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Heat Shock Restores Insulin Secretion after Injury by Nitric Oxide by Maintaining Glucokinase Activity in Rat Islets

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Heat shock protein (hsp), including hsp70, has been reported to restore the glucose-induced insulin release suppressed by nitric oxide (NO). However, the mechanism underlying this recovery remains unclear. In the present study, we examine the effects, in rat islets, of heat shock on insulin secretion inhibited by a small amount of NO and also on glucose metabolism, the crucial factor in insulin release. Exposure to a higher dose (15 U/ml) of interleukin-1β (IL-1β) abolished the insulin release by stimulation of glucose or KCl in both control and heat shocked islets. In rat islets exposed to a lower dose (1.5 U/ml) of IL-1β, insulin secretion in response to glucose, but not to glyceraldehydes (GA), ketoisocaproate (KIC), or KCl, was selectively impaired, concomitantly with lower ATP concentrations in the presence of 16.7 mM glucose, while such suppression of insulin secretion and ATP content was not observed in heat shock-treated islets. NO production in islets exposed to 1.5 U/ml IL-1β was significantly, but only partly, decreased by heat shock treatment. The glucose utilization rate measurement using [5-^{3}H]-glucose and [2-^{3}H]-glucose and the glucokinase activity *in vitro* were reduced in islets treated with 1.5 U/ml IL-1β. In heat shock-treated islets, glucose utilization and glucokinase activity were not affected by 1.5 U/ml IL-1β. These data suggest that heat shock restores glucose-induced insulin release inhibited by NO by maintaining glucokinase activity and the glucose utilization rate in islets in addition to reducing endogenous NO production.

Key Words: **heat shock; islets; interleukin-1β; nitric oxide; insulin secretion.**

Heat shock proteins including hsp70 are known to play a protective role in cellular survival and function (1, 2). In pancreatic islets, hsp70 protein itself has not been reported to influence protein synthesis and insulin secretion (3), but overexpression of hsp70 has been suggested to prevent rat islet and cell line RINm5F cells exposed to, an NO donor, sodium nitroprusside (SNP) from cell lysis (4, 5). In addition, the hsp70 introduced through liposomal delivery or expressed by heat shock restored the glucose-induced insulin release inhibited by IL-1β in rat and human islets (3, 6). Although the mechanism may include reduced iNOS expression (6), heat shock or hsp70 also has been reported to affect the cAMP concentration (7, 8), protein kinase C activity (9), and the regulation of intracellular free calcium concentration (10, 11). These reports suggest several possible mechanisms of the protective function of heat shock in pancreatic islets.

It is well known that nitric oxide (NO) inhibits insulin secretion, but the mechanism is thought to be dependent on the volume of NO. A larger amount of NO induces apoptosis, leading to cell death and the abolition of insulin release (12–14), but a relatively smaller concentration of NO induces a reversible deterioration of the cellular signal transduction system which suppresses insulin release. However, the identity of the sites disturbed by NO is controversial, and the enzymes including glucokinase (15–17), phosphofructokinase (18–20), and aconitase in the Krebs cycle (21) are all candidates. It has been reported that NO inhibits phospholipase C activity (22), cAMP synthesis (23), and insulin mRNA synthesis (24), and increases the production of free radicals (25) that results in impaired insulin secretion. In the present study, we examined the effect of heat shock treatment on glucose metabolism, the crucial factor in glucose-induced insulin release in islets exposed to 1.5 U/ml IL-1β or NO donors.

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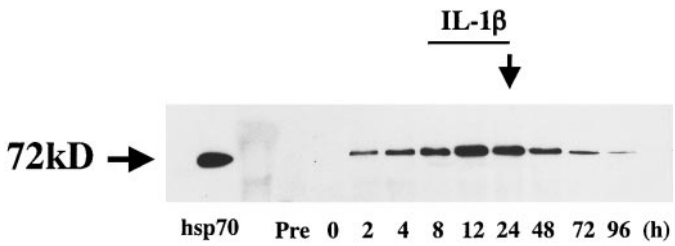

FIG. 1. Time course of hsp70 expression in rat islets after heat shock. Time course of hsp70 expression from 0 to 96 h after heat shock is illustrated. Positive control by human recombinant hsp70 (0.01 g/lane) shows most in the left column. The adjacent column (pre) indicates expression before heat shock treatment. The protein concentrations are 5 μg/lane. Arrow and bar indicate the experimental time and exposure period to IL-1β.

We found that heat shock maintains the glucose phosphorylation rate of glucose metabolism in islets subjected to NO.

MATERIALS AND METHODS

Materials. The nitrite assay kit was obtained from Cayman Chemical Company (Ann Arbor, MI). [5-^{3}H]-glucose and [2-^{3}H]-glucose were obtained from Amersham Int. (Buckinghamshire, UK). Recombinant human interleukin-1β was obtained from Pepro Tech Inc. (London, UK), and *S*-nitroso-*N*-acetyl-DL-penicilline (SNAP) and N^G-monomethyl-L-arginine (L-NMMA) were purchased from Tocris Cookson Inc. (Ballwin, MO). Luciferin-luciferase was obtained from Turner Designs (Sunnyvale, CA). SNP and other chemicals were obtained from Nakalai Tesque (Kyoto, Japan).

Islet isolation and heat shock treatment. Pancreatic islets were isolated from male Wister rats weighing 200–250 g by collagenase digestion as described previously (26). The islets in 15 ml Krebs-Ringer bicarbonated buffer (KRBB) medium consisting of (in mM) 129.4 NaCl, 4.0 KCl, 2.7 $CaCl_2$, 1.2 KH_2PO_4, 1.3 $MgSO_4$, and 24.8 $NaHCO_3$ containing 11.1 mM glucose and 0.1% BSA were incubated at 37°C (control) or 42°C (heat shock) for 30 min. The expression of hsp70 was confirmed by Western blotting method as illustrated in Fig. 1. Afterward, the isolated islets were cultured at 37°C in CMRL-1066 medium with 11.1 mM glucose, supplemented with 2 mM glutamine, 10% fetal calf serum, 100 U/ml penicillin, and 0.1 mg/ml streptomycin in an atmosphere of 95% air and 5% CO2 until use in the experiments (24 h). When necessary, 1.5 U/ml or 15 U/ml IL-1β was added to the medium 8 h after culture, and, in the case of the experiments using NO donors, islets were incubated with 100 μM SNP or 400 μM SNAP for 1 h after culture for 24 h.

Insulin release from isolated rat pancreatic islets (27). Insulin release from islets was monitored using batch incubation method. The control and treated islets were preincubated at 37°C for 30 min with KRBB medium supplemented with 3.3 mM glucose and 0.2% BSA. Groups of 5 islets were then incubated for 30 min at 37°C in 0.8 ml of the medium with test materials. At the end of the incubation period, islets were pelleted by centrifugation and aliquots of the buffer were sampled. The amount of immunoreactive insulin was determined by RIA, using rat insulin as standard. Experiments using the same protocol were repeated three times to ascertain reproducibility.

Determination of cell viability (4). The cell viability was evaluated by the Trypan blue exclusion assay. At least 200 dispersed control islet cells and heat shock-treated cells were examined microscopically.

Measurement of nitrite. The nitrite production was measured by colorimetric assay. Control islets were cultured in 3 ml CMRL-1066 with 1.5 U/ml IL-1β or with 1.5 U/ml IL-1β and 0.5 mM L-NMMA for 16 h, and the heat shock-treated islets were treated with 1.5 U/ml IL-1β for 16 h. Nitrate in the culture medium was reduced to nitrite by adding sulfanilamide, and, afterward, nitrite was converted to azo product by mixture with *N*-(1-Naphthyl)-ethylenediamine. The amount of nitrite was determined by photometric measurement of the absorbance of emission from azo products (at 546 nm), using BIO RAD model 550.

Measurement of ATP content in islets (28). After preincubation at 3.3 mmol/l glucose for 30 min, 10 cultured islets were incubated in 0.8 ml KRBB supplemented with 16.7 mM glucose and 0.2% BSA at 37 for 30 min. The reaction was stopped by the addition of trichloroacetic acid (TCA), the concentration of 5% at the time of examination. The reaction buffers were immediately mixed with vortex, and then sonicated at 4°C. 0.8 ml of supernatant obtained by centrifugation was mixed with 1 ml of water-saturated diethyl ether, and the ether phase containing TCA was removed repeatedly, and a fraction of the extracts was diluted with 0.1 ml 20 mM Hepes solution (pH 7.4 with NaOH). The ATP concentration in the solutions was measured by adding luciferin-luciferase solution using a bioluminometer (Luminometer Model 20e, Turner Designs, Sunnyvale, CA). To draw a standard curve, blanks and ATP standards were run through the entire procedure, including the extraction steps.

Glucose utilization in isolated islets (29). Cultured islets were preincubated in KRBB medium supplemented with 3.3 mM glucose and 0.2% BSA at 37°C for 30 min. Triplicate batches of 30 islets for each condition were placed into micro tubes to which were added 1.5 Ci of [5-^{3}H]-glucose (specific activity 91.7 mCi/mg) or [2-^{3}H]-glucose (specific activity 91.7 mCi/mg) to a final concentration of 16.7 mM glucose. These batches were incubated at 37°C for 120 min. Aliquots of the incubation medium (100 μl) and 20 μl of 1N HCl were transferred into small tubes, and placed into a glass vial containing 5 ml of H_2O. The capped vials were incubated overnight at 37°C to vaporize ^3H_2O from the solution. Afterward, the inner tube was lifted out, and the dpm of water-melting ^3H_2O in the vial was counted.

Measurement of glucokinase activity in vitro (30). The activity of glucokinase was spectrophotometrically measured. Control and treated islets were preincubated with KRBB medium supplemented with 3.3 mM glucose for 30 min at 37°C. The islets then were homogenized in solution consisting of 50 mM trisaminomethane and 250 mM saccharose (pH 8.0 with HCl) at 4°C, and the supernatants were obtained from the homogenates by centrifugation at 4°C. The protein content in the supernatants was assayed by the method reported by Lowry *et al.* (31). The enzyme reaction was performed in solution consisting of (in mM) 50 trisaminomethane, 6.0 $MgCl_2$, 100 glucose, and 5 ATP (pH 7.5 with HCl) at room temperature. Glucokinase activity was estimated as the increase in NADH through the following reaction: glucose-6-phosphate + NAD 6-phosphoglucono-lactone + NADH by NAD-dependent glucose-6-phosphate dehydrogenase. Correction for hexokinase activity was applied by subtracting the activity measured at 0.5 mM glucose from the activity measured at 100 mM glucose. One unit was defined as the amount of enzyme which catalyzes the formation of 1 μmol/l NADH/min under these conditions.

Statistical analysis. The statistical analysis of results and the significance of the differences were evaluated by unpaired Student's *t* test, and $P < 0.05$ was considered significant. Results were expressed as mean ± SE.

RESULTS

The Effect of Heat Shock Treatment on Insulin Secretion

Insulin release stimulated by 16.7 mM glucose was abolished in both control and heat shock-treated rat

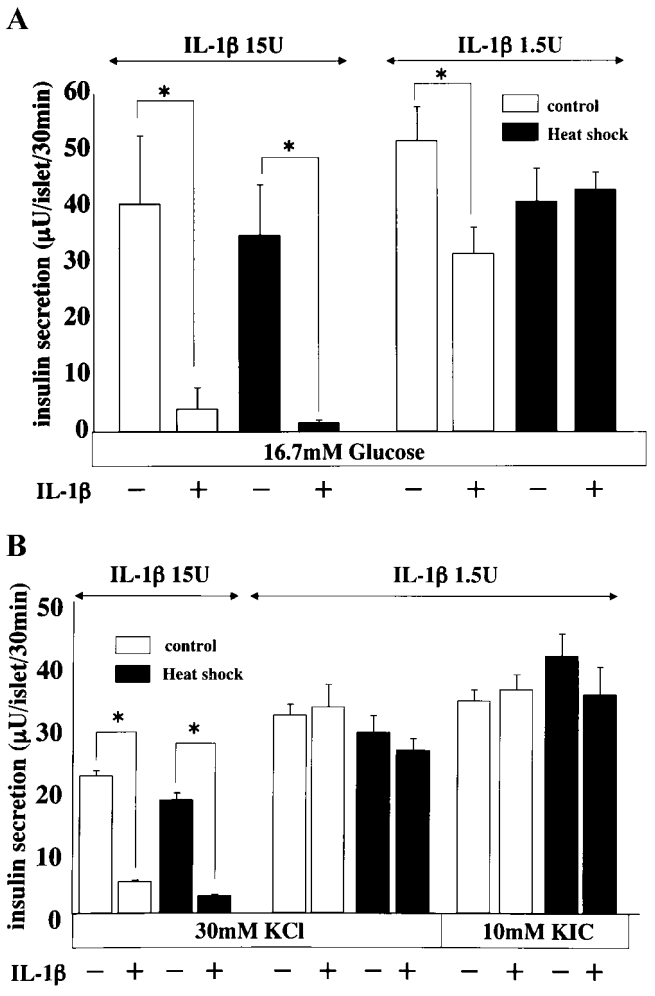

FIG. 2. Effect of exposure to 15 U/ml or 1.5 U/ml IL-1β on insulin release in control and heat shock-treated islets. (A) Effect on 16.7 mM glucose induced insulin secretion. (B) Effect on 30 mM KCl- or 10 mM KIC-induced insulin secretion. Open bars show control islets and hatched bars show heat shock-treated islets. Each bar shows mean ± SE of 6 observations. *$P < 0.01$.

islets treated with 15 U/ml IL-1β (Fig. 2A). On the other hand, insulin release in response to 16.7 mM glucose was partly suppressed in control islets exposed to 1.5 U/ml IL-1β, and this suppression was reversed to 101.2 ± 4.7% ($n = 7$) of control by the addition of 0.5 mM L-NMMA. In heat shock-treated islets, exposure to 1.5 U/ml IL-1β did not affect the insulin release enhanced by 16.7 mM glucose (Fig. 2A). 15 U/ml IL-1β also suppressed the 30 mM KCl-induced insulin release even in heat shock-treated islets, however, regardless of heat shock treatment, the insulin secretion stimulated by 30 mM KCl or a mitochondrial metabolite, 10 mM KIC, was not inhibited by exposure to 1.5 U/ml IL-1β (Fig. 2B). Smaller secretions in response to 10 mM glyceraldehydes (GA) in control (19.7 ± 2.8 μU/islet/30 min) and heat shock-treated islets (14.7 ± 1.2 μU/islet/30 min) was not changed by exposure to 1.5 U/ml IL-1β (18.8 ± 1.3 μU/islet/30 min in control and 15.3 ± 1.4 μU/islet/30 min in the heat shock group). In the islets exposed for 1 h to 0.1 mM SNP or 0.4 mM SNAP, the 16.7 mM glucose-induced insulin secretion was similarly inhibited to the level of 55.1 ± 5.7% or 74.6 ± 7.6% of control, while each of the secretions remained at levels similar to controls in heat shock-treated islets.

Cell Viability

The spontaneous lysis of control cells was in the range of 14.5 ± 1.2% ($n = 3$), and lysis of heat-treated cells was in the range of 11.9 ± 2.4% ($n = 3$). After treatment of rat islets for 24 h with 1.5 U/ml IL-1β, the spontaneous lysis of control cells was in the range of 8.7 ± 1.4% ($n = 3$), and lysis of heat-treated cells was in the range of 10.1 ± 2.6% ($n = 3$). These values were not significantly different.

IL-1β-Induced NO Production in Islets

As illustrated in Fig. 3, in the presence of 1.5 U/ml IL-1β NO production in heat shock-treated islets was 22.8 ± 0.6% ($n = 3$) less at 16 h than in control. The IL-1β-induced increase in NO production was completely suppressed in islets cultured with 0.5 mM L-NMMA, an iNOS inhibitor.

Effect of NO on Glucose Metabolism in Control and Heat Shock Treated Islet

The intracellular ATP content in the presence of 16.7 mmol/l glucose in islets exposed to IL-1β or 0.1 mM SNP was significantly less than in controls, while in islets with heat shock treatment, ATP contents were

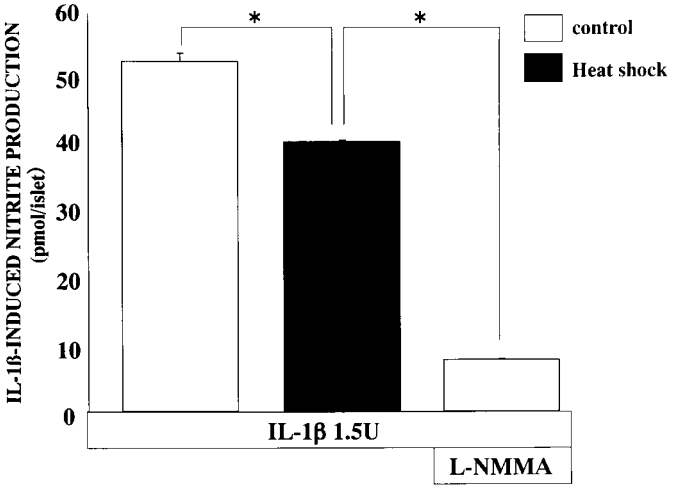

FIG. 3. NO production after exposure to IL-1β in rat control and heat shock-treated islets. NO production in control islets (open bar), heat shock-treated islets (black bar) and control islets with 0.5 mmol/l NMMA (hatched bar) for 16 h (from addition of IL-1β to experimental time) are shown. Each bar shows mean ± SE of 3 observations. *$P < 0.01$ between both bars.

TABLE 1

Effect of 1.5 U/ml IL-1β or SNP on ATP Content, Glucose Utilization, and Glucokinase Activity in Control and Heat Shock-Treated Islets

(A) IL-1β (1.5 U/ml)

	ATP content (pmol/islet) (n = 7)		[2-^{3}H]-glucose utization (pmol/islet/h) (n = 4)		[5-^{3}H]-glucose utilization (pmol/islet/h) (n = 4)		Glucokinase activity (U/mg protein) (n = 4)	
	(−)	(+)	(−)	(+)	(−)	(+)	(−)	(+)
IL-1β								
control islets	6.8 ± 0.4	3.5 ± 0.2*	269.8 ± 9.7	220.6 ± 11.1*	164.7 ± 19.0	108.8 ± 3.7*	0.41 ± 0.05	0.17 ± 0.03*
heat shock islets	5.6 ± 0.2	5.8 ± 0.4	183.1 ± 11.3	174.6 ± 7.6	122.2 ± 19.0	135.6 ± 9.5	0.45 ± 0.07	0.46 ± 0.06

(B) SNP (0.1 mM)

SNP								
control islets	4.8 ± 0.2	2.9 ± 0.2*	126.9 ± 0.5	106.6 ± 2.0*	178.4 ± 7.7	147.4 ± 1.5*	0.42 ± 0.01	0.33 ± 0.02*
heat shock islets	7.4 ± 0.6	7.4 ± 0.2	115.6 ± 8.3	126.1 ± 3.7	172.1 ± 7.4	172.3 ± 24.5	0.38 ± 0.05	0.39 ± 0.06

* $P < 0.01$ versus corresponding values without IL-1β or SNP.

almost identical regardless of exposure to IL-1β (Table 1). Similarly, the intraislet ATP content in controls was significantly reduced by treatment with 0.4 mM SNAP (5.0 ± 0.3 to 2.3 ± 0.2), while this was not the case in the heat shock-treated groups (6.8 ± 0.3 versus 6.3 ± 0.2). The glucose utilization rate using [5-^{3}H]-glucose or [2-^{3}H]-glucose is known to reflect the phosphofructokinase activity rate or the glucose phosphorylation rate, respectively. The glucose utilization rate using either tracer in islets exposed to IL-1β or SNP was significantly less than in the respective controls. However, there was little reduction in the glucose utilization rate by IL-1β or SNP in the islet groups treated by heat shock (Table 1).

The glucokinase activity in islets exposed to IL-1β or SNP was significantly reduced, compared with the activity in control islets. In the heat shock-treated groups, the glucokinase activity in islets treated with IL-1β or SNP was kept to the same level as in control (Table 1). The hexokinase activity in control and heat shock-treated islets was 0.14 ± 0.02 U/mg protein and 0.13 ± 0.02 U/mg protein, respectively, and was not significantly altered by exposure to NO.

DISCUSSION

Glucose-induced insulin secretion is well known to be inhibited by NO, but the inhibitory mechanism remains controversial. One reason is the degree of NO stimulation has not been uniformly reported among the various studies, and, therefore, the difference in the amount of IL-1β exposed to islets could determine the site responsible for the disturbed insulin secretion. A relatively lower dose (~5 U/ml) reportedly leads to decreased glucose utilization (16, 17), while a higher dose (~25 U/ml) suppresses the activity of aconitase in the Krebs cycle (21). In the present study, exposure to a higher dose (15 U/ml) of IL-1β is shown to inhibit insulin release in response to glucose or KCl, while GA-, KIC-, and KCl-induced insulin release is not affected in islets treated with a lower dose (1.5 U/ml) of IL-1β, suggesting that NO first suppresses glucose-induced insulin release by impairing early steps in glycolysis. To selectively investigate this initial deterioration by NO, 1.5 U/ml IL-1β was chosen in the present study. Indeed, the ATP content in islets exposed to 1.5 U/ml IL-1β was less than in control in the presence of 16.7 mM glucose. Furthermore, the glucose phosphorylation rate and phosphofructokinase rate was reduced using [2-^{3}H]-glucose and [5-^{3}H]-glucose with 16.7 mM glucose in NO-treated islets. Consistently with the glucose utilization findings, glucokinase activity *in vitro* was suppressed in islets exposed to IL-1β. These results concur with previous reports that glucokinase activity is suppressed by exposure to a low dose of NO (16, 17). Glucokinase is a rate-limiting enzyme in glycolysis, and its inhibition is known to result in impaired glucose-induced insulin secretion (32). Accordingly, our finding of glucokinase activity disturbed by NO implicates the mechanism of impaired glucose-induced insulin release in islets exposed to NO. Since little cell lysis was found in both the presence and absence of IL-1β, it is unlikely that the reduced glucokinase activity is due to enzyme leakage. However, in the present investigation, NO already had dissipated from the islets before measurement. This suggests that glucokinase is conformationally transformed during the exposure to NO, and this is confirmed by the suppressive glucokinase activity *in vitro*.

Several different mechanisms could explain the restoration of glucose-induced insulin secretion by heat shock. First, heat shock might eliminate NO from the islets. Scarim *et al.* have shown recently that heat shock suppresses the expression of iNOS, and leads to

marked reduction of NO production and to restored insulin release (6). We find that NO production is partly reduced in islets treated by heat shock. However, there is a complete restoration of insulin secretion in these islets even when NO is still produced in part, similarly to the case of NO production abolished by NMMA. This suggests another protective function of heat shock in addition to its suppression of iNOS expression in the recovery of glucose-induced insulin release. Second, heat shock might activate many of the sites responsible for the augmentation of insulin release. Heat shock has been reported to increase the intracellular cAMP level in other cells, such as human A-431 cells (7) and human thymocytes (8). A rise in cAMP concentration would stimulate insulin secretion, but there is no confirmation of this in cells. In addition, IL-1β reportedly inhibits phospholipase C activity (22) and Ca^{2+}-induced exocytosis (33) in rat islets. Our data show that Ca^{2+}-evoked insulin release induced by 30 mM KCl is not influenced by the expression of iNOS or by exposure to SNP in the presence or absence of heat shock treatment. This may not indicate any specific role of heat shock treatment on steps after Ca^{2+} influx, but, because the effects of heat shock on phospholipase C activity and Ca^{2+}-dependent exocytosis have not yet been determined, further investigation is required to clarify the function of heat shock on intracellular signal transduction in pancreatic β cells. Third, heat shock might facilitate repair of sites injured by NO. Heat shock-treated islets exposed to NO are similar to control islets in glucose-induced insulin secretion, ATP content in the presence of 16.7 mM glucose, glucose utilization rate, and glucokinase activity *in vitro.* These findings strongly suggest that heat shock protects glucokinase activity from NO attack, promoting glucose metabolism and insulin release in response to glucose. Ma *et al.* have reported that IL-1β maintains the mRNA content of glucokinase for several hours, and that it subsequently decreases in rat islets (16). In the present study, the effect of exposure to SNP for only 1 h on glucose utilization and glucokinase activity resembled the inhibitory efficacy of IL-1β. Accordingly, protection by heat shock probably is not mediated by the diminution of glucokinase mRNA. The possibility that glucokinase activity is directly, allosterically modulated seems unlikely since heat shock is not known to have an allosteric function and the glucokinase activity disturbed by NO did not recover in the presence of recombinant hsp70 *in vitro* (unpublished observation). Finally, heat shock probably has a protective function on structural transformation of the enzyme. Taken together, heat shock might protect glucose-induced insulin secretion from NO attack not only by reducing NO production, but also by maintaining glucokinase activity in pancreatic β cells.

The function of heat shock in islets under physiological conditions remains unknown. Because NO generated via iNOS may be related to cell lysis (34, 14), heat shock would protect against cell death through maintenance of glucose metabolism and other intracellular signal transduction. On the other hand, fever and glucose intolerance concomitant with hypoinsulinemia have been reported when IL-1β is injected in normal rats (35). IL-1β originating from an infectious focus simultaneously induces fever and the expression of iNOS in cells, resulting in impaired glucose-induced insulin release. Such impairment could well be ameliorated by heat shock administered during fever because of its protective role in transient suppression of the insulin secretion and its maintainence of glucokinase activity during fever or ischemic stress.

In rat islets exposed to a smaller dose of NO, the insulin release in response to glucose was selectively inhibited. In addition, there was a lower ATP concentration, reduced glucose utilization, and reduced glucokinase activity in the islets. NO generation via iNOS was in part somewhat reduced in islets treated by heat shock; in addition, heat shock reduced the scale of the alterations in glucose metabolism. Accordingly, heat shock might function a chaperone for the glycolytic enzyme, glucokinase, and so be crucial in the maintainence of as well as glucose-induced insulin secretion in pancreatic β cells.

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REFERENCES

1. Hirakawa, T., Rokutan, K., Nikawa, T., and Kishi, K. (1996) Geranylgeranylacetone induces heat shock proteins in cultured guinea pig gastric mucosal cells and rat gastric mucosa. *Gastroenterology* **111,** 345–357.
2. Suzuki, K., Sawa, Y., Kaneda, Y., Ichikawa, H., Shirakura, R., and Mutsuda, H. (1997) In vivo gene transfection with heat shock protein 70 enhances myocardial tolerance to ischemia-reperfusion injury in rat. *J. Clin. Invest.* **7,** 1645–1650.
3. Margulis, B. A., Sander, S., Eizirik, D. L., Welsh, N., and Welsh, M. (1991) Liposomal delivery of purified heat shock protein hsp70 into rat pancreatic islets as protection against interleukin 1β-induced impaired beta-cell function. *Diabetes* **40,** 1418–1422.
4. Bellmann, K., Wenz, A., Radons, J., Burkart, V., Kleemann, R., and Kolb, H. (1995) Heat shock induces resistance in rat pancreatic islet cells against nitric oxide, oxygen radicals and streptozotocin toxicity in vitro. *J. Clin. Invest.* **95,** 2840–2845.
5. Bellmann, K., Jäättelä, M., Wissing, D., Burkart, V., and Kolb, H. (1996) Heat shock protein hsp70 overexpression confers resistance against nitric oxide. *FEBS Lett.* **391,** 185–188.
6. Scarim, A. L., Heitmeier, M. R., and Corbett, J. A. (1998) Heat

shock inhibits cytokine-induced nitric oxide synthase expression by rat and human islets. *Endocrinology* **139,** 5050–5057.

7. Kiang, J. G., Wu, Y. Y., and Lin, M. C. (1991) Heat treatment induces an increase in intracellular cyclic AMP content in human epidermoid A-431 cells. *Biochem. J.* **276,** 683–689.
8. Lin, P. S., Kwock, L., Hefter, K., and Wallach, D. F. (1978) Modification of rat thymocyte membrane properties by hyperthermia and ionizing radiation. *Int. J. Radiat. Biol.* **33,** 371–382.
9. Dring, X. Z., Tsokos, G. C., and Kiang, J. G. (1998) Overexpression of HSP-70 inhibits the phosphorylation of HSF1 by activating protein phosphatase and inhibiting protein kinase C activity. *FASEB J.* **12,** 451–459.
10. Kiang, J. G., Koenig, M. L., and Smallridge, R. C. (1991) Heat shock increases cytosolic free Ca^{2+} concentration via Na^{+}-Ca^{2+} exchange in human epidermoid A 431 cells. *Am. J. Physiol.* **263,** C30–C38.
11. Kiang, J. G., and Koenig, M. L. (1996) Characterization of intra cellular calcium pools and their desensitization in thermotolerant cells. *J. Investig.* **44,** 352–361.
12. Bergmann, L., Kröncke, K. D., Suschek, C., Kolb, H., and Kolb-Bachofern, V. (1992) Cytotoxic action of IL-1β against pancreatic islets is mediated via nitric oxide formation and is inhibited by N^{G}-monomethyl-L-arginine. *FEBS Lett.* **299,** 103–106.
13. Ankarcrona, M., Dypbukt, J. M., Brüne, B., and Nicotera, P. (1994) Interleukin-1β-induced nitric oxide production activates apoptosis in pancreatic RINm5F cells. *Exp. Cell Res.* **213,** 172–177.
14. Kröncke, K. D., Kolb-Bachofern, V., Berschick, B., Burkart, V., and Kolb, H. (1991) Activated macrophages kill pancreatic syngeneic islet cells via arginine-dependent nitric oxide generation. *Biochem. Biophys. Res. Commun.* **175,** 752–758.
15. Beggs, M., Beresford, G., Clarke, J., Mertz, R., Espinal, J., and Hammonds, P. (1990) Interleukin-1β inhibits glucokinase activity in clonal HIT-T15 beta-cells. *FEBS Lett.* **26,** 217–220.
16. Ma, Z., Landt, M., Bohrer, A., Ramanadham, S., Kipnis, D. M., and Turk, J. (1997) Interleukin-1 reduces the glycolytic utilization of glucose by pancreatic islets and reduces glucokinase mRNA content and protein synthesis by a nitric oxide-dependent mechanism. *J. Biol. Chem.* **272,** 17827–17835.
17. Park, C., Kim, J. R., Shim, J. K., Kang, B. S., Park, Y. G., Nam, K. S., Lee, Y. C., and Kim, C. H. (1999) Inhibitory effects of streptozotocin, tumor necrosis factor-α, and interleukin-1β on glucokinase activity in pancreatic islets and gene expression of GLUT2 and glucokinase. *Arch. Biochem. Biophys.* **362,** 217–224.
18. Ma, Z., Ramanadham, S., Kempe, K., Hu, Z., Ladenson, J., and Turk, J. (1996) Characterization of expression of phosphofructokinase isoforms in isolated rat pancreatic islets and purified beta cells and cloning and expression of the rat phosphofructokinase-A isoform. *Biochem. Biophys. Acta* **1308,** 151–163.
19. Tsuura, Y., Ishida, H., Hayashi, S., Sakamoto, K., Horie, M., and Seino, Y. (1994) Nitric oxide opens ATP-sensitive K^{+} channels through suppression of phosphofructokinase activity and inhibits glucose-induced insulin release in pancreatic beta cells. *J. Gen. Physiol.* **104,** 1079–1098.
20. Tsuura, Y., Ishida, H., Shinomura, T., Nishimura, M., and Seino, Y. (1998) Endogenous nitric oxide inhibits glucose-induced insulin secretion by suppression of phosphofructokinase activity in pancreatic islets. *Biochem. Biophys. Res. Commun.* **252,** 34–38.
21. Welsh, N., Eizirik, D. L., Bendtzen, K., and Sandler, S. (1991) Interleukin-1β-induced nitric oxide production in isolated rat pancreatic islets requires gene transcription and may lead to inhibition of the Krebs cycle enzyme aconitase. *Endocrinology* **129,** 3167–3173.
22. Vadakekalam, J., Rabaglia, M. E., and Metz, S. A. (1997) Interleukin-1β inhibits phospholipase C and insulin secretion at sites apart from K_{ATP} channel. *Am. J. Physiol.* **273,** E942–E950.
23. Green, I. C., Delaney, C. A., Cunningham, J. M., Karmiris, V., and Southern, C. (1993) Interleukin-1β effects on cyclic GMP and cyclic AMP in cultured rat islets of Langerhans-arginine-dependence and relationship to insulin secretion. *Diabetologia* **36,** 9–16.
24. Eizirik, D. L. (1991) Interleukin-1β induces an early decrease in insulin release, (pro) insulin biosynthesis and insulin mRNA in mouse pancreatic islets by a mechanism dependent on gene transcription and protein synthesis. *Autoimmunity* **10,** 107–113.
25. Sumoski, W., Baquerizo, H., and Rabinovitch, A. (1989) Oxygen free radical scavengers protect rat islet cells from damage by cytokines. *Diabetologia* **32,** 792–796.
26. Sutton, R., Peters, M., Mcshane, P., Gray, D. W. R., and Morris, P. J. (1986) Isolation of rat pancreatic islets by ductal injection of collagenase. *Transplantation* **42,** 689–691.
27. Fujimoto, S., Ishida, H., Kato, S., Okamoto, Y., Tsuji, K., Mizuno, N., Ueda, S., Mukai, E., and Seino, Y. (1998) The novel insulinotropic mechanism of pimobendan: direct enhancement of the exocytotic process of insulin secretory granules by increased Ca^{2+} sensitivity in beta-cells. *Endocrinology* **139,** 1133–1140.
28. Fujimoto, S., Tsuura, Y., Ishida, I., Tsuji, K., Mukai, E., Kajikawa, M., Hamamoto, Y., Takeda, T., Yamada, Y., and Seino, Y. (2000) Augmentation of basal insulin release from rat islets by preexposure to a high concentration of glucose. *Am. J. Physiol. Endocrinol. Metab.* **279,** E927–E9440.
29. Ashcroft, S. J. H., Weerasinghe, L. C. C., Bassett, J. M., and Randle, P. J. (1972) The pentose cycle and insulin release in mouse pancreatic islets. *Biochem. J.* **126,** 525–532.
30. Shimizu, T., Parker, J. C., Najafi, H., and Matschinsky, F. M. (1988) Control of glucose metabolism in pancreatic beta-cells by glucokinase, hexokinase, and phosphofructokinase. *Diabetes* **37,** 1524–1530.
31. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) Protein measurement with the folin phenol reagent. *J. Biol. Chem.* **193,** 265–275.
32. Grupe, A., Hultgren, B., Ryan, A., Ma, Y. H., Bauer, M., and Stewart, T. A. (1995) Transgenic knockouts reveal a critical requirement for pancreatic beta cell glucokinase in maintaining glucose homeostasis. *Cell* **83,** 69–78.
33. Wolf, B. A., Hughes, J. H., Florholmen, J., Turk, J., and McDaniel, M. L. (1989) Interleukin-1 inhibits glucose-induced Ca^{2+} uptake by islets of Langerhans. *FEBS Lett.* **248,** 35–38.
34. Corbett, J. A., and McDaniel, M. L. (1992) Does nitric oxide mediate autoimmune destruction of beta-cells? Possible therapeutic interventions in IDDM. *Diabetes* **41,** 897–903.
35. Reimers, J. I., Bjerre, U., Mandrup-Poulsen, T., and Nerup, J. (1994) Interleukin 1β induces diabetes and fever in normal rats by nitric oxide via induction of different nitric oxide syntheses. *Cytokine* **6,** 512–552.