, may provide more thiol groups in the membrane of the β-cell via GSH, which may be necessary to sensitize the β-cell to the triggering action of glucose, leucine, or glucose plus leucine.

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