Caerulein pancreatitis increases mRNA but reduces protein levels of rat pancreatic heat shock proteins

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Caerulein pancreatitis increases mRNA but reduces protein levels of rat pancreatic heat shock proteins. Am. J. Physiol. 273 (Gastrointest. Liver Physiol. 36): G937–G945, 1997.—We have recently reported that preconditioning through hyperthermia induces expression of pancreatic heat shock proteins (HSPs) and protects against caerulein pancreatitis. Here, we investigate caerulein-mediated effects on pancreatic HSPs without prior hyperthermia. Caerulein time and dose dependently increased pancreatic mRNA levels of the constitutive isoform of HSP70 (HSC70). However, pancreatic HSC70 protein levels were decreased, as were HSP60 protein levels. Also, in contrast to hyperthermia preconditioning, caerulein did not induce measurable levels of mRNA or protein of the inducible isoform of HSP70. Thus the pancreas reacts to different kinds of stress (hyperthermia vs. hyperstimulation) with differential induction of HSP mRNAs. Clearly, hyperthermia leads to induction of HSP protein expression, whereas caerulein treatment does not. Therefore, our current study further supports the idea that hyperthermia-induced protection against caerulein pancreatitis is mediated through increased protein levels of pancreatic HSPs. It is further tempting to hypothesize that failure to appropriately increase HSP protein levels in response to high doses of caerulein might be a factor in the development of pancreatitis.

HSP70; HSC70; pancreatic stress reaction

Increased expression in response to thermal stress has led to the discovery of the so-called heat shock proteins (HSPs) (13). Expression of these proteins, however, is inducible by almost any kind of stress, and they are therefore also referred to as stress proteins. The clinical importance of these stress proteins is currently a matter of intense research (3). HSPs are ubiquitous and comprise several structurally unrelated protein families. These are named according to the apparent molecular weights of their respective members, such as HSP60 or HSP70 (5, 7, 20). HSPs not only function as part of the cellular stress-response machinery but also fulfill important physiological functions under normal conditions. Thus HSP70 members play an important role in the correct folding of nascent peptides during translation as well as on transport across membranes of different cellular organelles. It further appears that HSP70 and HSP60 act in concert to help peptides assume their proper three-dimensional structure by carrying them through intermediate states during folding.

We are interested in characterizing the pancreatic stress response and have recently shown that preconditioning through hyperthermia can induce expression of pancreatic HSPs both in vitro and in vivo (17). We have further found that hyperthermia preconditioning also induces protection against caerulein pancreatitis and that both induction of HSP expression as well as reduction of pancreatic organ damage through hyperthermia correlate well with respect to time-course and peak effect. It thus appears that heat shock pretreatment can protect the pancreas against subsequent episodes of stress, possibly mediated through induction of HSP expression.

It has previously been reported that stimulation with caerulein loads too small to induce pancreatitis (0.25 µg·kg⁻¹·h⁻¹ for 12 h) as well as feeding with the protease inhibitor FOY-305, which increases serum cholecystokinin concentrations, can increase pancreatic HSC70 mRNA levels two- to threefold (8). Protein levels of pancreatic HSPs were not measured in that study (8). Although it has further been reported that hyperstimulation with caerulein can increase mRNA levels of HSP70 in the pancreas (19), no effect on HSP70 protein levels has been observed. In that study (19), isoforms of HSP70 were not separately investigated and HSP60 expression was not studied at all. In contrast to the study by Hensel et al. (8), caerulein amounts not inducing pancreatitis (0.1 µg·kg⁻¹·h⁻¹ for 12 h) were further found to have no effect on HSP70 expression in the pancreas (19).

However, a thorough investigation of caerulein-mediated effects on pancreatic HSP mRNA and protein levels has so far not been performed. Therefore, we have now investigated the effects of submaximal and supramaximal stimulation with caerulein on expression of mRNA and protein levels of rat pancreatic HSPs without prior preconditioning through hyperthermia. Interestingly, although caerulein markedly increased pancreatic mRNA levels of the constitutive isoform of HSP70 (HSC70), in contrast to hyperthermia preconditioning, protein levels of HSC70 were actually reduced after caerulein. HSP60 protein levels were also reduced by caerulein. Also, in contrast to hyperthermia, caerulein treatment did not appear to induce expression of either mRNA or protein of the inducible isoform of HSP70. Thus the pancreas apparently responds to different kinds of stress (hyperstimulation vs. hyperthermia) with expression of different subsets of HSPs. It also appears that the attempted increase of HSC mRNA expression does not translate into an appropri-
ate increase of HSC protein levels. Thus, in addition to
the positive correlation between hyperthermia-induced
HSP expression and protection against caerulein pan-
creatitis (17), the inverse correlation of caerulein dos-
age to pancreatic HSP protein levels provides addi-
tional evidence for a possible causal relation between
pancreatic HSP levels and the development of cae-
rulein pancreatitis.

MATERIALS AND METHODS

Chemicals and antibodies. Mouse monoclonal antibodies
against HSP70 and HSP60 were from Sigma Chemical (St.
Louis, MO). Isoform-specific antibodies against inducible
HSP70 and HSC70 were from StressGen (Victoria, BC,
Canada). Detection reagents for enhanced chemilumines-
cence (ECL) and horseradish peroxidase-coupled anti-mouse
and anti-rabbit antibodies were from Amersham (Arlington
Heights, IL). Sodium dodecyl sulfate (SDS), polyacrylamide,
and molecular weight standards were from Bio-Rad (Herc-
ules, CA). Nitrocellulose membranes were from Schleicher
and Schuell (Keene, NH). All other chemicals were from Sigma
Chemical.

Induction of pancreatitis. Pancreatic homogenates used for
Western analysis of pancreatic HSP expression came from
rats treated with a total of 80 µg/kg caerulein given in two
divided intravenous injections 30 min apart. For all other
experiments conducted to assess the time course and dose
response of caerulein hyperstimulation-mediated effects on
mRNA and protein expression of pancreatic HSPs, edematous
pancreatitis was induced by intraperitoneal application of
caeerulein five times at hourly intervals, according to our
previously published protocol (17). For time course studies,
animals received 10 µg·kg⁻¹·h⁻¹ caerulein and were then
killed at the indicated times (0–96 h), after the last inapcri-
teronal injection of caerulein. Pancreata were then quickly
removed, and 100-mg tissue aliquots were immediately fro-
zen in liquid nitrogen and stored at −80°C for mRNA
preparation. Some of the pancreatic tissue was homogenized
in 0.9% saline and stored at −80°C for further processing and
Western analysis. The remainder was used for histological
evaluation. Blood samples were also kept for analysis of
lipase and amylase levels. For each group, three control rats
were killed after the last intraperitoneal injection of caerulein.

Preparation of RNA. RNA from whole pancreas was pre-
pared according to the method of Chomczynski and Sacchi (2)
as previously described (16), with the following modifications.
We pulverized 100 mg of frozen pancreatic tissue in liquid
nitrogen. The pulverized tissue was homogenized on ice using
an Ultra Turrax homogenizer (Tekmar Instruments, Cincinn-
ati, OH) at full speed for 40 s in a buffer containing 4 M
guanidinium thiocyanate and 0.1 M 2-mercaptoethanol. After
addition of 25 mM sodium citrate, pH 4.0, and 0.5% sarcosyl,
a phenol-chloroform extraction was performed after centri-
fugation at 10,000 g for 30 min at 4°C. After an overnight
precipitation of the upper aqueous phase with isopropanol,
RNA was recovered by centrifugation at 10,000 g for 30 min at
4°C. The resulting RNA pellet was washed twice with ethanol,
dissolved in Tris-EDTA buffer, pH 8.0, and stored at
−80°C. Quality of RNA was confirmed by running samples
with size standards on agarose gels and staining with ethidium
bromide. Sharp 28S and 18S ribosomal bands were taken as
indicators for intact RNA. Total RNA was quantified spectro-
photometrically.

Northern transfer and probing with cDNA. For Northern
analysis, 50 µg of RNA were electrophoresed on 1% agarose
gels containing 1.8% formaldehyde at 42°C in 0.1 ml/cm² hybridization buffer [30% formamide, 1
M EDTA, 70 µg/ml phosphate buffer (740
M Na₂HPO₄ + 260 mM NaH₂PO₄) pH 7.2] containing 10
µg/ml bovine serum albumin and 100 µg/ml denatured salmon
testes DNA. We then added 50 ng α-²P-labeled cDNA probe
(specific activity 5 × 10⁶ cpm/µg DNA), and hybridization
continued overnight. Probes were labeled using random
hexamer primers. Membranes were then washed sequen-
tially with increasing stringency up to a final concentration of
0.5× SPE (1× SPE is 0.18 M NaCl, 0.01 M sodium phos-
phate, 1 mM EDTA) containing 0.1% SDS at 68°C for 20 min.
Membranes were exposed to X-ray films (X-Omat, Kodak,
Rochester, NY) at −80°C for 24–36 h using intensifying
screens. The probes used were specific for the inducible
isoform of HSP70 and HSC70 in the pancreas, as previously
shown (8, 17). For HSC70, nucleotides 66–1,360 of the cDNA,
as described by Bautz et al. (1), denoted in PUC12, were
generously provided by G. Hensel and cut out for labeling and
hybridization with EcoR I. For the inducible isoform, nucleo-
tides 2,165–3,292 of the cDNA, as described by Lowe and
Moran (14), denoted in PUC18, were also generously provided
by G. Hensel and cut out for hybridization with EcoR I and
BamHI I. Equal loading of total RNA was assessed by ethidium
bromide staining and hybridization with 28S rRNA.

Light microscopy and histological evaluation. For histo-
logical evaluation, freshly removed pancreata were Formalin
(4%) fixed, ethanol dehydrated, and embedded in paraffin.
Six-micrometer slices were then stained with hematoxylin
and eosin and subjected to conventional light microscopy.
Caerulein-induced pancreatic organ damage was assessed by
two independent pathologists, who were blinded to the exper-
imental protocol as previously described (17).

Densitometry and quantitative analysis of HSP expression.
Densitometry of pancreatic HSP expression was performed as
described (6, 17). To normalize for differences in efficiency of
the batch of antibodies used and the Western or Northern
transfer, as well as time of exposure for Western or Northern analysis, the appropriate control samples were included in each gel. The signals obtained through Western analysis and autoradiography were then expressed as the percentage increase in density compared with controls not exposed to hyperthermia.

Statistical analysis. Densitometrical data are presented as means ± SE and were obtained from at least three animals for each data point. Significance (P < 0.05) was evaluated by unpaired Student’s t-test.

RESULTS

Intraperitoneal administration of 80 µg/kg caerulein reduces rat pancreatic HSP60 and HSP70 protein levels. We have recently reported that preconditioning though hyperthermia can induce both pancreatic expression of HSPs as well as protection against caerulein pancreatitis (17). Because stimulation with supramaximal amounts of secretagogues had previously been reported to increase pancreatic HSP expression (19), we have thus far not thoroughly investigated the effects of caerulein treatment alone, without prior hyperthermia, on expression of pancreatic HSPs. However, when assessing pancreatic HSP expression in pancreatic tissue homogenates provided by another group (F. Fiedler), we found, in contrast to our expectations, that caerulein treatment did not increase but instead strongly decreased pancreatic protein levels of HSC70, as well as of HSP60 (Fig. 1, A and C). The investigated samples were obtained in the course of a study that was performed independently from our own investigations and designed to investigate the effects of pre-conditioning with high-dose caerulein treatment on survival after taurocholate-induced hemorrhagic pancreatitis (unpublished results). The samples we investigated for expression of HSPs (Fig. 1) came from rats treated intraperitoneally with 80 µg/kg to induce pancreatitis and represent controls not treated with taurocholate. Although the reduction of amylase protein levels during caerulein pancreatitis (Fig. 1B) corresponds well to earlier reports (9), the apparently pronounced decrease in pancreatic HSC70 and HSP60 protein levels (Fig. 1, A and C) came as a surprise. Although preliminary, the data also indicated a time-dependent recovery of HSP levels in the pancreas. Nonspecific degradation of the proteins investigated through Western analysis, for example through activation of pancreatic peptidases during caerulein pancreatitis, appeared unlikely since Coomassie blue stains of Western transfers did not reveal significant differences in the protein patterns of pancreatic homogenates from control animals compared with animals treated with 80 µg/kg caerulein (data not shown). Therefore, we decided to investigate whether this effect could also be seen in our own experimental settings (see MATERIALS AND METHODS and Ref. 17).

Supramaximal amounts of caerulein, inducing pancreatitis, reduce pancreatic HSP protein levels dose dependently. We first investigated the effects of different amounts of caerulein given five times intraperitoneally at hourly intervals on pancreatic HSP protein expression. When animals were killed after the last caerulein injection (time 0), a pronounced reduction of pancreatic HSP protein levels was apparent, thus confirming that high-dose caerulein treatment can reduce pancreatic HSP protein levels (Fig. 2, bottom, and Fig. 4A). All gels included three controls treated with NaCl five times intraperitoneally to account for differences in protein loading, Western transfer efficiency, amount of antibody added, and quality of different batches of antibody used (Fig. 2). No difference in protein levels of pancreatic proteins was found comparing treatment with 0.9% NaCl administered five times intraperitoneally and no treatment at all (data not shown). Interestingly, submaximal amounts of caerulein (0.1 µg/kg) failed to reduce pancreatic HSP levels (Fig. 2, top). Instead, there seemed to be a tendency toward increased levels of pancreatic HSC70 and HSP60 protein after low-dose caerulein treatment, which was, however, not statistically significant (Figs. 4A and 9A). Low-dose caerulein also failed to induce pancreatitis, with no apparent histological changes compared with controls and normal serum amylase and lipase levels (data not shown). Although Fig. 2 shows representative Western blots after treatment with 0.1 (Fig. 2, top) and 25 µg/kg (Fig. 2, bottom) caerulein, reduction of pancreatic HSC70 and HSP60 protein was also apparent at
5 and 10 μg/kg (Figs. 4A and 9A). It is important to note that these amounts of caerulein when given five times intraperitoneally at hourly intervals also induced edematous pancreatitis, as judged from both histological alterations as well as increased serum amylase and lipase levels (data not shown). The antibody, recognizing all isoforms of HSP70 (Fig. 2B), revealed similar effects of caerulein on HSP expression compared with the isoform-specific anti-HSC antibody (compare Fig. 2B with Fig. 2A). The former visualizes at least two bands, but one-dimensional analysis does not allow distinction between HSC70 and the inducible isoform of HSP70, which both run at 72 kDa (17). The lower, 70-kDa band likely represents another unidentified isoform of HSP70 (17). Therefore, among pancreatic HSP70 isoforms, only HSC70 expression was densitometrically quantified (Figs. 4 and 7). Pancreatic amylose protein levels were also dose dependently affected (not shown), similar to the effects seen with 80 μg/kg iv caerulein (Fig. 1B). The strongest reduction of protein expression was observed for HSP60 (Fig. 2C, bottom, and Fig. 9B), again similar to the data shown in Fig. 1. Generally, the data on the dose-response analysis of the effects of caerulein on pancreatic protein expression as shown in Figs. 2, 3, 4A, and 9B represent the effects observed directly after the last injection of caerulein (time 0). To further confirm our observations, the same dose-response analysis was also carried out 12 h after the last caerulein injection, showing almost identical effects (not shown). The 12-h time point was chosen because the time-course analysis using 10 μg·kg⁻¹·h⁻¹ caerulein, as shown in Figs. 5, 6, 7, and 9A, revealed that pancreatic HSP levels were still reduced at that time point.

Caerulein dose dependently increases pancreatic HSC70 mRNA levels. In contrast to the effects of caerulein on HSP protein levels, pancreatic HSC mRNA levels were dose dependently increased after caerulein treatment (Figs. 3 and 4B). Pancreatic HSC70 mRNA levels were assessed by Northern analysis (Fig. 3) from the same animals also used for Western analysis of HSP protein levels (Figs. 2 and 4A). This revealed a striking difference between caerulein-mediated increase of HSC70 mRNA (up to 8-fold compared with untreated controls) and the observed decrease of HSC70 protein of up to 50% (Fig. 4). Without pancreatitis, after 0.1 μg/kg caerulein, both pancreatic HSC70 mRNA and protein levels appeared to follow the same pattern, showing a tendency toward increased expression. Concomitant with the induction of pancreatitis, the divergent effects of caerulein hyperstimulation on pancreatic HSP mRNA and protein levels became apparent. Again, effects of caerulein on pancreatic HSP mRNA levels assessed directly after the last injection (time 0) (Figs. 3 and 4B) were almost identical to effects observed 12 h later (time 12), with the exception that

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**Fig. 2. Western analysis reveals dose-dependent reduction of pancreatic HSP protein levels.** For investigation of caerulein effects on pancreatic HSP expression in our own experimental settings (see MATERIALS AND METHODS), pancreatic homogenates were subjected to Western analysis using a variety of antibodies. Shown here are representative Western blots using anti-HSC antibody (A), an antibody recognizing all isoforms of HSP70 (B), and an anti-HSP60 antibody (C). Directly after the last caerulein injection (time 0), 5 μg of pancreatic protein were subjected to SDS-polyacrylamide gel electrophoresis and Western blot analysis. Membranes were then probed with indicated antibodies, and visualization was performed by enhanced chemiluminescence. Top: samples from rats treated with a submaximal caerulein dose (0.1 μg·kg⁻¹·h⁻¹) not inducing pancreatitis. Bottom: tissue homogenates from rats treated with 25 μg·kg⁻¹·h⁻¹. For densitometry and complete dose-response data, see Figs. 4A and 9B.
mRNA levels were then maximally five- to sixfold increased after 25 µg/kg caerulein compared with eight- to tenfold at time 0 (not shown).

Time course of caerulein hyperstimulation-mediated effects on pancreatic HSC70 protein and mRNA levels. Because we had so far found a reduction of pancreatic HSP protein levels in samples from two independent experimental settings (Figs. 1 and 4), we also wanted to investigate the time course of caerulein-mediated effects on pancreatic HSP mRNA and protein expression.

mRNA levels were then maximally five- to sixfold increased after 25 µg/kg caerulein compared with eight- to tenfold at time 0 (not shown).

Fig. 3. In contrast to effects on protein levels, caerulein dose dependently increases HSC70 mRNA levels. Top: mRNA levels of pancreatic HSC directly after intraperitoneal treatment with saline (NaCl) or increasing amounts of caerulein (0.1–25 µg/kg). Caerulein moderately increased HSC mRNA two- to threefold already at 0.1 µg/kg caerulein, which does not induce pancreatitis. With increasing amounts of caerulein and development of pancreatitis, HSC mRNA levels are more strongly (8- to 10-fold) increased (see also Fig. 4B). Bottom: same blot rehybridized with 28S mRNA as a loading control. Data represent 3 experiments.

Again, 10 µg·kg⁻¹·h⁻¹ caerulein reduced pancreatic HSC70 levels to 50%, lasting for up to 24 h. HSC protein levels then started to normalize, reaching 75% of controls at 48 h and 100% at 96 h (Figs. 5 and 7A). In contrast, HSC70 mRNA levels were strongly increased (8- to 10-fold) directly after induction of pancreatitis (Figs. 6 and 7B). Over time, HSC70 mRNA levels declined but remained elevated two to threefold above basal at 6 h, mRNA then resurfaced to fivefold elevation at 12 and 24 h followed by a return to the low basal levels at 96 h after caerulein treatment. This increase of HSC70 message at 12 and 24 h after the last injection of caerulein was observed in all animals (n = 3, P, 0.05). Apparently, in the resting pancreas, very low levels of HSC70 mRNA, which are barely detectable (Figs. 3 and 6, NaCl-treated controls), go along with significant amounts of HSC70 protein. During pancreatitis, HSC70 gene transcription seems strongly acti-
In contrast to hyperthermia preconditioning, caerulein fails to induce mRNA or protein expression of the inducible isoform of HSP70. Because we had previously shown that hyperthermia leads to induction of the inducible isoform of HSP70, not expressed in the resting pancreas (17), we also investigated the effects of caerulein on expression of this HSP70 isoform. However, submaximal amounts of caerulein (0.1 µg·kg⁻¹·h⁻¹) did not reduce HSP60 protein but rather revealed a tendency for increased expression of HSP60 (Fig. 9A). However, maximal reduction of pancreatic HSP60 protein was slower in onset, occurring at 24 h for HSP60 compared with 0 h for HSC70 after induction of pancreatitis (compare Fig. 9A with Fig. 7A), but of greater magnitude (25% of basal vs. 50% of basal). Complete recovery of HSP60 to basal levels also appeared to be prolonged and did not occur within 96 h (Fig. 9A) but was evident 7 days after caerulein treatment (Fig. 1C).

At present, we can only speculate on HSP60 mRNA levels in the pancreas after caerulein treatment, since for all experimental conditions we were unable to obtain a detectable HSP60 signal in our Northern blots using a commercially available cDNA probe (Stress-Gen).

DISCUSSION

In our study, we have investigated the time course and dose response of caerulein on pancreatic HSP protein and mRNA levels. Caerulein dose dependently increased HSC70 mRNA levels, producing a detectable twofold increase of HSC70 message already at submaximal amounts (0.1 µg·kg⁻¹·h⁻¹ for 5 h) and a further dose-dependent increase to a maximum of eight- to tenfold compared with basal after 25 µg·kg⁻¹·h⁻¹ caerulein for 5 h.

Interestingly, we did not observe an increase in pancreatic expression of HSC70 protein despite the strong induction of HSC70 mRNA levels after caerulein hyperstimulation. Instead, caerulein led to a 50% reduction of HSC70 protein levels. In addition, HSP60...
protein levels were also reduced after caerulein hyperstimulation, whereas submaximal amounts of caerulein had no such effect. Apart from investigating the dose-response relationship between pancreatic HSP mRNA and protein levels, we also studied the time course after caerulein hyperstimulation and induction of pancreatitis. Here, we observed that HSC70 mRNA levels showed a biphasic response. Whereas induction of pancreatic HSC70 mRNA was maximal directly after induction of pancreatitis and then declined to two- to threefold above basal levels, mRNA levels resurfaced again, exhibiting a second peak at 12 and 24 h with fivefold increased HSC70 message compared with controls. Again, HSC70 protein levels during caerulein pancreatitis were reduced despite elevated HSC70 mRNA. Whether this is due to decreased mRNA stability or reduced translation efficiency, for example through a block of the protein translation machinery during caerulein pancreatitis, is currently unknown. From our data it nevertheless appears that the pancreas tries to respond to hyperstimulation stress with increased expression of HSC70. The strong increase of HSC mRNA depending on the amount of caerulein given might also explain why the reduction of HSC70 protein is not as clearly dose dependent as is the increase in HSC70 mRNA (Fig. 4). It may be that stronger increases of HSC70 mRNA at 25 µg/kg caerulein administered five times partially overcome translational insufficiency, avoiding a further reduction of HSC70 protein levels after 25 µg/kg caerulein administered five times compared with 5 and 10 µg/kg caerulein, each administered five times. Possibly, the stronger increase in HSC70 mRNA could thus prevent an even further reduction of HSC70 protein levels. Only 12–24 h after caerulein treatment, when pancreatitis, judged from serum amylase and lipase levels as well as from histological appearance (data not shown), started to resolve, the now fivefold increased HSC70 mRNA levels then appeared to enable the pancreas to synthesize more HSC70, allowing HSC70 protein levels to resurge. Again, whether this represents increased mRNA stability or recovery of the acinar translational machinery function is unknown.

With respect to our observation that caerulein hyperstimulation reduces HSP protein levels in the pancreas, our data differ from a previously published study (18). In that study (18), caerulein hyperstimulation in vitro increased HSP70 mRNA but not protein levels, whereas hyperstimulation in vivo (10 µg·kg⁻¹·h⁻¹ iv for 4–12 h) appeared to increase HSP70 mRNA and protein levels. The reasons for this discrepancy are not totally clear, particularly since the reported increase of HSP70 mRNA after caerulein hyperstimulation was very similar to our own observation. We feel that...
the slight differences in experimental protocol (intravenous vs. intraperitoneal caerulein) and animal species (Sprague-Dawley vs. Wistar rats) are unlikely to fully account for this discrepancy. However, we believe that our observation that caerulein hyperstimulation reduces HSP70 protein levels is very well substantiated for the following reasons.

First, the reduction of pancreatic HSP protein levels after caerulein hyperstimulation was seen not only in pancreatic tissue obtained after we induced caerulein pancreatitis (Figs. 4 and 9) but was similarly apparent when investigating pancreatic homogenates from an independent group treated with caerulein to induce pancreatitis (Fig. 1). Taken together, we have thus assessed HSP70 protein levels in pancreatic tissue after caerulein hyperstimulation of over 50 animals and have never observed an increase in HSP protein levels.

Second, regarding caerulein treatment not inducing pancreatitis, with respect to caerulein having no effect on the inducible isofrom of HSP 70 and to the biphasic response of HSC70 mRNA to caerulein, our results are in excellent accordance with data from a recent study (8) that reported a threefold increase of pancreatic HSC mRNA after 0.25 μg·kg⁻¹·h⁻¹ iv caerulein for 12 h. This again differs from the above-mentioned study (19), which did not report a similar effect by submaximal caerulein (0.1 mg·kg⁻¹·h⁻¹ iv, for 12 h) on pancreatic HSP70 mRNA or protein and did not separately investigate different isoforms of HSP70 at all. This indicates that our assay systems may reflect pancreatic HSP70 mRNA and protein levels more appropriately.

Third, nonspecific protein degradation, for example, through nonspecific activation of digestive enzymes within acinar cells, during induction of pancreatitis appears unlikely since protein band patterns visualized by Coomassie blue did not differ between controls and caerulein-treated animals (not shown). Moreover, if intracellular activation of digestive enzymes was strong enough to digest pancreatic proteins, one would expect that pancreatic mRNA, through intracellular activation of pancreatic ribonuclease concomitantly with activation of other digestive enzymes such as proteases, might also be significantly degraded after induction of caerulein pancreatitis. mRNA prepared from edematous pancreata did, however, not appear to be significantly degraded (see Figs. 3 and 6, bottom, 28S RNA loading controls).

HSP60 protein levels were also strongly reduced after induction of caerulein pancreatitis. Here, reduction was somewhat slower in onset, reaching minimal levels only 24 h after induction of pancreatitis, but was even more pronounced compared with HSC70. In addition, HSP60 protein levels needed more time to fully recover to basal levels. In contrast to HSC70, we were unable to obtain a measurable signal of HSP60 mRNA. We therefore assume that basal levels of pancreatic HSP60 mRNA are very low, similar to HSC70 mRNA levels, and might be below the sensitivity threshold of our Northern analysis. Therefore, no definite conclusions about its regulation through caerulein can be made. However, whereas hyperthermia weakly increases pancreatic HSP60 protein expression (17), caerulein treatment does not. Differential regulation of individual subsets of HSPs according to different kinds of stress has previously been found in the pancreas and in other systems (4, 10, 12, 21). In the pancreas, induction of mRNA expression of the inducible isofrom of HSP70 through hyperthermia but not through submaximal caerulein stimulation has thus been reported (8).

Although speculative, it seems possible that the failure to appropriately increase HSP protein levels in response to hyperstimulation stress might be a factor for the development of edematous pancreatitis in our system since the reduction in pancreatic HSC70 and HSP60 protein levels only occurred when caerulein amounts were sufficient to induce pancreatitis. Furthermore, hyperthermia preventing caerulein-mediated pancreatitis induces both mRNA and protein expression of HSC70 as well as the inducible isofrom of HSP70 (17). Taken together, our study provides further evidence for a causal relationship between the hyperthermia-induced increase of pancreatic protein levels and the hyperthermia-induced protection against caerulein-mediated pancreatitis as reported previously (17) and opens up the possibility that the low levels of pancreatic HSPs observed only after induction of caerulein pancreatitis might be involved in the development of pancreatitis in our system.

In conclusion, we investigated time course and dose response of mRNA and protein levels of pancreatic HSPs, demonstrating induction of different subsets of pancreatic HSPs by different kinds of stress. We also found that during caerulein pancreatitis, increased HSC70 mRNA levels are uncoupled from protein synthesis and that HSP protein levels in the pancreas are in fact reduced during caerulein-induced pancreatitis.

We feel that further research investigating the signal transduction pathway underlying caerulein, as well as heat-shock-mediated induction of different HSP mRNA species in the pancreas, is needed and might aid our understanding of the molecular mechanisms underlying caerulein-mediated pancreatitis.

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REFERENCES
