Syncollin is required for efficient zymogen granule exocytosis

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Syncollin is a 13 kDa protein that is present in the exocrine pancreas, where the majority of the protein is tightly attached to the luminal surface of the zymogen granule membrane. We have addressed the physiological role of syncollin by studying the phenotype of syncollin KO (knockout) mice. These mice show pancreatic hypertrophy and elevated pancreatic amylase levels. Further, secretagogue-stimulated amylase release from pancreatic lobules of syncollin KO mice was found to be reduced by about 45 % compared with wild-type lobules, and the delivery of newly synthesized protein to zymogen granules was delayed, indicating that the mice have a pancreatic secretory defect. As determined by two-photon imaging, the number of secretagogue-stimulated exocytotic events in acini from syncollin KO mice was reduced by 50 %. This reduction was accounted for predominantly by a loss of later, 'secondary' fusion events between zymogen granules and other granules that had already fused with the plasma membrane. We conclude that syncollin is required for efficient exocytosis in the pancreatic acinar cell, and that it plays a particularly important role in compound exocytosis.

Key words: acinar cell, exocrine pancreas, exocytosis, syncollin.

INTRODUCTION

Regulated exocytosis in the exocrine pancreatic acinar cell involves fusion of the membranes of zymogen granules both with the apical domain of the plasma membrane and with each other [1–4]. This latter process, known as compound exocytosis, occurs in only a few cell types. In the acinar cell, it is likely to be an adaptation designed to increase the efficiency of digestive enzyme secretion at the spatially restricted apical pole of the cell. As for most other membrane fusion events [5,6], exocytosis in the acinar cell depends on SNAREs (soluble *N*-ethylmaleimide-sensitive fusion protein attachment protein receptors [7–9]). Syntaxin 2, the apical membrane t-SNARE (target membrane SNARE) [7], is required for granule–plasma-membrane fusion *in vitro* [9]. Further, syntaxin 3, present on the granule membrane, mediates granule–granule fusion, and may be involved in compound exocytosis [9]. Both endobrevin [VAMP 8 (vesicle-associated membrane protein 8)] and synaptobrevin 2 are present on the granule membrane [9,10]. The phenotype of an endobrevin KO (knockout) mouse indicates that this protein acts as the major v-SNARE (vesicle SNARE) for zymogen granule exocytosis [10]. In contrast, synaptobrevin 2 appears to play only a minor role in granule exocytosis [9].

A search for syntaxin-binding proteins on the zymogen granule membrane yielded syncollin [11], which is localized within the granule. Syncollin has several unusual biochemical properties. Although it has no recognizable transmembrane domain, it is associated predominantly with the luminal surface of the zymogen granule membrane [12] in the form of a homo-oligomer, most likely a hexamer [12–14]. It resists extraction from the granule membrane in high-salt buffer solutions, unlike typical peripheral membrane proteins, but is removed under alkaline conditions (pH >10), when it becomes monomerized [12]. Syncollin associates with GP-2, the major glycoprotein present in the granule membrane, which in turn interacts with components of the granule matrix [15]. Imaging of syncollin bound to supported lipid bilayers by atomic force microscopy has revealed doughnut-shaped structures with a central depression of diameter approx. 5 nm [14]. Purified native syncollin is able to permeabilize both liposomes [14] and erythrocytes [16], suggesting that it might have pore-forming properties.

The physiological role of syncollin is still unclear. This issue has been addressed in a previous study of syncollin KO mice [17]. Abnormalities on the secretory pathway in the exocrine pancreas were detected, but amylase secretion was reported to be normal. In the present study, we have re-examined the phenotype of the syncollin KO mouse. In contrast with the results reported previously [17], we find that pancreatic secretion in this mouse is significantly compromised, and that compensatory changes, such as pancreatic hypertrophy, occur. Our results indicate that syncollin is required for efficient exocytosis in the exocrine pancreas.

EXPERIMENTAL

Animals

Homozygous WT (wild type) and syncollin KO mice were used [17]. Mice were inbred down to three generations on a mixed genetic background (129Ola/C57Bl6). For each experiment, mice from at least two different litters and with different sires were used. The mice had access *ad libitum* to water and standard dry laboratory food at all times.

Measurement of amylase release from pancreatic lobules

Dulbecco's modified Eagle's medium, containing 25 mM Hepes, pH 8.0, was injected into the connective tissue surrounding the pancreatic tissue, and the separated lobules were excised. Lobules (25–30 per sample) were washed three times in incubation medium [120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 1 mM NaH_2PO_4, 1 mM $NaHCO_3$, 11 mM D-glucose, 4 mM D-*myo*-inositol, 2 % (v/v) basal medium Eagle amino acids,

Abbreviations used: KO, knockout; SNARE, soluble *N*-ethylmaleimide-sensitive fusion protein attachment protein receptor; t-SNARE, target membrane SNARE; VIP, vasoactive intestinal peptide; v-SNARE, vesicle SNARE; WT, wild type.

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2 mM L-glutamine and 25 mM Hepes, pH 7.2], and pre-incubated in this medium for 10 min at 37 °C with gentle agitation. A sample (100 μl) of medium was removed, and lobules were incubated for 1 h at 37 °C in either the absence or the presence of secretagogue. Oxygen was supplied every 15 min. At the end of the incubation, a further 100 μl of supernatant was removed. Amylase secreted during the 1 h incubation period (corrected for the amount of amylase present initially in the bathing medium) was assayed [18] and expressed as a percentage of total amylase, determined after solubilization of the lobules with Triton X-100 (1 %). The viability of the lobules was monitored by measuring the release of lactate dehydrogenase into the medium, using an assay kit (Sigma).

Time course of delivery of newly synthesized proteins to zymogen granules

Lobules were washed three times in methionine- and cysteine-deficient medium (ICN Pharmaceuticals), and then pre-incubated in the same medium (5 ml) for 15 min. The lobules were pulse-labelled by the addition of TRAN^{35}S-label ($\sim$ 500 μCi; ICN Pharmaceuticals) for 2.5 min. They were then washed three times in chase medium (incubation medium supplemented with a 10-fold excess of unlabelled methionine and cysteine), and incubated further for various times, with oxygenation every 15 min. Lobules were washed with ice-cold Hepes-buffered saline, pH 7.6, and zymogen granules were prepared by homogenization followed by centrifugation [19]. Both the granule pellet and the supernatant were retained. Granules were lysed by freeze–thawing. Protein in fractions (50 μl) of supernatant and granules was precipitated on to filters (Whatman) by incubation for 30 min in ice-cold 10 % (w/v) trichloroacetic acid, with agitation. Protein-bound radioactivity in the zymogen granules was expressed as a percentage of total protein-bound radioactivity.

Preparation of acini

Pancreatic acini were prepared by digestion with CLSPA collagenase (Worthington) at 37 °C for 6 min, as described previously [20].

Imaging of exocytosis

Acini were viewed with a Leica TCS-SP1-MP microscope, running Leica software. Acini were selected in phase contrast using an infinity-corrected ×63 water immersion, 1.2 numerical aperture, plan apochromatic lens with a cover glass correction collar and a 225 μm working distance. Calcium Green™-1 dextran (potassium salt; 10 000 Da, anionic; Molecular Probes) was excited by laser light from a solid-state Millenia V-pumped Physics Tsunami Ti/sapphire laser tuned to 850 nm, with a pulse width of 1.3 ps and a repetition rate of 82 MHz. Emitted light was captured at 500–560 nm with a spectrophotometer detector. The confocal pinhole was set to 2 airy disk equivalents for the objective lens. Acini were incubated at room temperature and exocytosis was triggered by the addition of 1 μM acetylcholine. Sixty frames were collected at 10 s intervals. Image size was 512 × 512 pixels, at 0.077 μm/pixel. Individual exocytotic events occurring during the time sequence were identified, expressed as number of events per cell and placed in 20 s bins. Cumulative exocytotic data were also generated.

Data analysis

Data are presented as means ± S.E.M. The statistical significance of differences was assessed by means of an unpaired two-tailed Student's *t* test.

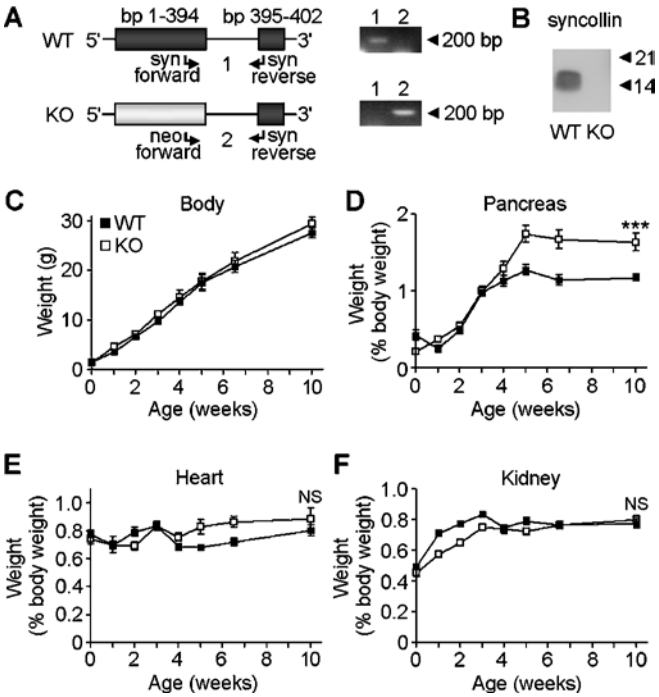

Figure 1 Basic phenotype of syncollin KO mice

(**A**) Confirmation of the genotypes of WT and syncollin KO mice. Genomic DNA from tail biopsies was used as a template for PCR with the following primers: syn forward, 5′-ACTGGTCCGACTCCATCTCTGCCCTC-3′; neo forward, 5′-GAGCGCGCGCGGCGGAGTTGTTGAC-3′; syn reverse, 5′-GTGCACTCAGAGATGGGACCTCCAGG-3′. (**B**) Immunoblot analysis of samples of zymogen granule membrane, showing the absence of syncollin from the syncollin KO mouse. Syncollin was detected using a mouse monoclonal anti-syncollin antibody (87.1 [12]) followed by a horseradish peroxidase-conjugated goat anti-mouse secondary antibody (Bio-Rad), with enhanced chemiluminescence visualization (Pierce). (**C**) Age-dependent increase in body weight of WT (■) and syncollin KO (□) mice. (**D**–**F**) Age-dependent increases in weight of the pancreas (**D**), heart (**E**) and kidney (**F**), expressed as a percentage of body weight, of WT and syncollin KO mice. (***$P < 0.001$, $n = 14$; NS, not significant.)

RESULTS

Syncollin KO mice, produced by replacement of exon 1 of the syncollin gene with a neomycin resistance cassette by homologous recombination, have been described previously [17]. Here, the genotypes of normal and syncollin KO mice were confirmed using PCR, with forward primers based on sequences in exon 1 of syncollin and in the neomycin resistance cassette respectively, together with a reverse primer based on a sequence in exon 2 of syncollin (Figure 1A). For both strains of mice, the appropriate pair of primers led to the production of an amplified band of the expected size ($\sim$ 200 bp). The absence of syncollin from the pancreas of the syncollin KO mice was also confirmed by immunoblotting (Figure 1B). The syncollin KO mice were healthy and fertile, and grew at the same rate as WT mice (Figure 1C). However, the gross appearance of the pancreas in the KO mice was different from that of WT mice. Specifically, the pancreas from KO mice typically had a pale, cream-coloured appearance, in contrast with the normal pink colour (results not shown). Further, the mean weight of the pancreas in syncollin KO mice, expressed as a percentage of total body weight, was significantly greater than that of WT mice from 5 weeks onwards (Figure 1D). At 10 weeks, the pancreas in the syncollin-deficient mice was hypertrophied by 39 % (WT, 1.17 ± 0.04 % of body weight; syncollin KO, 1.63 ± 0.11 % of body weight; $P < 0.001$, $n = 14$). In contrast, there was no significant difference in the relative weight of the

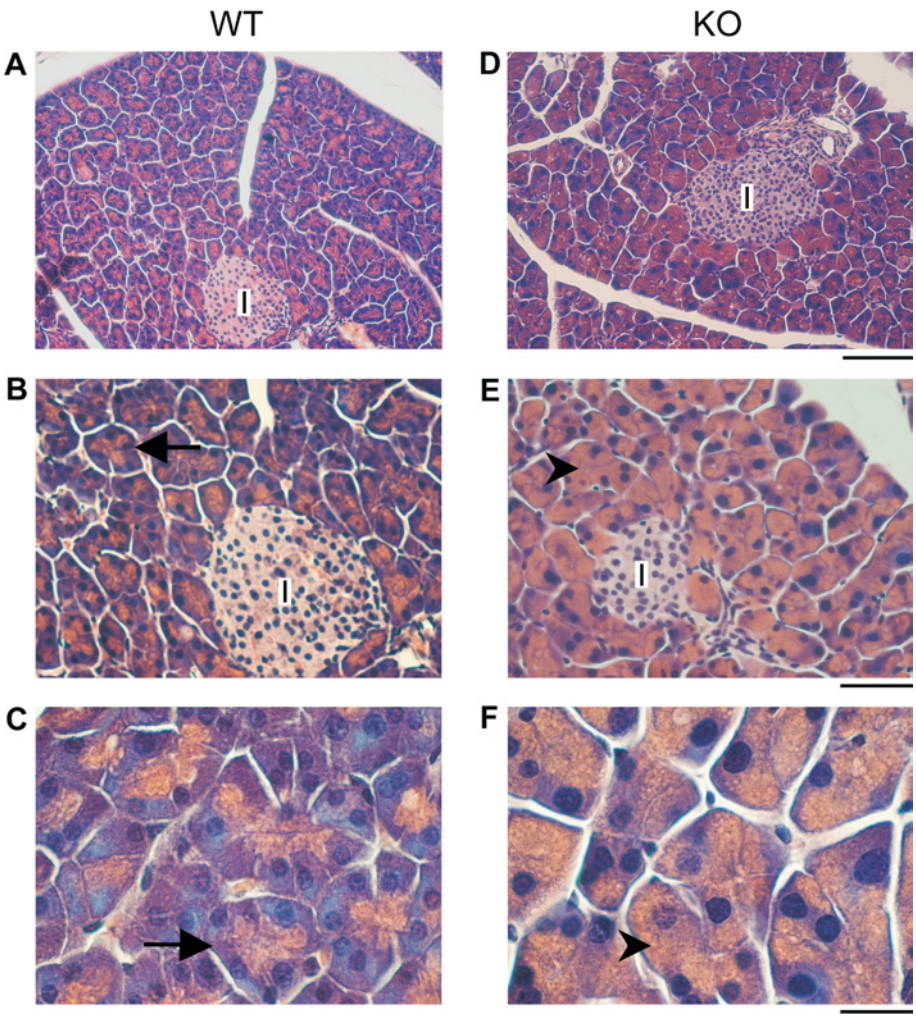

Figure 2 The exocrine pancreas in syncollin KO mice shows histological abnormalities

Haematoxylin and eosin stains of 6 μm sections of formalin-fixed, paraffin-embedded pancreas from adult WT (**A**–**C**) and syncollin KO (**D**–**F**) mice are shown. Arrows indicate acinar structures; arrowheads indicate highly eosinophilic, zymogen granule-rich areas. Islets of Langerhans are labelled 'I'. Scale bars indicate 100 μm (**A**, **D**), 50 μm (**B**, **E**) or 20 μm (**C**, **F**).

heart (Figure 1E) or the kidney (Figure 1F) between WT and syncollin KO mice at 10 weeks of age.

In addition to the pancreatic hypertrophy in syncollin KO mice, there was also an increase in the amount of the digestive enzyme amylase per unit weight of pancreatic tissue. The pancreatic amylase content of WT and syncollin-deficient adult mice was 65.0 ± 3.7 and 86.8 ± 8.3 units/mg wet tissue respectively ($n = 6$), representing a 34% increase in the syncollin KO pancreas ($P < 0.05$). Since there was no significant change in the DNA content of a given amount of pancreatic tissue in the KO mouse (results not shown), this difference in amylase content indicates an increase in the number of zymogen granules per acinar cell (and possibly also in the amount of amylase present upstream in the secretory pathway). Since packed granules are white in colour [19], an increase in granule density is consistent with the cream-coloured appearance of the pancreas in the syncollin KO mice.

The increase in amylase content in the pancreas of syncollin KO mice was also reflected in the different appearance of the tissue after histological staining. Figure 2 shows typical images, at various magnifications, of pancreatic sections stained with haematoxylin and eosin. In tissue from WT mice (Figures 2A–2C), the nuclei were localized near the basolateral poles of the acinar cells (blue staining) and zymogen granules were towards the apical poles (pink staining). The arrows in Figures 2(B) and 2(C) indicate the normal acinar structure of the tissue. In contrast, the acinar cells in tissue from KO mice (Figures 2D–2F) had much more extensive pink staining, indicative of an expansion of the zymogen granule-rich areas, and there was evidence of a breakdown of the normal structure of the acini (arrowheads in Figures 2E and 2F). Interestingly, endobrevin KO mice, which also exhibit a defect in exocrine pancreatic secretion, show similar abnormalities in pancreatic morphology; for instance, the pancreas of the endobrevin KO mouse also has a creamy appearance, and the acinar cells are packed with zymogen granules, as judged by both haematoxylin and eosin staining and electron microscopy [10].

To compare the abilities of pancreas from WT and syncollin KO mice to secrete digestive enzymes, pancreatic lobules were prepared from both groups and stimulated for 1 h at 37 °C with the secretagogues carbachol (500 nM) and/or VIP (vasoactive intestinal peptide; 100 nM). These two secretagogues were chosen because carbachol acts primarily through release of Ca^{2+} from intracellular stores, whereas VIP acts primarily through mobilization of cAMP [21]. As shown in Figure 3(A), there was a small amount of basal release of amylase from unstimulated lobules. Addition of carbachol to WT lobules caused a significant

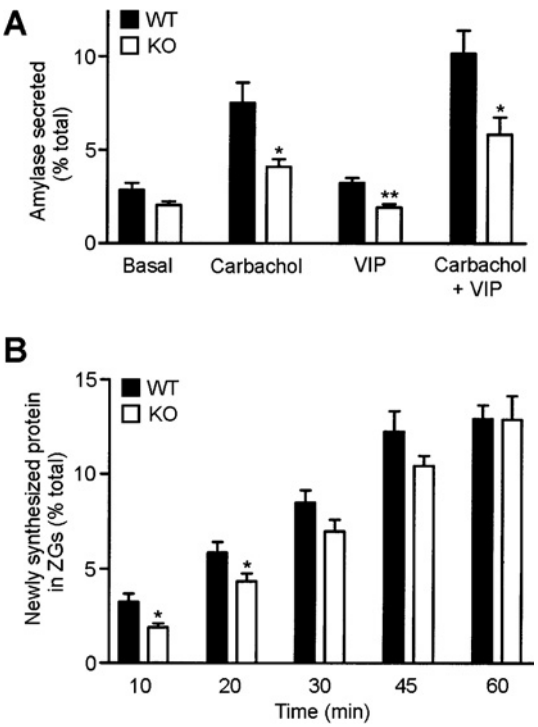

Figure 3 Amylase secretion and delivery of proteins to the zymogen granules are defective in syncollin KO mice

(**A**) Secretagogue-stimulated secretion of amylase from pancreatic lobules from WT (■) and syncollin KO (□) mice. Lobules were incubated for 1 h at 37 °C with no secretagogue (basal), carbachol (500 nM), VIP (100 nM) or both carbachol and VIP. Amylase secretion is expressed as a percentage of total tissue amylase. (*$P < 0.05$, **$P < 0.01$; $n = 7$). (**B**) Time course of delivery of newly synthesized proteins into zymogen granules (ZGs). Newly synthesized proteins in pancreatic lobules prepared from either WT (■) or syncollin KO (□) mice were pulse-labelled with [^{35}S]methionine and [^{35}S]cysteine for 2.5 min and then chased for various times at 37 °C. Zymogen granules were prepared from the lobules, and granule-associated radiolabelled protein was expressed as a percentage of the total tissue content. (*$P < 0.05$, $n = 5$–7).

stimulation of amylase release. VIP had no significant effect when used alone, but had a synergistic effect when used together with carbachol. Basal amylase release from lobules taken from WT and syncollin KO mice was not significantly different. In contrast, the extent of secretion in the presence of carbachol, VIP or both carbachol and VIP was significantly lower (by 41–45 %) from the lobules from syncollin KO mice than from lobules from WT mice.

We speculated that the reduced secretory capacity of the pancreas from syncollin KO mice might result in a 'backing-up' effect on the secretory pathway. We therefore pulse-labelled newly synthesized digestive enzymes by incubation of lobule preparations with [^{35}S]methionine and [^{35}S]cysteine, and then determined the amount of labelled protein reaching zymogen granules after various times of chase. As shown in Figure 3(B), the percentage of total labelled protein present in the zymogen granules was significantly reduced in the lobules taken from syncollin KO mice at early chase times (10 and 20 min), but at later times (30–60 min) delivery of the proteins to the granules in these lobules had 'caught up' with delivery to zymogen granules in lobules from WT mice.

We next studied the exocytotic process directly in isolated acini using two-photon imaging. The membrane-impermeant dye Calcium Green dextran was added to the medium bathing the acini and the cells were imaged during stimulation with the secretagogue acetylcholine (1 μM). When zymogen granules fused with the apical surface of the acinar cell plasma membrane, the dye entered the fusing granule through the fusion pore, enabling the detection of single exocytotic fusion events [3,4]. A typical response of a WT acinus is illustrated in the time sequence shown in Figure 4(A), and in Supplemental Movie 1 (http://www.BiochemJ.org/bj/385/bj3850721add.htm). There was no detectable activity for the 30 s before application of acetylcholine; however, shortly after agonist application, omega-shaped features began to appear with an approximate diameter of 1 μm, consistent with the filling of zymogen granules with dye. As reported previously using the same technique [3,4], two types of fusion events could be distinguished: those where the granule fused directly with the plasma membrane (primary fusion event; Figure 4A, arrow), and those where a granule lying deeper within the cell fused with a granule that had itself already opened to the exterior (secondary fusion event; arrowhead). Granules usually did not collapse rapidly into the target membrane after fusion, but remained as distinct structures for some time. For instance, the granule indicated by the arrowhead in Figure 4(A) appears at 60 s and is still discernible at the end of the time sequence (300 s). Although not studied here, the eventual fate of a fused granule is to be budded away gradually, most probably in the form of small vesicles [4]. Similar exocytotic features were seen in acini from syncollin KO mice; however, as shown in the time sequence in Figure 4(B) and in Supplemental Movie 2 (http://www.BiochemJ.org/bj/385/bj3850721add.htm), the response of the syncollin KO acinus was typically smaller and more delayed than that of the WT acinus, and the number of secondary fusion events appeared to be particularly reduced. Note that although Figure 4(B) appears to show a delocalization of exocytotic fusion away from the apical pole of the cell, overall there was no change in the location of the exocytotic response.

The numbers of fusion events occurring during each frame of the imaging sequence (total time 10 min) were assigned to 20 s time bins (excluding data from acini that did not give a detectable response). The profiles of the exocytotic responses in acini from WT and syncollin KO mice are shown in Figure 5(A). It can be seen that shortly after the addition of acetylcholine to a WT acinus there was a burst of exocytosis that reached a peak 20–40 s after agonist application, which would include a period of agonist equilibration through the incubation chamber. The exocytotic burst was followed by a gradual decline in the rate of exocytosis over the 10 min time course. Acini from syncollin KO mice showed fewer exocytotic events, and the exocytotic burst in particular was much reduced. Cumulative traces for the combined exocytotic responses are shown in Figure 5(B). The total number of exocytotic events in the acini from syncollin KO mice was 50 % lower than in acini from WT mice, similar to the reduction in carbachol-stimulated amylase release from lobules (41–45 %). Since the reduction in amylase release is occurring against a background of higher amylase levels per cell (and, most probably, higher zymogen granule numbers), one might expect that the percentage reduction in the number of exocytotic events would be smaller than the reduction in amylase release. However, it should be borne in mind that only a small minority of the granules within a cell (approx. 10 %) are competent to undergo fusion at any time, and the size of this population might not be directly related to total cellular amylase content.

To assess whether syncollin might play a role specifically in either primary or secondary fusion events, separate profiles of the two types of event were prepared (Figures 5C–5F). Assignment of fusion events into the primary and secondary categories was carried out independently by two observers, with very similar results. As would be expected, the primary events in the WT acini reached a peak earlier than the secondary responses (20 s compared with 40 s; Figures 5C and 5E). It is also clear,

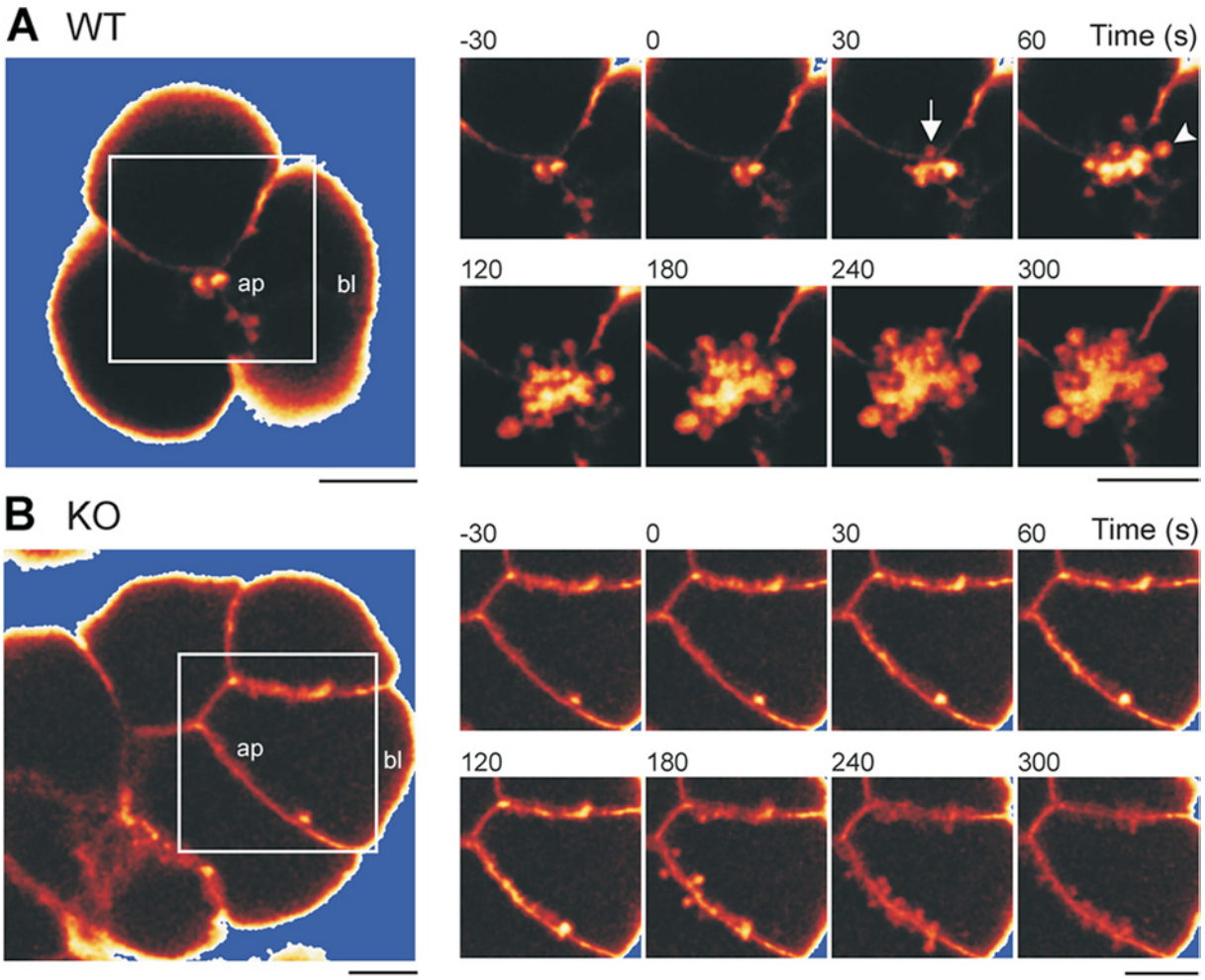

Figure 4 Exocytotic responses in acini from WT and syncollin KO mice

(**A**) Response of a WT acinus. The left panel shows a two-photon image of an acinus, and the right panel shows a sequence of images of the area defined by the box. Acetylcholine (1 μM) was added at time 0. The arrow indicates the fusion of a zymogen granule directly with the plasma membrane (primary fusion event); the arrowhead indicates the fusion of a granule with another granule that was already in contact with the plasma membrane (secondary fusion event). See also Supplemental Movie 1 (http://www.BiochemJ.org/bj/385/bj3850721add.htm). (**B**) Response of a syncollin KO acinus. See also Supplemental Movie 2. Apical (ap) and basolateral (bl) regions of a representative cell in each acinus are indicated. Scale bars, 10 μm.

particularly from the cumulative data (Figures 5D and 5F), that the decrease in the number of secondary events in syncollin KO acini was greater than the decrease in primary events. Specifically, the difference between the numbers of secondary events (WT, 3.09 ± 0.56 events/cell, $n = 16$; KO, 1.10 ± 0.32 events/cell, $n = 15$) was highly significant ($P < 0.01$), whereas the difference between the numbers of primary events (WT, 3.06 ± 0.51 events/cell, $n = 16$; KO, 1.97 ± 0.33 events/cell, $n = 15$) did not reach statistical significance ($P = 0.086$). The reduction in the number of secondary events in the syncollin KO acini was especially pronounced during the early stages of the response, with very little activity occurring during the first 3 min. This difference in the exocytotic profiles of WT and syncollin KO acini is also apparent in the image sequences shown in Figure 4.

DISCUSSION

The number of exocytotic events in acinar cells from syncollin KO mice was reduced by approx. 50 % compared with cells from WT mice. In WT mice, primary and secondary granule fusion events make approximately equal contributions to this response, and the change seen in the KO mice consisted of a 36 % reduction in primary fusion events and a 64 % reduction in secondary fusion events. This result suggests that syncollin is involved primarily in maintaining compound exocytosis. Since compound exocytosis occurs in only very few cell types, this proposed function of syncollin would be consistent with its highly restricted tissue distribution [11].

If syncollin does indeed play a role in exocytosis in the exocrine pancreas, then one might expect to observe compensatory changes in the syncollin KO mice, such as increases in the total amount of pancreatic tissue and/or in the amount of digestive enzymes within a given amount of tissue. Further, if exocytosis from the acinar cells is compromised, one might also expect to see a reduction in the efficiency with which newly synthesized digestive enzymes are delivered to the granules. All of these changes were detected in our study. Hence the characteristics of the pancreas in syncollin KO mice are entirely consistent with a reduction in the efficiency of exocytosis.

Abnormalities in pancreatic function in syncollin KO mice have been reported previously [17]. For instance, the necrotic response to hyperstimulation with caerulein was shown to be more severe in the KO mouse than in the WT mouse. In addition, the rate of synthesis of new proteins was reduced and the time course of their

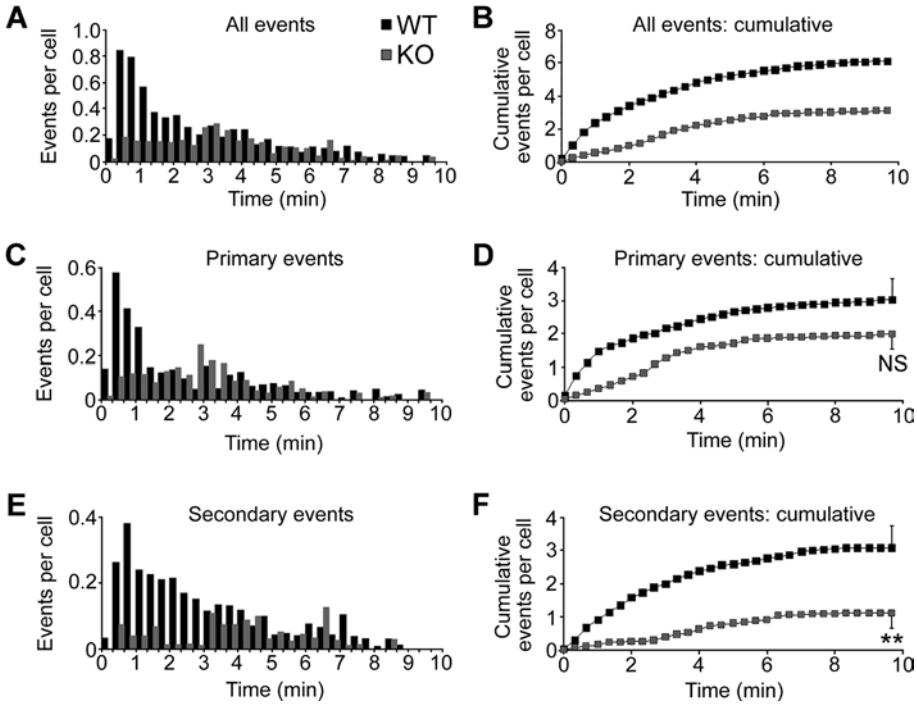

Figure 5 The exocytotic response is abnormal in syncollin KO mice

(**A**) Exocytotic profiles for acini from WT (black bars) and syncollin KO (grey bars) mice. Twenty acini from seven mice were analysed; of these, 16 acini gave detectable exocytotic responses. Twenty-six acini from seven syncollin KO mice were analysed, of which 15 gave detectable responses. Sixty frames (as shown in Figure 3) were collected at 10 s intervals. Exocytotic events occurring during the time sequence were expressed as number of events per cell and placed in 20 s bins. (**B**) Cumulative exocytotic responses of WT (black symbols) and syncollin KO (grey symbols) acini. (**C**) Profile of primary exocytotic events for WT and syncollin KO acini. (**D**) Cumulative primary exocytotic responses of WT and syncollin KO acini. (**E**) Profile of secondary exocytotic events. (**F**) Cumulative secondary exocytotic responses of WT and syncollin KO acini. (**$P < 0.01$, $n = 15$–16; NS, not significant.)

release from the acinar cells under conditions of stimulation was more protracted. In contrast with our results, however, it was reported that there was no change in tissue amylase levels [17], although protein levels were normalized to DNA content, and no attempt was made to determine the total amount of pancreatic tissue. Pancreatic histology was considered to be normal, despite the appearance of increased eosin staining, similar to that shown in the present study. Secretagogue-stimulated amylase secretion from lobules was reported to be unaffected; however, the viability of the lobules was not determined, and basal secretion was high (10 % after 2 h), suggesting that the integrity of the lobules might have been compromised. It was proposed [17] that syncollin might play a role in the packaging of proteins into the zymogen granules rather than in exocytosis. In contrast, our data clearly indicate that syncollin plays a significant role in exocytosis.

How might syncollin act during compound exocytosis in the pancreatic acinar cell? There is evidence that syncollin is able to permeabilize both lipid bilayers [14] and biological membranes [16], and its structure indicates that it might have pore-forming properties [14]. Recently we showed that syncollin expressed exogenously in AtT-20 cells was targeted to corticotropin ('ACTH')-containing dense-core granules, and that it reduced both regulated corticotropin secretion and the number of dense-core granules within the cells by approx. 50 % [16]. This ability of syncollin to destabilize the dense-core granules might be a result of uncontrolled pore formation following its expression in a 'foreign' cell line, although this has not been demonstrated directly. Secondary fusion between zymogen granules requires the initial primary fusion of a granule with the plasma membrane, and there is a lag of approx. 10–20 s between the primary and secondary fusion events [3,4]. To account for this behaviour, it has been proposed that secondary fusion requires the recruitment of components of the fusion machinery (e.g. syntaxin 2) from the plasma membrane [3]. However, it has been shown that there is no lipid mixing between the granule membrane and the plasma membrane during fusion [4], raising the question of whether plasma membrane t-SNAREs can move into the granule membrane. Secondary fusion might also be triggered in response to a change in the luminal milieu of the granule that has undergone primary fusion. One such change is an elevation of luminal pH from the 'resting' value of approx. 5.0 [22,23] to the extracellular value of 7.4. It is possible that such a change might activate syncollin's membrane-permeabilizing properties. However, it should be emphasized that the pH-sensitivity of the behaviour of syncollin has not yet been investigated.

An alternative possibility is that syncollin has a more structural role, through its ability to interact with GP-2 and, indirectly, with the granule matrix [15]. It is likely that 'secondary' granules are closely apposed to the 'primary' granules, so that fusion occurs efficiently when the primary granules become primed [1–4]. This positioning of the granule might depend on its physical properties, such as its elasticity, and this in turn could be influenced by changes in the organization of the granule content. Finally, syncollin might, in theory, stabilize the fused primary granule so that secondary fusion can occur. According to this idea, secondary fusion events would be selectively lost in the syncollin KO mice because the primary fusion events are too transient. However, although we have not carried out a systematic study of the lifetimes of fused vesicles, it is clear from our images of acini from KO mice (e.g. Figure 4B) that the primary fusion events persist for much longer than the average time interval between primary and secondary events (10–20 s).

The fact that syncollin KO mice are healthy and develop normally begs the question of why syncollin is necessary, even if it does improve the efficiency of pancreatic exocytosis. The exocrine pancreas normally provides a bolus of secreted digestive enzymes in response to occasional feeding sessions. It is possible, therefore, that the ability of syncollin KO mice to thrive under standard housing conditions may depend on constant access to food. We speculate that the KO mice may fare less well under natural conditions, where food might be only sporadically available.

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