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Regulation of translation elongation and phosphorylation of eEF2 in rat pancreatic acini

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Abstract

While pancreatic protein synthesis and the initiation of translation are regulated by hormones and neurotransmiters, whether the elongation process is also regulated is unknown. Stimulatory doses of cholecystokinin (CCK) (100 pM), bombesin (10 nM), and carbachol (10 μM) increased elongation rates (measured as ribosomal half-transit time) in pancreatic acini in vitro. At the same time these secretagogues reduced elongation factor 2 (eEF2) phosphorylation, the main factor known to regulate elongation, and increased the phosphorylation of the eEF2 kinase. The mTOR inhibitor rapamycin reversed the dephosphorylation of eEF2 induced by CCK, as did treatment with the p38 MAPK inhibitor SB202190, the MEK inhibitor PD98059, and the phosphatase inhibitor calyculin A. Neither rapamycin, SB202190, PD98059 nor calyculin A had an effect on CCK mediated eEF2 kinase phosphorylation. Translation elongation in pancreatic acinar cells is likely regulated by eEF2 through the mTOR, p38, and MEK pathways, and modulated through PP2A.

Keywords: Cholecystokinin; Ribosomal half-transit times; Rapamycin; PP2A; eEF2 kinase

The protein translation elongation process defines the rate at which amino acids from amino acyl-tRNAs are added sequentially to the growing peptide in the order dictated by the mRNA bound to the ribosome. To coordinate overall protein synthesis, the elongation rate needs to increase/decrease with changes in initiation rates [1]. However, elongation of peptide chains can also be regulated independently of translation initiation in order to avoid missense errors or premature termination due to high levels of elongation [2]. Translation initiation is highly regulated and has been more extensively studied in different cell types [3–5], including pancreatic acinar cells [6–10], than the translation elongation process [2,11].

The process of peptide-chain elongation requires elongation factors and these factors are regulated via phosphorylation/dephosphorylation events in response to different stimuli [2]. Eukaryotic elongation factor 2 (eEF2) mediates the translocation step in which the ribosome moves along the mRNA by the equivalent of one codon during translation [11]. eEF2 belongs to the superfamily of GTP hydrolases and when phosphorylated on Thr-56 reduces its activity and its binding to ribosomes [2]. Different stimuli, usually associated with cellular stress, including increases in cytoplasmic calcium levels [12], nutrient and amino acid withdrawal [13], depletion of cellular ATP [14], and increase of cAMP [15] and AMP levels [16], increase eEF2 phosphorylation. Mitogenic and hormonal stimuli that increase elongation rates can induce eEF2 dephosphorylation through activation of the mTOR pathway [11,17] and associated phosphatases like PP2A [11]. Many of these eEF2 phosphorylation/dephosphorylation events are also dependent on the activity of eEF2 kinase, a calcium/calmodulin (CaM)-dependent enzyme that was formerly called Ca/CaM-kinase III [18], and has eEF2 as its only known substrate [11]. The regulation of eEF2 kinase is primarily based on the phosphorylation status of several of its serine residues. Increases of intracellular calcium [2,11], activation of the cAMP-dependent protein kinase [19] or activation of the

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AMP-activated protein kinase [16,20] will activate eEF2 kinase. Signaling events dependent upon activation of mTOR [2], MEK/ERK pathway [21], and SAPK/p38 MAP kinases [22,23] will phosphorylate eEF2 kinase in different serine residues and inhibit its activity [2].

Recent studies have begun to elucidate the signaling pathways regulating translational control in the pancreas. Cholecystokinin (CCK), a major gastrointestinal hormone regulator of exocrine pancreatic function [24], activates the assembly of the initiation-factor complex eIF4F [6,25,26] and the activity of the 70 kDa ribosomal protein S6 kinase (p70 S6K or S6K) [7]. However, it is not known whether elongation and elongation factors can be also regulated in pancreatic acinar cells. In the present study, therefore, we first demonstrate that CCK and other pancreatic secretagogues can stimulate peptide elongation and regulate elongation factor (eEF) 2 phosphorylation. Second, we characterize the different pathways that can be involved in the phosphorylation of eEF2 and its kinase eEF2K by CCK stimulation in isolated pancreatic acini.

Experimental procedures

Preparation of pancreatic acini. Pancreatic acini were prepared by collagenase (Crescent Chemical; Islandia, NY) digestion of pancreas from 125 to 150 g male Sprague–Dawley rats [7,27]. Acini were suspended in incubation buffer, consisting of a *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid-buffered Ringer (HR) solution supplemented with 11.1 mM glucose, Eagle's minimal essential amino acids (GIBCO; Grand Island, NY), 0.1 mg/ml soybean trypsin inhibitor (SBTI) (Sigma Chemical; St. Louis, MO), and 1 mg/ml BSA and equilibrated with 100% O_2. In some experiments, acini were pre-incubated 1 h with the inhibitors rapamycin, SB202190, PD98059, and calyculin A (all from Calbiochem–Novabiochem; San Diego, CA) at the specified concentrations.

Elongation rate measurements. Elongation rate measurements in pancreatic acinar cells were performed following an adaptation of the protocol used by Kimball and Jefferson [28] and Fan and Penman [29]. Briefly, after pre-incubation, acini were incubated with either sulfated cholecystokinin octapeptide (CCK) (Research Plus; Bayonne, NJ), bombesin (Bachem; Torrance, CA), carbamylcholine chloride (carbachol) (Sigma Chemical; St. Louis, MO) or vasoactive intestinal peptide (VIP) (Bachem; Torrance, CA) for 45 min at 37 °C in a buffer containing leucine at the normal rat plasma concentration (160 μM); other amino acids were present at 10 times their normal rat plasma concentrations [30]. [^{3}H]Leucine (Perkin–Elmer Life & Analytical Sciences; Boston, MA) (173 Ci/mmol) was added to the acinar solution and 2 ml aliquots of acini were taken after 6, 8, 10, and 12 min. Samples were homogenized at 4 °C in 50 mM Tris–HCl buffer, pH 7.6, containing cycloheximide (10 μg/ml), Triton X-100 (1%, vol/vol), and sodium deoxycholate (1% wt/vol). The homogenate was maintained at 4 °C and centrifuged at 12,000 rpm for 10 min in an Eppendorf F-45-30-11 rotor to obtain the post-mitochondrial supernatant (PMS) and then at 90,000 rpm for 2 h in a Beckman TLA 100.4 rotor to obtain the post-ribosomal supernatant (PRS). Thirty microliters of aliquots of PMS and PRS samples was spotted on to Whatman filter papers # 540 (Whatman International; Kent, UK) and the filters were placed in ice-cold 10% TCA + leucine (10×) for 10 min, washed with ice-cold 5% TCA, boiled in 5% TCA, and washed again with 5% TCA. The filters were then rinsed once in ethanol and once in ether and air-dried before placing them in scintillation vials. Proteins were solubilized from the filters with Solvable (Perkin–Elmer Life & Analytical Sciences; Boston, MA) and their radioactivity was determined by liquid scintillation counting. Radioactivity incorporated in the PMS (which would include the counts in mature and nascent proteins) and PRS (which would include only the counts in mature proteins) as a function of time was determined as described previously [28]. These data were used to obtain ribosomal half-transit times according to the method of Fan and Penman [29]. The ribosomal half-transit time is an average measurement of the length of time required for a ribosome, after it becomes attached to an mRNA, to complete translation and release a finished polypeptide, and it is determined from the displacement in time between the two lines corresponding to the PMS and PRS data plotted as a function of time. In fact, polyribosomes of many sizes contribute to such a measurement and the measured transit time is the weighted-average of different transit times. This parameter is used to measure elongation rates; when elongation is activated, the ribosomal half-transit time decreases.

Evaluation of the phosphorylation state of the elongation factor 2 (eEF2) and its associated kinase (eEF2K). The phosphorylation state of these proteins was determined by the relative amount of protein in the phosphorylated form, quantified by protein immunoblot analysis using affinity-purified antibodies that specifically recognize the phosphorylated forms of eEF2 at Thr-56 and eEF2K at Ser-366 (Cell Signaling; Beverly, MA). To ensure equal loading, the same membranes were stripped and reproved for the total amount of the proteins, using polyclonal antibodies diluted 1:1000 for both, eEF2 and eEF2K (Cell Signaling; Beverly, MA).

Results

CCK stimulates peptide elongation

Previous studies have demonstrated that CCK maximally stimulates total protein synthesis at 100 pM in rat pancreatic acini in vitro [6,9]. In order to determine whether CCK also stimulates translation elongation, we determined translation elongation rates by measuring the ribosomal half-transit time. As explained in Experimental procedures, we measured the incorporation of [^{3}H]leucine into proteins of the post-mitochondrial supernatant (PMS), and the post-ribosomal supernatant (PRS) at different time points (Fig. 1). In basal acini the separation between the incorporation into the PMS and PRS (the ribosomal half-transit time) was 3.1 ± 0.6 min (Figs. 1A and C), that would correspond to a 6.2 min average transit time. In 100 pM CCK-stimulated acini the ribosomal half-transit time was reduced, compared to basal acini, and the separation between PMS and PRS was only of 1.2 ± 0.3 min (Figs. 1B and C); corresponding to an average transit time of 2.4 min. Thus, 100 pM CCK stimulates the translation elongation process in rat pancreatic acini, by reducing the ribosomal half-transit time to $37.8 \pm 7.8\%$ of basal.

CCK inhibits eEF2 phosphorylation in a dose-dependent manner

After establishing that CCK was in fact stimulating protein elongation in acinar cells, we studied whether

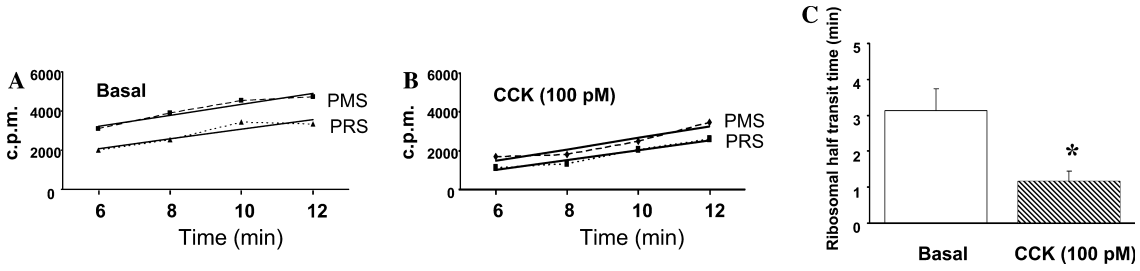

Fig. 1. Representation of the radioactivity incorporated in the post-mitochondrial supernatant (PMS) and the post-ribosomal supernatant (PRS) as a function of time to obtain the ribosomal half-transit time, as described in Experimental procedures. (A,B) Representative plots of results from basal and stimulated acini with the cholecystokinin octapeptide (CCK) at 100 pM, respectively. (C) Average of the ribosomal half-transit time values ± SE obtained after calculating the separation (in time) between the PMS and PRS for each group from five independent experiments. *$p < 0.05$ vs basal.

CCK had any effect on eEF2, the major regulatory factor of translation elongation. For this the phosphorylation levels of eEF2 on Thr-56, which can be correlated with its activation status, were analyzed at different doses of CCK. The results show that eEF2 was phosphorylated in basal acini (Fig. 2A), and that this phosphorylation was decreased by 10 pM CCK with a maximal decrease in response to CCK concentrations of 100 pM–10 nM (Fig. 2A). The reduction of eEF2 phosphorylation is associated with its activation and is consistent with an increase in peptide chain elongation. To test whether these phosphorylation changes on eEF2 were correlated with changes in its kinase (eEF2K), the phosphorylation levels of eEF2K on Ser-366 at different doses of CCK were evaluated. CCK from 10 pM to 10 nM increased eEF2K phosphorylation (Fig. 2B), which is known to be associated with its inhibition, and, in turn, correlated with the reduced phosphorylation of eEF2 on Thr-56 (Fig. 2A).

Other pancreatic secretagogues stimulate translation elongation and inhibit eEF2 phosphorylation

To test whether other pancreatic secretagogues, at doses that are known to stimulate total protein synthesis [6], would have any effect on translation elongation we measured their effect on elongation rates, and analyzed their effect on the phosphorylation status of eEF2 and eEF2K. The incubation of acini with stimulatory doses of bombesin (a synthetic analog of the gastrin-releasing peptide (GRP) [31,32]), at 10 nM and carbachol (a cholinergic agonists [31]), at 10 μM reduced the ribosomal half-transit time to 43.9 ± 15.0% and 45.5 ± 14.8% of basal, respectively ($n = 4$). These secretagogues also reduced eEF2 phosphorylation comparable to CCK at 100 pM (Fig. 3A). As expected, both secretagogues also increased eEF2K phosphorylation on Ser-366 (Fig. 3B) consistent with inhibition of the kinase activity and reduction of eEF2 phosphorylation. By contrast, the pancreatic secretagogue (VIP), which acts via cAMP, did not increase the elongation rate (data not shown) and did not modify the phosphorylation status of eEF2 or eEF2K (Fig. 3), consistent with a lack of major effect on pancreatic protein synthesis [7].

Regulation of eEF2 phosphorylation by intracellular mediators

Next we analyzed which intracellular mediators were responsible for the secretagogue effects. Acini were incubated with the ionophore A23187 at 2 μM, the synthetic cAMP analog 8-(4-chlorophenylthio)adenosine 3′,5′-cyclic monophosphate (CPT-cAMP) at 100 μM and the phorbol ester PMA at 10 nM. PMA reduced eEF2

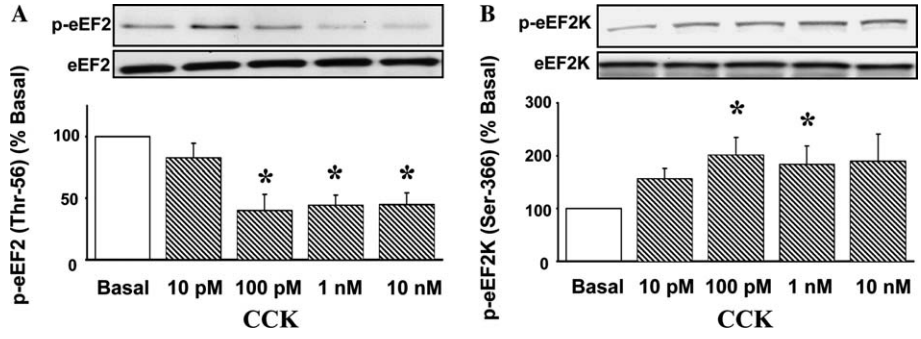

Fig. 2. Effect of CCK on eEF2 phosphorylation (A) and eEF2K phosphorylation (B). Acini were incubated for 60 min with Cholecystokinin octapeptide (CCK) at different concentrations and processed as described under Experimental procedures for eEF2 and eEF2K phosphorylation. In (A,B) the results were expressed as a percentage of basal levels. Each column represents the means ± SE of 4–8 different experiments. *$p < 0.05$ vs basal. Blots are representative of phosphorylated and total eEF2 and eEF2K levels.

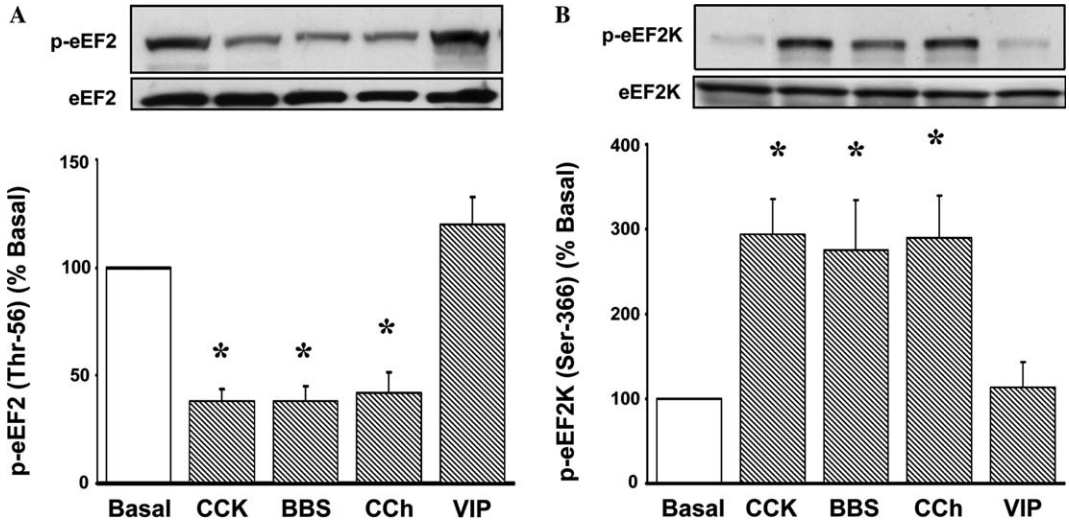

Fig. 3. Effect of stimulatory doses of CCK, bombesin (BBS), carbachol (CCh), and the vasoactive intestinal peptide (VIP) on eEF2 (A) and eEF2K (B) phosphorylation. Acini were incubated for 60 min with CCK at 100 pM, BBS at 10 nM, CCh at 10 μM, and VIP at 100 nM, and processed as described in Experimental procedures. Results are expressed as a percentage of basal levels. Each column represents the means ± SE of 4–8 different experiments. $*p < 0.05$ vs basal. Blots are representative of phosphorylated and total eEF2 and eEF2K levels.

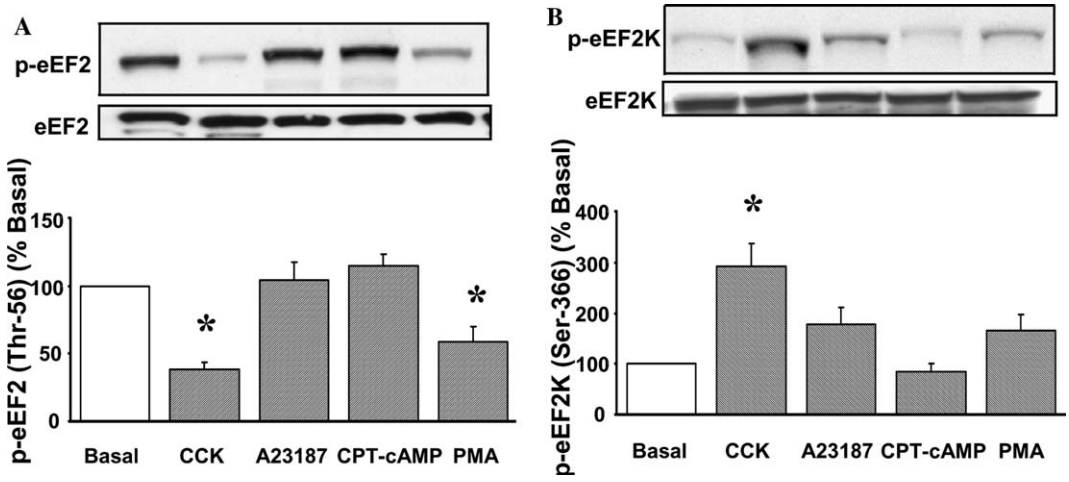

Fig. 4. Effects of different intracellular mediators on eEF2 (A) and eEF2K (B) phosphorylation. Acini were incubated for 60 min with 100 pM CCK, 2 μM A23187, 100 μM CPT-cAMP, and 10 nM phorbol ester analog PMA, and processed as described in Experimental procedures. Results are expressed as a percentage of basal levels. Each column represents the means ± SE of 5–10 different experiments. $*p < 0.05$ vs basal. Blots are representative of phosphorylated and total eEF2 and eEF2K levels.

phosphorylation (Fig. 4A). Surprisingly, the ionophore A23187 did not modify the phosphorylation status of eEF2, compared to basal acini (Fig. 4A). CPT-cAMP did not have any effect on the phosphorylation of eEF2 (Fig. 4A), implying that cAMP is not involved in the regulation of eEF2. None of the intracellular mediators had any effect on the phosphorylation status of eEF2K on Ser-366 (Fig. 4B).

eEF2 phosphorylation is regulated through different intracellular pathways

To further characterize the phosphorylation of eEF2 in acinar cells, we analyzed the effects of different specific inhibitors for the major pathways implicated in the phosphorylation and activation of eEF2 and eEF2K [2,11]. Acini were incubated with the mTOR inhibitor rapamycin (100 nM), the MEK inhibitor PD98059 (50 μM), and the SAPK/p38 inhibitor SB202190 (20 μM); all three partially reversed the dephosphorylation of eEF2 induced by CCK (Fig. 5A). When the effects of these inhibitors on eEF2K phosphorylation at Ser-366 were analyzed, only the inhibitor PD98059 reduced eEF2K phosphorylation, increased by CCK (Fig. 5B). All these results taken together demonstrate that the dephosphorylation of eEF2 on Thr-56 is downstream of the mTOR, SAPK/p38, and MEK pathways but it is only through the MEK pathway that

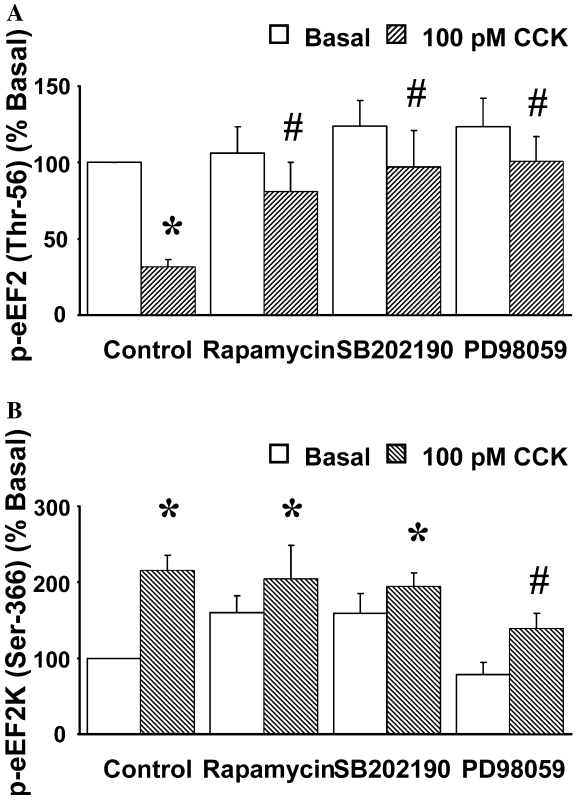

Fig. 5. Effects of different inhibitors on basal and CCK-stimulated eEF2 (A) and eEF2K (B) phosphorylation. Before treatment with 100 pM CCK, acini were pre-incubated for 60 min in the presence/absence of these inhibitors. Rapamycin, the inhibitor of the mTOR pathway, was assayed at 100 nM; SB202190, the inhibitor of the SAPK/p38 MAP kinase pathway at 20 μM, and the inhibitor of the MEK/ERK pathway, PD98059 at 50 μM. Results are expressed as a percentage of basal control levels. Each column represents the means ± SE of 4–10 different experiments. $^{*}p < 0.05$ vs basal control. $^{\#}p < 0.05$ vs CCK-stimulated control.

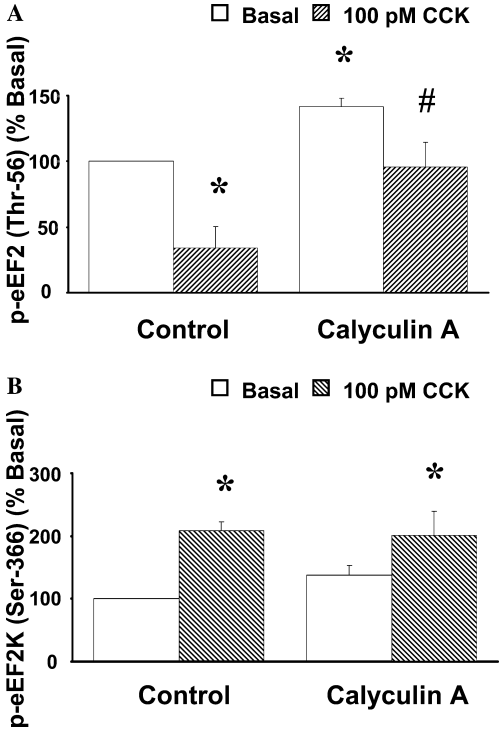

Fig. 6. Effects of the phosphatase inhibitor calyculin A on basal and CCK-stimulated eEF2 (A) and eEF2K (B) phosphorylation. Before treatment with 100 pM CCK, acini were pre-incubated for 60 min in the presence/absence of calyculin A at 100 nM. Results are expressed as a percentage of basal control levels. Each column represents the means ± SE of 5–6 different experiments. $^{*}p < 0.05$ vs basal control. $^{\#}p < 0.05$ vs CCK-stimulated control.

the phosphorylation (and inactivation) of its kinase eEF2K on Ser-366 is involved.

Finally, because the phosphorylation status of eEF2 on Thr-56 can be also modified by the action of a phosphatase [11], we used the phosphatase inhibitor calyculin A (100 nM) to test whether a phosphatase could be activated by CCK in pancreatic acini to reduce the phosphorylation of eEF2 on Thr-56. The results shown in Fig. 6A demonstrate that the CCK-induced eEF2 dephosphorylation is significantly reversed by calyculin A. As expected, this phosphatase inhibitor did not have any effect on the phosphorylation status of the eEF2K on Ser-366 after CCK stimulation (Fig. 6B), although it increased eEF2K phosphorylation in basal acini (Fig. 6B). Thus, the CCK-induced dephosphorylation of eEF2 in pancreatic acinar cells seems to be dependent both on the inhibition of its associated kinase (eEF2K) by increased phosphorylation on Ser-366 and the activation of a phosphatase.

Discussion

In the present study we demonstrated that the pancreatic secretagogues cholecystokinin (CCK), bombesin, and carbachol stimulated translation elongation (measured as a decrease in the ribosomal half-transit times [28,29]) in pancreatic acinar cells, at concentrations that are known to stimulate total pancreatic protein synthesis and translation initiation factors [6,8]. These results are consistent with the hypothesis that all the intermediate regulatory steps (translation initiation, elongation, and termination) need to be coordinately regulated in order to induce an overall increase of protein synthesis [1]. We also demonstrated that the increase in translation elongation rates was correlated with a decrease of elongation factor 2 phosphorylation.

There is a growing evidence that the activities of elongation factors are subjected to acute control by phosphorylation [11]. Thus, we analyzed the phosphorylation levels of eEF2 on Thr-56, and its kinase eEF2K on Ser-366 at different concentrations of CCK and stimulatory doses of bombesin and carbachol. There was a strong correlation between eEF2 dephosphorylation (consistent with its activation) and eEF2K phos-

phorylation (consistent with its inhibition) [1,2,11] from 100 pM to 10 nM CCK (Fig. 2). There was also a correlation between increased elongation rates and decreased eEF2 phosphorylation by different secretagogues. All data were consistent with increased activation of eEF2 and an increase in peptide chain elongation, strongly suggesting a direct regulation of translation elongation through the dephosphorylation of eEF2. All three secretagogues (CCK, bombesin, and carbachol) act through the stimulation of their G-protein coupled receptors with similar intracellular mediators [33] and similar effects at maximal stimulatory doses on pancreatic secretion [27] and protein synthesis [6]. Because of this similarity, common intracellular mediators (increase in intracellular Ca^{2+}, PKC activation, and cAMP formation) that are known to regulate eEF2 and eEF2K phosphorylation [2,11] were used to further characterize the regulation of eEF2 and eEFK in pancreatic acini.

eEF2K is a Ca^{2+}/calmodulin-dependent kinase that becomes autophosphorylated and active in the presence of calcium ions [2,11], thereby, phosphorylating and inactivating eEF2. It is also known that a variety of secretagogues and agents that raise cytoplasmic Ca^{2+} levels induce an increase on eEF2 phosphorylation [11]. We therefore might expect a similar effect of the ionophore A23187 on eEF2 phosphorylation, but our results did not show any significant effect of A23187 on eEF2 or its kinase (Fig. 4). The increase in intracellular Ca^{2+} levels by A23187, that inhibits pancreatic protein synthesis [9] therefore, does not likely regulate either eEF2 or eEF2K phosphorylation. Rather, the inhibitory action of A23187 seems to be mediated through an increase on eIF2α phosphorylation and a decrease in eIF2B activity in acinar cells [9].

Activation of the protein kinase C (PKC) is another intracellular mediator involved in pancreatic secretion [24,34] that has been implicated in the activation (dephosphorylation) of eEF2 [11]. Stimulation of acinar cells with the phorbol ester PMA reduced eEF2 phosphorylation without a significant effect on the phosphorylation of eEF2K, implying that eEF2 was activated and translation elongation increased through a PKC-related mechanism that did not involve the phosphorylation of eEF2K on Ser-366. This contrasts to several other cell types where PKC has been shown to be involved in the phosphorylation of Ser-366 on eEF2K [2].

Stimulation with the vasoactive intestinal peptide (VIP), the predominating neuropeptide in the pancreas that stimulates pancreatic secretion via a pathway involving activation of adenyl cyclase [31,33], had no effect on either eEF2 or eEF2K phosphorylation (Fig. 4). It has also been shown that VIP has no significant effect on the activity of $p70^{S6K}$ (or S6K), another translation regulatory factor, in pancreatic acinar cells [7]. The lack of effects of the cAMP analog CPT-cAMP, an intracellular mediator of the VIP stimulated pathway, on the phosphorylation levels of eEF2 on Thr-56 and eEF2K on Ser-366 confirms that cAMP is not involved in the regulation of the elongation factor 2 phosphorylation in pancreatic acinar cells. These results are in agreement with the lack of stimulatory effect on elongation rates (data not shown) and other translational machinery [7] in acini. However, in some other cell types, cAMP can regulate eEF2 phosphorylation and peptide elongation [15,19,35].

Different pathways have been implicated in the inhibition of the eEF2K through phosphorylation on several of its serine residues, leading to a dephosphorylation (and activation) of eEF2 [23,36,37]. The use of different inhibitors that block specific pathways demonstrated that the mTOR, SAPK/p38, and MEK pathways are partially involved in the eEF2 dephosphorylation induced by 100 pM CCK. However, only the eEF2 dephosphorylation through the MEK pathway involved participation of eEF2K by increased phosphorylation on its Ser-366 residue as has been seen in other cell types [36,37]. This effect could be mediated through activation of the $p90^{RSK}$ which CCK activates in pancreatic acinar cells [38]. Because PKC is upstream of MEK signaling, the effects induced by PMA could also be part of the same signaling pathway. The effects of rapamycin, reversing only the dephosphorylation of eEF2 (without any effect on its kinase) indicate that the mTOR pathway, through $p70^{S6K}$ activation, might be implicated in regulating an eEF2 phosphatase, as it has been previously described in other cell types [11]. A similar analogy could account for the effects through the SAPK/p38 pathway; where the lack of effect on the eEF2 kinase indicates the possibility for phosphatase activation through SAPK/p38.

Finally, because the treatment with the phosphatase inhibitor calyculin A reversed the dephosphorylation induced by 100 pM CCK, there is an indication that PP2A might be involved in this process, as it has been demonstrated in other cell types [11]. As expected, calyculin A does not have any effect on the phosphorylation of eEF2K on Ser-366. The partial effect of calyculin A on the CCK-induced dephosphorylation may be explained by the fact that another phosphatase, calcineurin, can be involved in this process [26].

In conclusion, translation elongation is activated in pancreatic acinar cells by different secretagogues, presumably brought about by the dephosphorylation of eEF2, which is known to be activated by dephosphorylation. CCK-induced dephosphorylation of eEF2 can involve signaling from PKC and the participation of the mTOR, SAPK/p38, and MEK pathways in part through eEF2K.

Acknowledgments

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References

[1] W. Merrick, J. Nyborg, The protein biosynthesis elongation cycle, in: N. Sonenberg, J.W. Hershey, M. Mathews (Eds.), Translational Control of Gene Expression, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 2000, pp. 89–125.

[2] G.J. Browne, C.G. Proud, Regulation of peptide-chain elongation in mammalian cells, Eur. J. Biochem. 269 (2002) 5360–5368.

[3] L.E. Campbell, X. Wang, C.G. Proud, Nutrients differentially regulate multiple translation factors and their control by insulin, Biochem. J. 344 (1999) 433–441.

[4] J.W. Hershey, W.C. Merrick, Pathway and mechanism of initiation of protein synthesis, in: N. Sonenberg, J.W. Hershey, M. Mathews (Eds.), Translational Control of Gene Expression, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 2000, pp. 33–88.

[5] A. Hinnebusch, Translational regulation of yeast GCN4. A window on factors that control initiator-tRNA binding to the ribosome, J. Biol. Chem. 272 (1997) 21661–21664.

[6] M.J. Bragado, G.E. Groblewski, J.A. Williams, Regulation of protein synthesis by cholecystokinin in rat pancreatic acini involves PHAS-I and the p70 S6 kinase pathway, Gastroenterology 115 (1998) 733–742.

[7] M.J. Bragado, G.E. Groblewski, J.A. Williams, p70s6k is activated by CCK in rat pancreatic acini, Am. J. Physiol. 273 (1997) C101–C109.

[8] M.D. Sans, J.A. Williams, Translational control of protein synthesis in pancreatic acinar cells, Int. J. Gastrointest. Cancer 31 (2002) 107–115.

[9] M.D. Sans, S.R. Kimball, J.A. Williams, Effect of CCK and intracellular calcium to regulate eIF2B and protein synthesis in rat pancreatic acinar cells, Am. J. Physiol. Gastrointest. Liver Physiol. 282 (2002) G267–G276.

[10] M.D. Sans, M.J. DiMagno, L.G. D'Alecy, J.A. Williams, Caerulein-induced acute pancreatitis inhibits protein synthesis through effects on eIF2B and eIF4F, Am. J. Physiol. Gastrointest. Liver Physiol. 285 (2003) G517–G528.

[11] C.G. Proud, Control of the elongation phase of protein synthesis, in: N. Sonenberg, J.W. Hershey, M. Mathews (Eds.), Translational Control of Gene Expression, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 2000, pp. 719–739.

[12] A.C. Nairn, M. Matsushita, K. Nastiuk, A. Horiuchi, K. Mitsui, Y. Shimizu, H.C. Palfrey, Elongation factor-2 phosphorylation and the regulation of protein synthesis by calcium, Prog. Mol. Subcell. Biol. 27 (2001) 91–129.

[13] X. Wang, L.E. Campbell, C.M. Miller, C.G. Proud, Amino acid availability regulates p70 S6 kinase and multiple translation factors, Biochem. J. 334 (Pt. 1) (1998) 261–267.

[14] L.E. McLeod, C.G. Proud, ATP depletion increases phosphorylation of elongation factor eEF2 in adult cardiomyocytes independently of inhibition of mTOR signalling, FEBS Lett. 531 (2002) 448–452.

[15] K.B. Gutzkow, H.U. Lahne, S. Naderi, K.M. Torgersen, B. Skalhegg, M. Koketsu, Y. Uehara, H.K. Blomhoff, Cyclic AMP inhibits translation of cyclin D3 in T lymphocytes at the level of elongation by inducing eEF2-phosphorylation, Cell. Signal. 15 (2003) 871–881.

[16] S. Horman, G. Browne, U. Krause, J. Patel, D. Vertommen, L. Bertrand, A. Lavoinne, L. Hue, C. Proud, M. Rider, Activation of AMP-activated protein kinase leads to the phosphorylation of elongation factor 2 and an inhibition of protein synthesis, Curr. Biol. 12 (2002) 1419–1423.

[17] C.G. Proud, R.M. Denton, Molecular mechanisms for the control of translation by insulin, Biochem. J. 328 (Pt. 2) (1997) 329–341.

[18] K. Mitsui, M. Brady, H.C. Palfrey, A.C. Nairn, Purification and characterization of calmodulin-dependent protein kinase III from rabbit reticulocytes and rat pancreas, J. Biol. Chem. 268 (1993) 13422–13433.

[19] T.A. Diggle, T. Subkhankulova, K.S. Lilley, N. Shikotra, A.E. Willis, N.T. Redpath, Phosphorylation of elongation factor-2 kinase on serine 499 by cAMP-dependent protein kinase induces Ca^{2+}/calmodulin-independent activity, Biochem. J. 353 (2001) 621–626.

[20] G.J. Browne, S.G. Finn, C.G. Proud, Stimulation of the AMP-activated protein kinase leads to activation of eukaryotic elongation factor 2 kinase and to its phosphorylation at a novel site, J. Biol. Chem. 398 (2004).

[21] L. Wang, X. Wang, C.G. Proud, Activation of mRNA translation in rat cardiac myocytes by insulin involves multiple rapamycin-sensitive steps, Am. J. Physiol. Heart Circ. Physiol. 278 (2000) H1056–H1068.

[22] A. Knebel, N. Morrice, P. Cohen, A novel method to identify protein kinase substrates: eEF2 kinase is phosphorylated and inhibited by SAPK4/p38delta, EMBO J. 20 (2001) 4360–4369.

[23] A. Knebel, C.E. Haydon, N. Morrice, P. Cohen, Stress-induced regulation of eukaryotic elongation factor 2 kinase by SB 203580-sensitive and -insensitive pathways, Biochem. J. 367 (2002) 525–532.

[24] J.A. Williams, Intracellular signaling mechanisms activated by cholecystokinin-regulating synthesis and secretion of digestive enzymes in pancreatic acinar cells, Annu. Rev. Physiol. 63 (2001) 77–97.

[25] M.J. Bragado, M. Tashiro, J.A. Williams, Regulation of the initiation of pancreatic digestive enzyme protein synthesis by cholecystokinin in rat pancreas in vivo, Gastroenterology 119 (2000) 1731–1739.

[26] M.D. Sans, J.A. Williams, Calcineurin is required for translational control of protein synthesis in rat pancreatic acini, Am. J. Physiol. (Cell Physiol.) (2004) Mar 24 [Epub ahead of print]. PMID: 15044154. 0:5342003-0.

[27] J.A. Williams, M. Korc, R.L. Dormer, Action of secretagogues on a new preparation of functionally intact, isolated pancreatic acini, Am. J. Physiol. 235 (1978) 517–524.

[28] S.R. Kimball, L.S. Jefferson, Mechanism of the inhibition of protein synthesis by vasopressin in rat liver, J. Biol. Chem. 265 (1990) 16794–16798.

[29] H. Fan, S. Penman, Regulation of protein synthesis in mammalian cells. II. Inhibition of protein synthesis at the level of initiation during mitosis, J. Mol. Biol. 50 (1970) 655–670.

[30] D.E. Peavy, J.M. Taylor, L.S. Jefferson, Time course of changes in albumin synthesis and mRNA in diabetic and insulin-treated diabetic rats, Am. J. Physiol. 248 (1985) E656–E663.

[31] J. Holst, Neural regulation of pancreatic exocrine function, in: V. Go, E. DiMagno, J. Gardner, E. Lebenthal, H. Reber, G. Scheele (Eds.), The Pancreas. Biology Pathobiology and Disease, Raven Press, New York, 1993, pp. 381–402.

[32] W. Chey, Hormonal control of pancreatic exocrine secretion, in: V. Go, E. DiMagno, J. Gardner, E. Lebenthal, H. Reber, G. Scheele (Eds.), The Pancreas. Biology, Pathobiology and Disease, Raven Press, New York, 1993, pp. 403–424.

[33] J.A. Williams, D.I. Yule, Stimulus-secretion coupling in pancreatic acinar cells, in: V. Go, E. DiMagno, J. Gardner, E. Lebenthal, H. Reber, G. Scheele (Eds.), The Pancreas. Biology,

Pathobiology and Disease, Raven Press, New York, 1993, pp. 167–189.
[34] J.A. Williams, M.D. Sans, M. Tashiro, C. Schafer, M.J. Bragado, A. Dabrowski, Cholecystokinin activates a variety of intracellular signal transduction mechanisms in rodent pancreatic acinar cells, Pharmacol. Toxicol. 91 (2002) 297–303.
[35] T.A. Diggle, N.T. Redpath, K.J. Heesom, R.M. Denton, Regulation of protein-synthesis elongation-factor-2 kinase by cAMP in adipocytes, Biochem. J. 336 (Pt. 3) (1998) 525–529.
[36] L. Wang, C.G. Proud, Regulation of the phosphorylation of elongation factor 2 by MEK-dependent signalling in adult rat cardiomyocytes, FEBS Lett. 531 (2002) 285–289.
[37] X. Wang, W. Li, M. Williams, N. Terada, D.R. Alessi, C.G. Proud, Regulation of elongation factor 2 kinase by p90(RSK1) and p70 S6 kinase, EMBO J. 20 (2001) 4370–4379.
[38] M.J. Bragado, A. Dabrowski, G.E. Groblewski, J.A. Williams, CCK activates p90rsk in rat pancreatic acini through protein kinase C, Am. J. Physiol. 272 (1997) G401–G407.