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Immunohistochemical localization and mRNA detection of Rab3D and/or Rab3B in rat von Ebner's glands, parotid gland, pancreas, and liver

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Summary

In examining the secretory mechanism of exocrine glands, we focused on the small GTP-binding proteins, Rab3D and Rab3B, which function in the final steps of exocytosis in non-neuronal tissues. These proteins were observed in von Ebner's glands by ^{32}P-GTP overlay. mRNA isolated from von Ebner's glands, the pancreas, parotid glands, and liver was subjected to reverse transcription PCR and probed with primers and nested primers for Rab3D and Rab3B. Rab3D was found in all three exocrine glands and the liver, while Rab3B was found in the liver. Utilizing immunofluorescence histochemistry, Rab3D was localized to hepatocytes of the liver and to secretory granules of the exocrine glands, and Rab3B to liver and pancreatic islets. Isoproterenol evoked decreases in α-amylase- and Rab3D-labelled parotid secretory granules, and pilocarpine stimulated decreases in secretory granules labelled for lingual lipase and Rab3D from von Ebner's glands, and amylase and Rab3D from pancreas. Neither secretagogue affected Rab3B in pancreatic islets. These observed parallel decreases in response to β-adrenergic (parotid) or cholinergic (von Ebner's and pancreas) secretagogues indicate that the function of Rab3D in exocytosis in these exocrine organs is similar and that the type of secretagogue does not determine the function.

Introduction

Small GTP-binding proteins (SGPs) have been implicated as important factors in the regulation of secretion and vesicular transport. Mammalian SGPs named Rab are GTPases of M_r <30 kDa that play a role in secretory and endocytic pathways. More than 40 Rabs involved in the targeting and fusion of transport vesicles with acceptor membranes have been found in a variety of tissues and species. Newly synthesized proteins that are destined for secretion are stored in secretory granules. Rab3 proteins are important in the fusion of secretory granule membranes with cell membranes prior to exocytosis, and thus are associated with the final steps of exocytosis (see reviews by Novick & Zerial 1997, Martinez & Goud 1998, Schimmöller *et al.* 1998). There are four Rab3 isoforms (see review by Fischer von Mollard *et al*. 1994b). Rab3A is expressed mainly in neurons and neuroendocrine cells localized on synaptic vesicles (Matteoli *et al.* 1991) and also on adrenal chromaffin granules (Darchen *et al.* 1995). Rab3C is associated with the brain and colocalizes with Rab3A on synaptic vesicles (Fischer von Mollard *et al.* 1994a). Rab3B is found in epithelial-derived tissue, both cultured and native, i.e. liver, small intestine, colon, and distal nephron (Weber *et al*. 1994). It also is found in pancreatic islets (Regazzi *et al.* 1996). Rab3D has been found in adipocytes, differentiated 3T3-L1 cells, lung (Baldini *et al*. 1992), and in secretory granules in cells that undergo exocytosis, namely, exocrine pancreas, acinar cells of lacrimal glands, apical granules of Paneth cells of the intestine (Ohnishi *et al.* 1996), parotid (Ohnishi *et al.* 1996, Raffaniello *et al.* 1999), stomach chief cells (Ohnishi *et al.* 1996, Raffaniello *et al.* 1996), and mast cells (Roa *et al.* 1997, Tuvim *et al.* 1999).

It is not yet known whether Rab3 isoforms are found in von Ebner's glands or whether they are involved in protein secretion. The von Ebner's glands, also known as lingual serous glands, are minor salivary glands located in the tongue beneath the vallate and folate papillae (Hand 1970). These glands secrete two digestive enzymes, lingual lipase and α-amylase (Field & Hand 1987, Field *et al.* 1989). Lingual lipase digests triacylglycerols at the acid pH of the stomach, producing diacyl- and monoacyl-glycerols and fatty acids. (Field & Scow 1983). Secretion of lingual lipase and amylase from rat von Ebner's glands is regulated principally by cholinergic stimulation similar to the pancreas (Field & Hand 1987), but in the parotid gland, protein secretion is regulated primarily by β-adrenergic stimulation (Putney 1986). This difference in regulation of secretion makes the comparison of the role of Rab3 isoforms in different exocrine glands of great interest. In addition, although the Rab3B isoform was found in the liver, no studies have been done on Rab3D in the liver.

The purpose of this study was to investigate the presence of the SGPs, Rab3D, and Rab3B, in von Ebner's glands of rats, and their role in enzyme secretion by von Ebner's glands, the pancreas, and the parotid gland. The following techniques were utilized: ^{32}P-overlay of SDS-PAGE of

homogenates of von Ebner's glands, immunofluorescence histochemistry with antibodies to Rab3D, Rab3B, lingual lipase, and amylase in von Ebner's gland, the parotid, and pancreas, prior to and after treatment with the secretagogues, isoproterenol (β-adrenergic) or pilocarpine (cholinergic). In addition, mRNA was purified from the above glands and subjected to reverse transcription-polymerase chain reaction (rtPCR) to confirm the presence of mRNA for Rab3D or Rab3B in these glands. Rat liver was also examined by immunofluorescence histochemistry with antibodies to amylase, Rab3D, Rab3B, and by rtPCR using mouse liver mRNA. This is the first report of the presence of Rab3D in the liver and von Ebner's glands.

Materials and methods

Materials

All the rats were Sprague-Dawley from Charles River Laboratories, Raleigh, NC, certified free of sialodacryoadenitis and rat corona viruses except for the rat used for the liver studies which was obtained from Harlan, Indianapolis, IN; α-^{32}P-GTP was purchased from NEN Life Science Products, Boston, MA; and pilocarpine, isoproterenol, and propranolol were from Sigma, St. Louis, MO. All other materials and chemicals not mentioned specifically were of reagent grade of the highest purity available.

Methods

Detection of small GTP-binding proteins

^{32}P overlay. Small GTP-binding proteins were detected in von Ebner's glands by α-^{32}P-GTP overlay using the method of Ambudkar *et al.* (1990). The von Ebner glands were dissected from the tongues of 4 female rats, 221–7 g, 9 weeks old, after being anaesthetized by intraperitoneal injection with sodium pentobarbital (50 mg/kg) and euthanized by exsanguination. They were combined and homogenized. The 850 g supernatant was dialyzed, lyophilized, and 22.7, 45.4, and 67.1 μg protein were applied to two 12.5% SDS-polyacrylamide gels. One gel was stained with Coomassie blue (not shown) and the other gel was blotted to nitrocellulose. The nitrocellulose membrane was incubated in α-^{32}P-GTP and radioactivity was detected with Kodak X-Omat AR film.

Rab3B and 3D detection by reverse transcription-polymerase chain reaction

Isolation of mRNA from von Ebner's and parotid glands and the pancreas. Micro Poly (A) pure mRNA isolation kit (Ambion, Inc., Austin, TX) was used to isolate mRNA from rat von Ebner's gland, parotid, and pancreas. Rigorous precautions were taken to maintain an RNAse-free environment. Deionized, distilled water, used for the preparation of all solutions, was treated with diethylpyrocarbonate and autoclaved. Seven or eight week old male rats, 269–295 g, were anaesthetized by intraperitoneal injection with sodium pentobarbital (50 mg/kg) and euthanized by exsanguination. The tongue, parotid gland, and pancreas were removed and von Ebner's glands were carefully dissected out of the tongue. All the tissues were trimmed carefully of extraneous tissue and homogenized in lysis buffer from the kit. The rest of the procedure was as found in the kit instructions. The total amount of tissue dissected from von Ebner's glands was 0.49 g, 0.80 g from the parotid gland and 5.65 g from the pancreas. mRNA used for rtPCR was 7.2 μg from von Ebner's gland, 4 μg from the parotid gland, and 4 μg from the pancreas.

Reverse transcription. rtPCR was employed to detect Rab3B and Rab3D mRNA in von Ebner's glands, the pancreas, parotid glands, and liver of the rat. The RETROscript First-Strand Synthesis kit (Ambion, Inc., Austin, TX) provided the method for this procedure, utilizing the oligo dT_{18} primer for rt. For the kit control, rt of template mRNA (mouse liver) and kit positive control PCR primers (361 bp) were utilized. rt of mouse liver total RNA from the kit (see above) with Rab3D primers for PCR was chosen to be the negative control for Rab3D. However, positive results were obtained (see Results). For the positive control, the kit mouse liver mRNA was reverse transcribed using Rab3B reverse (antisense) primer on one sample and nested reverse (antisense) primer on another. The product of rt with Rab3B reverse primer was used for primary and secondary PCR and the product of rt with Rab3B reverse nested primer was used for nested PCR.

Primers for Rab3D and Rab3B PCR. Rab3D and Rab3B primers were prepared by Operon Technologies, Inc., Alameda, CA. Rab3D primers (product is 590 bp) for primary and secondary PCR were designed from mouse Rab3D sequence (Ohnishi *et al.* 1996): forward, 5′ CGG AAT TCC CCT GCC AGC CCA A GA GAC G 3′; reverse 5′ CGG GAT CCG CTG TGG GGG TGG GGT ATC C 3′.

Nested Rab3D primers (product is 335 bp) were determined from a Rab3D sequence described by Roa *et al.* (1997) using Gene Jockey II software (BIOSOFT, Ferguson, MO) forward, 5′ AAA CAG CAG CGT GGC AAG ACC 3′; reverse, 5′ TCC TCC AGG TCA CAC TTG TTC C 3′.

Gene Jockey II software (BIOSOFT, Ferguson, MO) was used to determine the primers for Rab3B from the sequence described by Klengel *et al.* (1997) as follows: forward, 5′ ATC ATT GGC AAC AGC AGC GTC G 3′; reverse, 5′ TGA GTC AGA CAT CTT ATC GC 3′ (product 491 bp) and nested Rab3B: forward, 5′ ACC ATC ACC ACA GCC TAC TAC C 3′; reverse, 5′ CTG ATG TTC TCC TTG GCA CTG G 3′ 5′ CTG ATG TTC TCC TTG GCA CTG G 3′ (product 256 bp).

PCR

The polymerase chain reaction was performed using Taq DNA polymerase (Boehringer Mannheim, Indianapolis, IN) in a PTC-100 Thermal Programmable Controller (MJ Research Inc., Watertown, MA).

The protocol for Rab3B for both primary and secondary and primary and secondary nested PCRs was as follows: step 1 – 95°C for 5 min, step 2 – 95°C for 30 s, step 3 – decrease temperature to 45°C at 1°C/s, step 4 – 45°C for 30 s, step 5 – 72°C for 1 min, step 6 – repeat steps 2–5, 39 times, step 7 – 72°C for 5 min, step 8 – 4°C. For the positive control, the kit mouse liver mRNA was subjected to rt using Rab3B reverse primers on one sample and nested reverse primers on another. The product of rt with Rab3B reverse primers was used for primary and secondary PCR and the product of rt with Rab3B antisense nested primers was used for nested PCR.

For Rab3D, the PCR protocol was the same except that the annealing temperature (steps 3 and 4) was 55°C instead of 45°C for primary and secondary PCR and primary and secondary nested PCR.

As positive controls for PCR, plasmids were subjected to PCR as described above. The Rab3D plasmid was a gift from Dr. Michele Roa of the Pasteur Institute and the Rab3B plasmid was a gift of Dr. Kevin L. Kirk from the University of Alabama.

Samples were separated on 2.5% agarose gels (NuSieve Agarose, FMC BioProducts, Rockland, ME) containing 0.4% ethidium bromide. The standard was 100 bp DNA ladder (Gibco BRL, Life Technologies, MD). Running buffer was Tris-borate-EDTA. The gels were destained with 2 washes in deionized, distilled water for 1 h each. The base pair bands were visualized under UV light using a trans-illuminator and photographed using an orange filter.

Rab3B and 3D detection by immunofluorescence histochemistry antibodies

Antibodies to Rab3B and amylase (anti-human salivary α-amylase, which strongly reacts with rat salivary α-amylase) were commercially available (Santa Cruz Biotechnology, Inc., Santa Cruz, CA and Sigma Chemical Co., St. Louis, MO, respectively). Antibodies to Rab3D were a gift from Dr. Robert D. Raffaniello. FITC-labelled secondary antibodies were from Jackson Immuno-Research Laboratories, West Grove, PA.

Preparation of antibodies to lingual lipase. Lingual lipase was purified from rat tongue using the method of Field & Scow (1983). A 4-month-old New Zealand male rabbit was injected with twelve intradermal injections of 27.4 μg lingual lipase in Freund's complete adjuvant. Booster shots with 303.4 μg in Freund's incomplete adjuvant were done 2 weeks later. The rabbit was bled from the ear two months after the first injection of lingual lipase and the blood was combined with 3.8% sodium citrate (1:9 v/v citrate:blood). The cells were centrifuged, the plasma heated to 58°C, and the white flocculent fibrinogen was centrifuged. The remaining serum was approximately 1.5 ml. A major band representing lingual lipase was observed on a Western blot of a gradient SDS-PAGE of 4–20% with a 1:200 dilution of the serum in 0.5% Tween-20, 10% bovine serum albumin in phosphate-buffered saline (PBS).

Animals. Eight week old male rats, 227–257 g, were fasted overnight (19 h). Two rats were injected, intraperitoneally, with either saline, or the following secretagogues; 30 mg/kg pilocarpine combined with 5 mg/kg propranolol, or 30 mg/kg isoproterenol. Pilocarpine is a cholinergic agonist with some minor β-adrenergic properties (Schneyer & Hall 1965, Schneyer 1965). Propranolol is used to inhibit any β-adrenergic responses to pilocarpine. Isoproterenol is a β-adrenergic agonist.

Tissue preparation. One hour after the injection of saline or the secretagogues, rats were anaesthetized with 50 mg/kg sodium pentobarbital, euthanized by exsanguination, and the tongue, parotid gland, and pancreas were removed. The von Ebner glands were carefully dissected out of the tongue. Tissue was fixed with 1% glutaraldehyde −0.2% picric acid in 0.08 M phosphate buffer, pH 7.4, and embedded in LR White resin (London Resin Co., Ltd., Berkshire, England). One μm-thick sections were fixed to slides with 10 μg/ml poly-L-lysine bromide at 60°C then blocked 1–2 h with 50 μl StabilGuard (SG) (SurModics, Inc., Eden Prairie. MN). In addition, a lobe of liver was removed from one untreated, 190 g, 7 week old male rat fed *ad lib*. The tissue was fixed as described above. Some liver sections were stained with methylene blue-Azure II (Richardson *et al.* 1960) for visualizing mast cells.

Detection of Rab3B, Rab3D, lingual lipase, or amylase in von Ebner's and parotid glands, pancreas, and liver

Primary rabbit polyclonal antibodies to Rab3B, Rab3D, lingual lipase, or amylase, diluted 1:25 v/v in SG, were bound to the tissue at 4°C at least overnight. Secondary antibodies, mouse-anti-rabbit (1 μ/50 μl) followed by rabbit-anti-mouse (1.5 μl/50 μl), labelled with fluorescein isothiocyanate (FITC) were used for detection (Bernstein & Goldberg 1989). FITC has maximum absorption at 494 nm and an emission peak at 520 nm. A Nikon Optiphot photomicroscope (Melville, NY) equipped with a Bio-Rad (Bio-Rad Life Sciences, Hercules, CA) MRC-1000 laser confocal imaging system with a Krypton/Argon laser as light source was used to examine the tissues. All slides were viewed at ×100 magnification. An imaging Quantex Intel-Pentium II computer was used to print digitalized images at approximately ×675 magnification.

Results

Detection of small GTP-binding proteins

As seen in Figure 1, two small GTP-binding proteins of molecular weight of approximately 28.36 and 26.3 kDa were identified in von Ebner's glands, thus indicating the presence of small GTP-binding proteins.

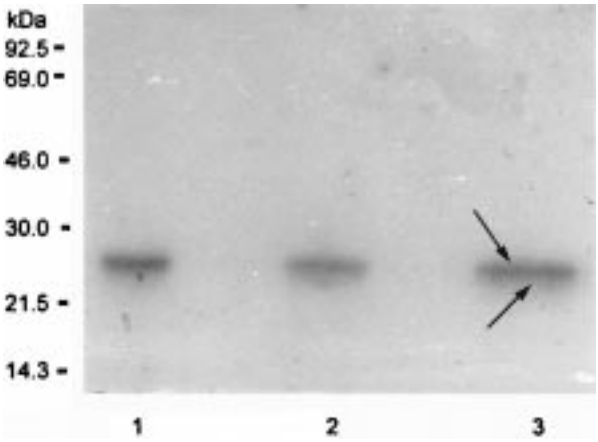

Figure 1. SDS-gel was blotted to nitrocellulose and probed with [α-^{32}P] GTP (21). Molecular weight markers are: lysozyme-14.3, trypsin inhibitor-21.5, carbonic anhydrase-30.0, ovalbumin-46.0, bovine serum albumin-69.0, and phosphorylase β-92.5. The arrows represent protein bands that bind to GTP, molecular weights 28.36 and 26.3 kDa. The gel was loaded with 22.7, 45.4, and 67.1 μg protein, in channels 1, 2, and 3, respectively.

Rab3B and 3D detection by reverse transcription-polymerase chain reaction

Using secondary nested PCR (after primary and secondary PCRs and primary nested PCR), Rab3D was detected in von Ebner's glands, the parotid and exocrine pancreas. As seen in Figure 2, there are bands in the appropriate place for the nested primers, 335 base pairs, for Rab3D. A band was also seen when PCR was performed on Rab3D plasmids. In addition and unexpectedly there was a band for Rab3D in the kit mouse liver mRNA. Since this negative control was found to be positive, we considered that the experiments shown in Figure 3 would constitute the negative control. The experiments in both figures were done within days of each other using the same reagents, solutions, and equipment. Three negative samples are seen in Figure 3 with no contamination or artifact showing in the area of the bands in Figure 2.

Bands for Rab3B were also detected by secondary nested PCR (261 base pairs) in kit mouse liver, and the Rab3B plasmid, but not in von Ebner's and parotid glands or the pancreas, as shown in Figure 3. Also, in the plasmid studies, there was no cross-reaction found with the Rab3D and Rab3B primers (not shown).

The kit control using template mRNA (mouse liver) on which rt was performed using kit 1st strand primers-oligo dT_{18} and kit PCR primers (361 bp) was positive (not shown).

In summary, the rtPCR studies reveal that Rab3B is in the liver, and Rab3D is in the liver, von Ebner's glands, parotid, and exocrine pancreas.

Rab3B and 3D detection by immunofluorescence histochemistry

The results of immunofluorescence histochemistry were very similar to the results of rtPCR. Figures 4a,c show positive responses to lingual lipase and Rab3D, respectively, in

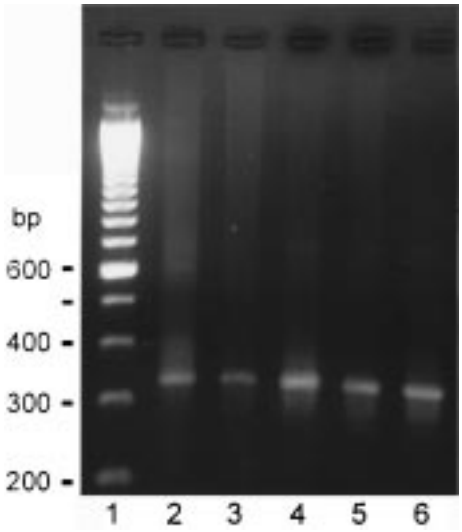

Figure 2. The products of secondary nested rtPCR using Rab3D nested primers (335 bp) were separated on 2.5% agarose gels containing ethidium bromide, viewed with a transilluminator, and photographed with an orange filter. Channel 1 – 100 bp standards; 2 – Rab3D plasmid; 3 – mouse liver from kit; 4 – pancreas; 5 – von Ebner's gland; 6 – parotid gland.

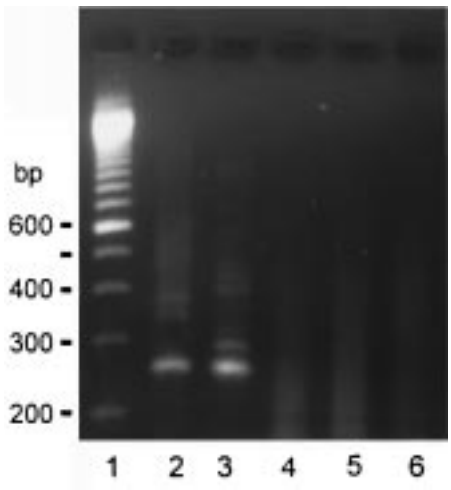

Figure 3. The products of secondary nested rtPCR using Rab3B nested primers (261 bp) were separated on 2.5% agarose gels containing ethidium bromide, viewed with a transilluminator, and photographed with an orange filter. Channel 1 – 100 bp standards; 2 – Rab3B plasmid; 3 – mouse liver from kit; 4 – pancreas; 5 – von Ebner's gland; 6 – parotid.

the saline-treated controls of von Ebner's glands. The fluorescence response was located primarily in the secretory granules. However, in some of the prints, most prominently in the pancreas, fluorescence also occurred in nuclei due to non-specific binding, a common phenomenon. The fluorescence response in the secretory granules which was diminished after pilocarpine treatment in both lingual lipase and Rab3D (Figures 4b,d) was not seen in other cellular components. Isoproterenol treatment did not effect lingual lipase or Rab3D response and Rab3B was negative (not shown). Similarly, the pancreas showed positive responses to amylase and Rab3D in the saline-treated controls (Figures 4e,g). After pilocarpine treatment, these responses were diminished

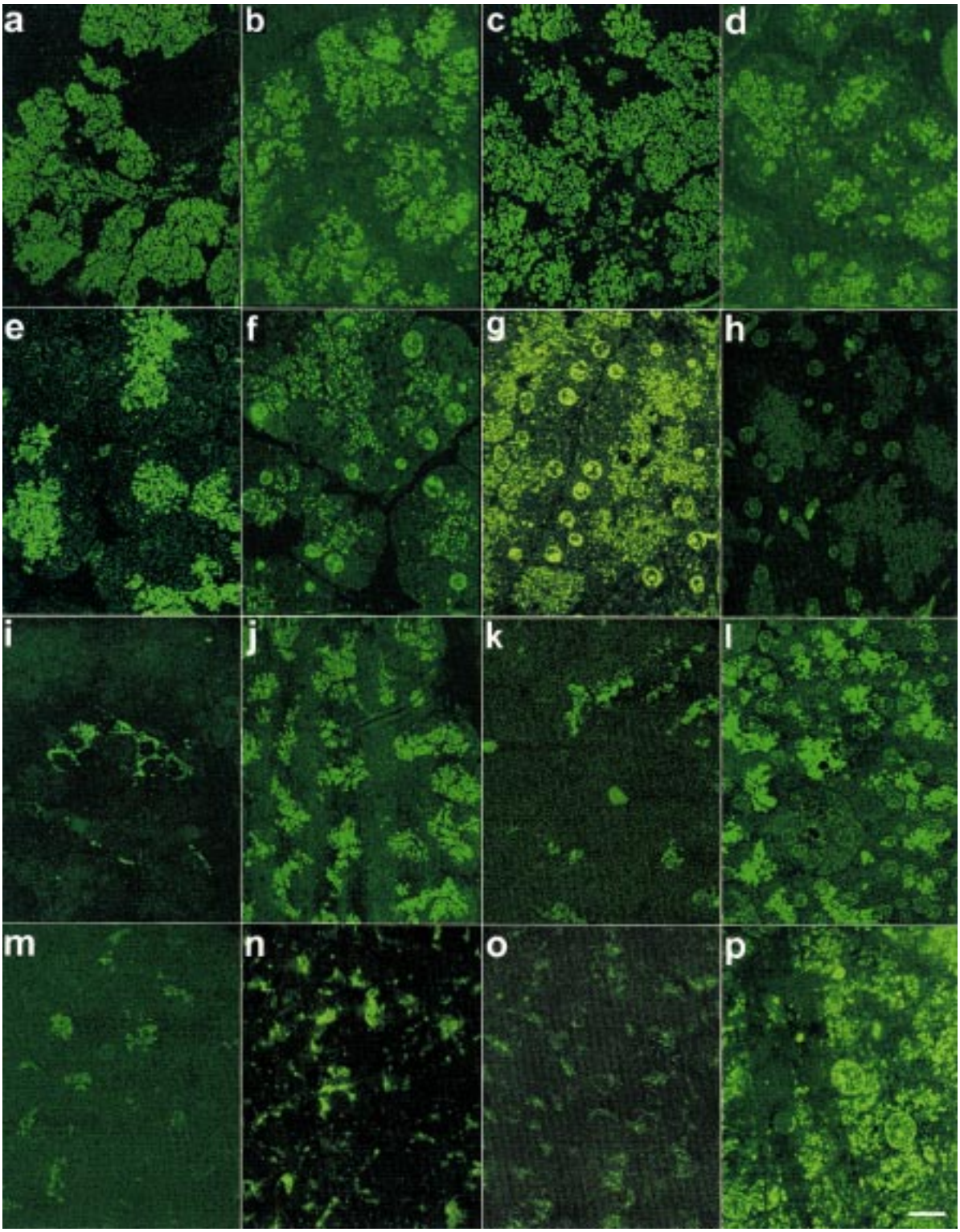

Figure 4. Immunofluorescence detection of antibodies in histologic slides of von Ebner's glands (a–d), pancreas (e–i), parotid (j–m), and liver (n–p). Rats were injected intraperitoneally with saline (control), pilocarpine (cholinergic), or isoproterenol (β-adrenergic), and one hour later von Ebner's glands, pancreas, and parotid were dissected. The liver samples were taken from untreated rats. Antibodies to lingual lipase in von Ebner's glands are shown in (a) (saline) and (b) (pilocarpine). Antibodies to Rab3D in von Ebner's glands are shown in (c) (saline) and (d) (pilocarpine). Antibodies to amylase in the pancreas are shown in (e) (saline) and (f) (pilocarpine). Antibodies to Rab3D in the pancreas are shown in (g) (saline) and (h) (pilocarpine). Antibodies to Rab3B in the pancreas are shown in (i) (saline). Antibodies to amylase in the parotid are shown in (j) (saline) and (k) (isoproterenol). Antibodies to Rab3D in the parotid are shown in (l) (saline) and (m) (isoproterenol). In liver samples (untreated rats), antibodies to amylase are seen in (n), Rab3D in (o), and Rab3B in (p).

as described above (Figures 4f,h), but response to isoproterenol was not changed (not shown). Seen also in Figure 4i, Rab3B was positive in the islets and no changes occurred due to the secretagogues (not shown). Figures 4j–m shows the results in the parotid gland. The saline-treated controls were positive to amylase, Figure 4j, and Rab3D, Figure 4l. Unlike von Ebner's and the pancreas, treatment with isoproterenol greatly diminished the response to amylase antibodies (Figure 4k) and Rab3D antibodies (Figure 4m) in secretory granules. Pilocarpine did not alter the fluorescence response in the parotid (not shown).

These results are expected since the parotid is stimulated to secrete protein by isoproterenol (β-adrenergic) (Putney 1986), whereas protein secretion in von Ebner's and the exocrine pancreas is stimulated by pilocarpine (cholinergic) (Case 1978, Field & Hand 1987). These findings indicate that Rab3D is involved in stimulated secretion.

The liver from an untreated rat showed a moderate response to amylase, a weak response to Rab3D, and a strong response to Rab3B, probably in the hepatocytes, Figures 4n–p, respectively. When the primary antibody was omitted, the response was negative. Examination of serial sections of liver for mast cells revealed the presence of 1 or 2 mast cells every 3–4 sections. These cells were found in the connective tissue around the large blood vessels and not in the vicinity of the hepatocytes.

Discussion

By both immunofluorescence histochemistry and rtPCR we have shown that Rab3D is found in von Ebner's glands, the parotid gland, the exocrine pancreas in the rat, and surprisingly, the liver in the rat and mouse. In previous studies utilizing rtPCR, Western blots, and immunocytochemistry, Rab3D was found in the rat parotid (Ohnishi *et al.* 1996, Raffaniello *et al*. 1999) and the rat pancreas (Ohnishi *et al.* 1996). Rab3D was also found in insulin secreting cells (Iezzi *et al.* 1999). In our experiments, the immunofluorescent response to Rab3D in the exocrine pancreas (Figure 3b) overwhelmed any visualization of islet response to Rab3D. Rab3B, as expected, was found in pancreatic islets (Regazzi *et al.* 1996) and the liver (Weber *et al*. 1994). Islets were readily visible in response to Rab3B, since Rab3B is not found in the exocrine pancreas. The current studies are the first to show the presence of Rab3D in von Ebner's glands and the liver. The fluorescence seen in the liver due to Rab3D was much weaker than Rab3B and weaker than amylase. It was considered that this signal might be due to mast cells which have been shown to contain Rab3D (Roa *et al.* 1997, Tuvim *et al.* 1999). Although mast cells are present in the liver in rat and humans in diseased states, very few mast cells are found in normal tissue (Rioux *et al.* 1996, Armbrust *et al.* 1997). In addition, when serial sections of the liver were stained for mast cells, it was ascertained that there were far too few mast cells to account for the positive effect of Rab3D. It is also possible that the fluorescent response to Rab3D might be an artifact, but a band indicating the presence of Rab3D mRNA was seen in the rtPCR experiments in the liver, reinforcing the observation that Rab3D is found in liver.

Immunofluorescence detection of amylase, using an antibody against human salivary amylase, was observed in the liver. Amylase, that is electrophoretically and immunologically identical to salivary amylase, has been found in rat and mouse liver. Genetic studies indicated that these two amylases were transcribed from the same gene by way of tissue-specific promotors (Meisler & Gumucio 1986).

An interesting finding was that no matter which type of secretory stimulation, β-adrenergic or cholinergic, was tested, or which tissue was involved, the immunofluorescence histochemistry results showed a decrease in the secretory granules of the secreted enzyme along with a decrease in Rab3D in the secretory granules. When the secretagogue did not stimulate secretion, no change in Rab3D was found.

Although Rabs were first found in rat brain and given the name Rab accordingly, many other tissues have one or more Rabs involved in the final steps of exocytosis (Yoshie *et al.* 2000). Understanding the role of Rabs in the exocytotic pathway will be clarified when the exact function of Rabs has been unravelled.

Acknowledgements

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References

Ambudkar IS, Horn VJ, Dai Y, Baum BJ (1990) Evidence against a role for a pertussis toxin-sensitive G protein in Ca^{2+} mobilization in rat parotid acinar cells. *Biochim Biophys Acta* **1055**: 259–264.

Armbrust T, Batusic D, Ringe B, Ramadori G (1997) Mast cells distribution in human liver disease and experimental rat liver fibrosis. Indications for mast cell participation in development of liver fibrosis. *J Hepatol* **26**: 1042–1054

Baldini G, Hohl T, Lin HY, Lodish HF (1992) Cloning of a Rab3 isotype predominately expressed in adipocytes. *Proc Natl Acad Sci USA* **89**: 5049–5052.

Bernstein JJ, Goldberg WJ (1989) Rapid migration of grafted cortical astrocytes from suspension grafts placed in host thoracic spinal cord. *Brain Res* **491:** 205–211.

Case RM (1978) Synthesis, intracellular transport and discharge of exportable proteins in the pancreatic acinar cell and other cells. *Biol Rev* **53**: 211–354.

Darchen F, Senyshyn J, Brondyk WH, Taatjes DJ, Holz RW, Henry J-P, Denizot J-P, Macara IG (1995) The GTPase Rab3a is associated with large dense core vesicles in bovine chromaffin cells and rat PC12 cells. *J Cell Sci* **108**:1639–1649.

Field RB, Hand AR (1987) Secretion of lingual lipase and amylase from rat lingual serous glands. *Am J Physiol* **253**: G217–225.

Field RB, Scow RO (1983) Purification and characterization of rat lingual lipase. *J Biol Chem* **258**: 14563–14569.

Field RB, Spielman AI, Hand AR (1989) Purification of lingual amylase from serous glands of rat tongue and characterization of rat lingual amylase and lingual lipase. *J Dent Res* **68**: 139–145.

Fischer von Mollard G, Stahl B, Khokhlatchev A, Südhof TC, Jahn R (1994a) Rab3C is a synaptic vesicle protein that dissociates from synaptic vesicles after stimulation of exocytosis. *J Biol Chem* **269**: 10971–10974.

Fischer von Mollard G, Stahl B, Li C, Südhof TC, Jahn R (1994b) Rab proteins in regulated exocytosis. *Trends Biochem Sci* **19**: 164–168.

Hand AR (1970) The fine structure of von Ebner's gland of the rat. *J Cell Biol* **44**: 340–353.

Iezzi M, Escher G, Meda P, Charollais A, Baldini G, Darchen F, Wolheim CB, Regazzi R (1999) Subcellular distribution and function of Rab3A, B, C, and D isoforms in insulin-secreting cells. *Mol Endo* **13**: 202–212.

Klengel R, Piiper A, Pittelkow S, Zeuzem S (1997) Differential expression of Rab3 isoforms during differentiation of pancreatic acinar cell line AR42J. *Biochem Biophys Res Commun* **236**: 719–722.

Martinez O, Goud B (1998) Rab proteins. *Biochim Biophys Acta* **1404**: 101–112.

Matteoli M, Takei K, Cameron R, Hurlbut P, Johnston PA, Südhof TC, Jahn R, De Camilli P (1991) Association of Rab3A with synaptic vesicles at late stages of the secretory pathway. *J Cell Biol* **115**: 625–633.

Meisler MH, Gumucio DL (1986) Salivary amylase: evolution and tissue-specific expression. In: Desnuelle P, Sjöström H, Norén O, eds. *Molecular and Cellular Basis of Digestion*. Amsterdam: Elsevier Science Publishers B.V. (Biomedical Division), pp. 457–466.

Novick P, Zerial M (1997) The diversity of Rab proteins in vesicle transport. *Curr Opin Cell Biol* **9**: 496–504.

Ohnishi H, Ernst SA, Wys N, McNiven M, Williams JA (1996) Rab3D localizes to zymogen granules in rat pancreatic acini and other exocrine glands. *Am J Physiol* **271**: G531–G538.

Putney, Jr. JW (1986) Identification of cellular activation mechanisms associated with salivary secretion. *Ann Rev Physiol* **48**: 75–88.

Raffaniello RD, Lin J, Schwimmer R, Ojakian GK (1999) Expression and localization of Rab3D in rat parotid gland. *Biochim Biophys Acta* **1450**: 352–363.

Raffaniello RD, Lin J, Wang F, J-P. Raufman J-P (1996) Expression of Rab3D in dispersed chief cells from guinea pig stomach. *Biochim Biophys Acta* **1311**: 111–116.

Regazzi R, Ravazzola M, Iezzi M, Lang J, Zahraoui A, Andereggen E, Morel P, Takai Y, Wolheim CB (1996) Expression, localization and functional role of small GTPases of the Rab3 family in insulin-secreting cells. *J Cell Sci* **109**: 2265–2273.

Richardson KC, Jarett L, Finke EH (1960) Embedding in epoxy resin for ultra thin sectioning in electron microscopy. *Stain Technology* **35**: 313–321.

Rioux KP, Sharkey KA, Wallace JL, Swain MG (1996) Hepatic mucosal mast cell hyperplasia in rats with secondary biliary cirrhosis. *Hepatology* **23**: 888–895.

Roa M, Paumet F, Le Mao J, David B, Blank U (1997) Involvement of the *ras*-like GTPase rab3d in RBL-2H3 mast cell exocytosis following stimulation via high affinity IgE receptors (FcεRI). *J Immunol* **159**: 2815–2823.

Schimmöller F, Simon I, Pfeffer SR (1998) Rab GTPases, directors of vesicle docking. *J Biol Chem* **273**: 22161–22164.

Schneyer CA (1965) Modification of the action of pilocarpine by adrenergic blocking agents. *Proc Soc Exp Biol Med* **120**: 230–232.

Schneyer CA, Hall HD (1965) Comparison of rat salivas evoked by auriculo-temporal and pilocarpine stimulation. *Am J Physiol* **209**: 484–488.

Tuvim MJ, Adachi R, Chocano JF, Moore RH, Lampert RM, Zera E, Romero E, Knoll BJ, Dickey BF (1999) Rab3D, a small GTPase, is located on mast cell secretory granules and translocates to the plasma membrane upon exocytosis. *Am J Respir Cell Mol Biol* **20**: 79–89.

Weber E, Berta G, Tousson A, St. John P, Green MW, Gopalokrishnan U, Jilling T, Sorscher EJ, Elton TS, Abrahamson DR, Kirk K (1994) Expression and polarized targeting of a Rab3 isoform in epithelial cells. *J Cell Biol* **125**: 583–594.

Yoshie S, Imai A, Nashida T, Shimomura H (2000) Expression, characterization, and localization of Rab 26, a low molecular weight GTP-binding protein, in the rat parotid gland. *Histochem Cell Biol* **113**: 259–263.