Secretin is not necessary for exocrine pancreatic development and growth in mice

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Sans MD, Sabbatini ME, Ernst SA, D’Aley LG, Nishijima I, Williams JA. Secretin is not necessary for exocrine pancreatic development and growth in mice. Am J Physiol Gastrointest Liver Physiol 301: G791–G798, 2011. First published August 18, 2011; doi:10.1152/ajpgi.00245.2011.—Adaptive exocrine pancreatic growth is mediated primarily by dietary protein and the gastrointestinal hormone cholecystokinin (CCK). Feeding trypsin inhibitors such as camostat (FOY-305) is known to induce CCK release and stimulate pancreatic growth. However, camostat has also been reported to stimulate secretin release and, because secretin often potentiates the action of CCK, it could participate in the growth response. Our aim was to test the role of secretin in pancreatic development and adaptive growth through the use of C57BL/6 mice with genetic deletion of secretin or secretin receptor. The lack of secretin in the intestine or the secretin receptor in the pancreas was confirmed by RT-PCR. Other related components, such as vasoactive intestinal polypeptide (VIP) receptors (VPAC1 and VPAC2), were not affected. Secretin increased cAMP levels in acini from wild-type (WT) mice but had no effect on acini from secretin receptor-deficient mice, whereas VIP and forskolin still induced a normal response. Secretin in vivo failed to induce fluid secretion in receptor-deficient mice. The pancreas of secretin or secretin receptor-deficient mice was of normal size and histology, indicating that secretin is not necessary for normal pancreatic differentiation or maintenance. When WT mice were fed 0.1% camostat in powdered chow, the pancreas doubled in size in 1 wk, accompanied by parallel increases in protein and DNA. Camostat-fed littermate secretin and secretin receptor-deficient mice had similar pancreatic mass to WT mice. These results indicate that secretin is not required for normal pancreatic development or adaptive growth mediated by CCK.

secretin; cholecystokinin; trypsin inhibitor; pancreatic growth

The adult exocrine pancreas maintains a considerable degree of plasticity. In addition to regulating the mix of digestive enzymes synthesized in response to dietary constituents (3), the pancreas can grow or atrophy in response to the need for digestive enzymes (34). Pancreatic growth occurs in response to adaptation to hyperphagia (23, 25), high-protein diets (13, 18), and ingestion of trypsin inhibitor (17, 26, 40). The latter has proven to be a valuable model for studying adaptive pancreatic growth and is dependent on the release of endogenous CCK when feedback of luminal trypsin activity is blocked. In mice or rats fed either soybean trypsin inhibitor or small chemical inhibitors such as camostat (FOY-305), plasma cholecystokinin (CCK) is elevated and the pancreas doubles in size over a 7- to 10-day period (17, 26, 28, 40). This growth is primarily hyperplasia and driven by mitogenesis of acinar cells but in some cases involves a component of hypertrophy where protein mass increases relative to DNA and cells become larger. In mice, the adaptive pancreatic growth in response to camostat is abolished in CCK or CCK receptor-deficient mice showing the primary importance of CCK (36, 40). CCK acts by increasing intracellular calcium, and recent work has shown that the calcium-activated phosphatase calcineurin is involved in protein synthesis (35) and growth (19, 39, 40).

Whether CCK is the only gastrointestinal hormone mediating the response to trypsin inhibitor is uncertain. The other major hormonal regulator of the pancreas is secretin, which is released in response to acid or fat in the duodenum and stimulates pancreatic secretion of water and bicarbonate (8). The secretin receptor was cloned in 1991; is a class II G protein-coupled receptor (27), and couples through the heterotrimeric G protein Gs to adenylyl cyclase (AC) to increase cAMP levels (15). Exogenous secretin is known to potentiate many effects of CCK in the pancreas, including pancreatic acinar cell enzyme secretion in some species (11, 37) and pancreatic growth (29, 38) in response to exogenous CCK or caerulein, a CCK analog. Moreover, one study in rats showed that intraduodenal administration of camostat increased plasma secretin and that pancreatic exocrine secretion stimulated by camostat was dependent on elevated plasma levels of both CCK and secretin (44).

In the last few years, a new approach to studying the importance of secretin in pancreatic growth has emerged with the development of secretin receptor (Sctr) and secretin (Sct)-deficient mice (7, 10, 22, 30, 46). While these mice have been used to study the effects of secretin on the brain and behavior, as well as on renal water absorption and food intake, they have not been used to study the effect of deleting secretin action on the pancreas. Therefore, we studied the effects of secretin on pancreatic growth and found that the pancreas develops normally in mice without secretin action. The response to exogenous secretin is lost both in vivo and in vitro experiments in the absence of the secretin receptor. Moreover, the pancreas is able to grow normally in response to feeding the synthetic trypsin inhibitor camostat. The lack of secretin involvement supports the concept that CCK is the primary relevant trophic hormone required for pancreatic growth.

MATERIALS AND METHODS

Materials. Collagenase was from Worthington (Lakewood, NJ), secretin and vasoactive intestinal polypeptide (VIP) were from American Peptide (Sunnyvale, CA), and forskolin, 3-isobutyl-1-methylxanthine (IBMX), soy bean trypsin inhibitor, and rabbit polyclonal anti-amylase antibody were from Sigma-Aldrich (St. Louis, MO). The
rat monoclonal anti-cytokeratin 19 antibody (TROMA III) was from the Developmental Studies HybriDioma Bank, University of Iowa. Camostat (FOY-305) was from Santa Cruz Biotechnology (Santa Cruz, CA), protease inhibitors were from Roche (Indianapolis, IN), and DMEM media were from GIBCO (Invitrogen, Carlsbad, CA). Vetbond tissue adhesive was from 3M Animal Care Products (St. Paul, MN). Other chemical reagents were obtained from Sigma Chemical.

**Animals and care.** Secretin (Sct)- and secretin receptor (Sctr)-deficient mice were prepared as described (22, 30, 46). Male Sct−/− and Sctr−/− mice were bred in our facilities from heterozygotes and were maintained on a 12:12-h light-dark cycle with free access to water and chow (5001 Rodent Diet; PMI Nutrition International, St. Louis, MO). Littermate wild-type (WT) mice were used as controls. All animals were killed by exsanguination under carbon dioxide asphyxiation. The University of Michigan Committee on Use and Care of Animals approved the animal facilities and the experimental protocol used in these studies.

**RT-PCR.** The expression of secretin, secretin receptor, and VPAC1 and VPAC2 receptors was assessed by RT-PCR. Total RNA was isolated from intestine, brain, and pancreas using TRIzol reagent (Invitrogen) from secretin and secretin receptor WT and knockout (KO) mice to synthesize first-strand cDNAs with the TaqMan RT-PCR kit (Applied Biosystems, Branchburg, NJ). Intactness of RNA was assessed by optical density ratio (260 to 280 nm) and agarose gel electrophoresis. One microgram of cDNA was used in each PCR reaction unless otherwise indicated. Amplification with Taq DNA Polymerase from the Expand High Fidelity Enzyme Mix kit (Roche Diagnostics) was conducted using specific primers for secretin (46), secretin receptor (30), and VPAC1 and VPAC2 receptors (2) (Table 1).

**Isolation of pancreatic acini and measurement of amylase secretion and cAMP levels.** Pancreatic acini from WT, Sct−/−, and Sctr−/− mice were prepared by enzymatic digestion with collagenase followed by mechanical shearing. Amylase secretion was determined as previously described (32). Acini were stimulated with either secretin (0.1, 1, 10, and 100 nM) or VIP (0.1, 1, 10, and 100 nM) in 1-ml aliquots in plastic blood dilution vials at 37°C for 30 min. Samples were centrifuged for 30 s in a microcentrifuge, and the supernatant was freeze-dried and stored at −20°C until assayed for amylase activity using Phadebas reagents (Magle Life Sciences, Land, Sweden). Results were expressed as a percentage of the initial acinar amylase content.

CAMP generation was determined as previously described (33). Acini were preincubated for 30 min in DMEM without phenol red and then for 3 min in fresh DMEM without phenol red containing 1 mM IBMX. Acini were stimulated with secretin (0.1, 1, 10, and 100 nM), VIP (10 nM), or forskolin (20 μM) for 12 min. CAMP was extracted in absolute ethanol and measured using a CAMP colorimetric enzyme immunoassay kit according to the instructions provided by the manufacturer (Cayman Chemical, Ann Arbor, MI). Results were expressed as picojoules per milligram protein.

**Measurement of pancreatic fluid secretion in vivo.** Mice were anesthetized with isoflurane. The common bile duct was exposed and cannulated (PE/01; Scientific Commodities, Lake Havasu City, AZ) to divert bile. A second cannula (PE/08; Scientific Commodities) was placed at the distal end of the common bile pancreatic duct near the duodenum and fixed in place with a tissue adhesive to collect pure pancreatic secretion. A 31-gauge needle (TSK Laboratory; Air-Tite Products, Virginia Beach, VA) was inserted in the left jugular vein. This needle was connected to a syringe containing secretin via PE-10 tubing. Pancreatic secretion was allowed to flow for 15 min after which pancreatic secretion was collected for 30 min previous to intravenous stimulation with secretin (2 μg/kg). Because of the flow rate, the volume of secretion was calculated from the length of the column of fluid. Results were expressed as nanoliters per 30 min per gram of body weight.

**Quantitation of pancreatic growth.** In these experiments, animals (6–8 wk of age) were acclimated to chow in powdered form for 2 days before experimental manipulation. Animals were then divided into groups provided with either standard powdered chow or powdered chow containing 0.1% camostat for 7 days. Mice were killed under carbon dioxide, and the pancreas was quickly excised and weighed. Portions of the pancreas were either processed immediately or frozen in liquid nitrogen for DNA and protein analysis. Following determination of total pancreatic wet weight, a frozen portion was weighed and homogenized in 0.3 M NaOH (2 ml/100 mg pancreas) and subsequently sonicated for 15 s. Protein was determined spectrophotometrically using Bio-Rad protein assay reagent. DNA was assayed using a DNA Quantitation Kit (Sigma-Aldrich) and a Perkin-Elmer LS 55 luminescence spectrometer (Perkin-Elmer Instruments, Norwalk, CT). Total pancreatic protein and DNA were then calculated. Results were expressed as milligram per gram of body weight.

**Histology.** Small blocks of pancreas were fixed with 10% formalin, embedded in paraffin blocks, processed for hematoxylin and eosin staining, and examined with an Olympus BX-51 light microscope equipped with a digital camera. For immunohistochemistry, standard procedures were used and cryostat sections of tissue fixed for 30 min with 1% formaldehyde (freshly prepared from paraformaldehyde) (6, 31). The primary antibody dilutions were 1:1,000 for the polyclonal anti-amylase and 1:15 for the rat monoclonal anti-TROMA III antibody. Secondary antibodies were anti-rat-Alexa 594 and anti-rabbit-Alexa 488. Prolong Gold with 4,6-diamino-2-phenylinodole was added to mounting medium to counterstain nuclei. Fluorescence images were taken with an Olympus BX-51 microscope and processed with Adobe Photoshop.

**Statistical analysis.** Results are expressed as means ± SE, which were obtained from 3–5 different experiments with a total of 9–14 mice per each condition. Statistical analysis was carried out by ANOVA followed by Student-Newman-Keuls or Dunnet’s post hoc testing performed by Instat Graphpad software. *P* ≤ 0.05 was considered to be the minimal level of statistical significance.

**Table 1. Amplification data**

<table>
<thead>
<tr>
<th>Primers Sequence</th>
<th>Annealing Temperature, °C</th>
<th>Product Sizes, bp</th>
</tr>
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<tbody>
<tr>
<td>Secretin Forward</td>
<td>5'–CGGACCCCAAGACACTGAGCAG-3'</td>
<td>60</td>
</tr>
<tr>
<td>Secretin Reverse</td>
<td>5'–GTCTAGTCCACCTCCTGAGGATGTC-3'</td>
<td>60</td>
</tr>
<tr>
<td>Secretin receptor Forward</td>
<td>5'–TCGGATGGGTCTCTCCAGAC-3'</td>
<td>61</td>
</tr>
<tr>
<td>Secretin receptor Reverse</td>
<td>5'–CTGATGCTGAGGGGCGCTTGCGT-3'</td>
<td>55</td>
</tr>
</tbody>
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VPAC1, vasoactive intestinal polypeptide (VIP) receptor 1; VPAC2, VIP receptor 2.
RESULTS

Mice. WT, heterozygous, and Sct\(^{-/-}\) and Sctr\(^{-/-}\) mice were born at the expected 1:2:1 Mendelian ratio from the heterozygous parents, suggesting that no embryonic lethality was associated with KO mice, similar to previous descriptions (30, 46). Sct\(^{-/-}\) and Sctr\(^{-/-}\) mice showed similar body weights at 6–9 wk compared with those of their WT littermates, indicating no growth abnormality in mice, as previously indicated (30, 46).

RT-PCR analysis of expression of secretin and secretin receptor. To document the expression of secretin, RNA was obtained from the intestine of WT and Sct\(^{-/-}\) mice. RT-PCR analysis showed expression of secretin mRNA in WT mice but not in Sct\(^{-/-}\) mice (Fig. 1A), confirming the lack of secretin expression in those mice. The expression of secretin receptor was analyzed in brain and whole pancreas from WT and Sctr\(^{-/-}\) mice. RT-PCR products yielded a band of the expected size (402 bp) only in tissues from WT mice (Fig. 1B). The expression of VPAC receptors (VPAC\(_1\) and VPAC\(_2\)) was similar in Sct\(^{-/-}\) mice (Fig. 1C), confirming the specificity of the Sctr\(^{-/-}\) mice. Both VPAC\(_1\) and VPAC\(_2\) were expressed in brain, but only VPAC\(_2\) was expressed in pancreas, as previously shown (21).

The lack of secretin receptor does not affect the development of the pancreas. In addition to the KO mice being normal sized, the weight of the pancreas was also normal, being 11.1 ± 0.5 mg/g body wt in WT mice, 11.5 ± 0.6 in Sct\(^{-/-}\) mice, and 10.1 ± 0.1 in Sctr\(^{-/-}\) mice.

In addition, the structure of the pancreas, including ducts and centroacinar cells, appeared normal in cryostat sections of mice. The expression of VPAC receptors (VPAC\(_1\) and VPAC\(_2\)) was not affected in Sctr\(^{-/-}\) mice (Fig. 1C), confirming the specificity of the Sctr\(^{-/-}\) mice. Both VPAC\(_1\) and VPAC\(_2\) were expressed in brain, but only VPAC\(_2\) was expressed in pancreas, as previously shown (21).

Pancreatic secretion in vivo induced by secretin is absent in Sctr\(^{-/-}\) mice. A decrease in basal pancreatic secretion was observed in Sctr\(^{-/-}\) mice (2.0 ± 1.2 vs. 0.4 ± 0.4 nl·30
Although variability in those mice prevented determining if there was a statistically significant difference. The administration of secretin (2 U/kg iv) increased pancreatic fluid from secretin receptor WT mice up to eightfold, but not from Sctr−/− mice (Fig. 5).

Secretin receptor deletion does not affect camostat-induced pancreas growth. Camostat feeding effects on pancreas were also analyzed in Sct−/− mice. Pancreas weight (corrected by body wt) was increased in the WT mice after 7 days of camostat feeding compared with control mice. Values are means ± SE; n = 7 (A) and 3 (B). *P < 0.05 and **P < 0.01 vs. control.

Secretin deletion does not modify camostat-induced pancreas growth. Camostat feeding effects on pancreas were also analyzed in Sct−/− mice. Pancreas weight (corrected by body wt) was increased in the WT mice after 7 days of camostat feeding compared with control mice. Values are means ± SE; n = 5 mice. *P < 0.05 vs. basal WT mice. #P < 0.01 vs. secretin receptor WT mice.

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Fig. 3. Secretin fails to induce cAMP generation in secretin receptor-deficient mice while vasoactive intestinal polypeptide (VIP) and forskolin (FSK) stimulate normally. Fresh pancreatic acini from WT or Sctr−/− mice were stimulated with different concentrations of secretin (A), VIP (B), or the direct adenyl cyclase (AC) stimulator forskolin (B). The content of cAMP was determined using an enzymatic immunoassay kit and expressed as pmol/mg protein. Values are means ± SE; n = 5 mice. *P < 0.05 and **P < 0.01 vs. control. #P < 0.05 and ##P < 0.01 vs. secretin receptor WT mice.

Fig. 4. The increase in amylase secretion induced by secretin is reduced from Sctr−/− mice. Fresh pancreatic acini from WT or Sctr−/− mice were stimulated with different concentrations of either secretin (A) or VIP (B). Amylase release was expressed as a percentage of total amylase. In pancreatic acini from Sctr−/− mice, only the stimulatory effect of secretin was absent. Values are means ± SE; n = 7 (A) and 3 (B). *P < 0.05 and **P < 0.01 vs. control.

Fig. 5. Secretin-stimulated pancreatic secretion is abolished from secretin receptor-deficient mice. Anesthetized WT or Sctr−/− mice were prepared with a cannula in the bile duct near the liver and another one near the ampulla of Vater to collect pure pancreatic fluid. The stimulation of iv secretin (2 U/kg) induced a significant increase in pancreatic secretion from secretin receptor WT mice but not Sctr−/− mice. Values are means ± SE; n = 5 mice. *P < 0.05 vs. basal WT mice. #P < 0.01 vs. secretin receptor WT mice.
feeding, and the lack of secretin did not modify the camostat-induced increase in pancreas weight (Fig. 7A). The camostat-induced increases on total protein and DNA were also not affected by secretin deficiency (Fig. 7, B–D). All of these results indicate that secretin signaling is not required for the camostat-induced pancreas growth.

**Secretin and secretin receptor deletion do not modify pancreas morphology.** Camostat feeding for 7 days induced an increase in acinar cell size, as already seen in previous studies (40) (Fig. 8B), compared with the control pancreas (Fig. 8A). Secretin receptor deletion did not induce any change on pancreatic morphology of control (Fig. 8C) or camostat-fed (Fig. 8D) mice. Deletion of secretin similar to deletion of the secretin receptor did not induce any change either on pancreatic morphology of control or camostat-fed mice (data not shown). These results indicate that secretin signaling deficiency in the pancreas does not cause any histological damage or change compared with WT mice.

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**Fig. 6.** Secretin receptor deletion does not affect pancreas growth. Pancreas wt/body wt (PW/BW) in WT and secretin deletion (Sctr-/-) mice fed control (CTL) or 0.1% camostat (CAM) diet (A), as well as total protein (B), total DNA (C), and the protein-to-DNA ratio (D), were analyzed after 7 days of camostat feeding. Values are means ± SE; n = 9–11 mice. *P < 0.05 vs. control WT mice.

**Fig. 7.** Secretin deletion does not modify pancreas growth. PW/BW in WT and Sct-/- mice fed CTL or 0.1% CAM diet (A), as well as total protein (B), total DNA (C), and the protein-to-DNA ratio (D), were analyzed after 7 days of camostat feeding. Values are means ± SE; n = 9–11 mice. *P < 0.05 vs. control WT mice.
DISCUSSION

This study demonstrates that secretin signaling is not necessary for normal mouse pancreatic development and adaptive growth in adult mice. The pancreas of both Sct\(^{-/-}\) and Sctr\(^{-/-}\) mice develops normally, as indicated by normal pancreas histology and immunohistochemistry of the ductal and acinar cells (Fig. 2). Pancreatic adaptive growth induced by feeding camostat to increase plasma CCK is not affected either by the lack of secretin or the secretin receptor in the pancreas, as indicated by pancreas weight and by the analysis of total pancreatic DNA and protein (Figs. 6 and 7).

Secretin was originally identified in dogs where an extract of duodenal mucosa was shown to increase pancreatic fluid secretion in the absence of neural connections. Later, secretin was isolated as a 27-amino-acid polypeptide and shown to be produced and secreted by a particular class of enteroendocrine cells. Secretin was also identified in the brain. The secretin receptor is present in the pancreas, brain, liver (cholangiocytes), and stomach. In the pancreas, the secretin receptor has been localized to duct and acinar cells by receptor autoradiography (42).

While the primary function of secretin in the pancreas is to stimulate ductal secretion, exogenous secretin has been shown to potentiate some actions of CCK and acetylcholine on acinar cells. Secretin action has been most studied with regard to digestive enzyme secretion, and, in fact, isolated pancreatic acini have been a primary target used to study secretin action due in large part to their ready availability. In isolated acini, secretin stimulates cAMP formation and thereby potentiates amylase secretion induced by CCK, which signals through calcium (4, 11, 14). Whether this action is part of the physiological in vivo response to a meal is unclear (37). Similarly, secretin administration is known to potentiate the growth response to exogenous CCK in rats (29, 38). However, the present study shows that secretin is not necessary for adaptive pancreatic growth in mice. That difference could be because large doses of secretin and CCK were administered in those previous studies, and, for that reason, the physiological role of secretin was uncertain.

The pancreatic growth response to feeding camostat is believed to be mediated by CCK and to involve activation of the calcineurin-nuclear factor of activated T cells (19, 20), mammalian target of rapamycin pathways (12), and possibly the mitogen-activated protein kinase pathways. cAMP, the primary intracellular mediator of secretin in acini, does not significantly regulate these pathways although it could synergize. Rather, in exocrine pancreas, cAMP activates protein kinase A and increases GTP-Rap1 levels (5, 32); at present, neither of these has been reported to be involved in pancreatic growth. The importance of the cAMP signaling pathways in the pancreas can also be inferred from a study of the deletion of G\(_{\alpha}\) by using a Pdx1-Cre (45). Interestingly, these mice had reduced \(\beta\)-cells and were hyperglycemic, but the overall pancreas size was increased relative to body weight, which was ascribed to ductal enlargement. Further study of such a model to look specifically at acinar or ductal cells would be useful.

Fig. 8. Secretin receptor deletion does not change pancreas morphology. Pancreas hematoxylin and eosin histological samples of WT CTL, WT CAM, Sctr\(^{-/-}\) CTL, and Sctr\(^{-/-}\) CAM were analyzed after 7 days of camostat feeding. No effects of secretin receptor deletion were observed in the control and camostat-fed mice.
Similar to the pancreas, in the liver, secretin is also known to stimulate bicarbonate secretion by biliary ductules by a comparable mechanism involving cAMP and the cystic fibrosis transport receptor. Bile ducts are known to proliferate in response to ligation of the main duct as part of what is believed to be a compensatory response. Recently, this proliferative response in large cholangiocytes was shown to be absent in Sctr−/− mice. Whether this response is triggered by increased pressure or cholestasis is unclear. These findings support the lack of secretin effect in the pancreatic development seen in this study. Interestingly, by contrast to the liver, pancreatic duct ligation induces either pancreatitis (24, 41) or atrophy (1, 43).

The characterization of the Sctr−/− mice also provided valuable information regarding secretin and its exocrine function. The basal pancreatic juice flow from Sctr−/− mice was reduced, and there was no stimulation by secretin in vivo (Fig. 5). Supporting that secretin plays a central role in the maintenance of basal secretion. Secretin stimulation of cAMP generation (Fig. 3A) and amylase release in vitro (Fig. 4A) were also reduced in the Sctr−/− mice. At the same time, we have seen this reduction of the effects of secretin does not affect cAMP levels in acinar cells in response to VIP or forskolin (Fig. 3) or the stimulation of amylase release in vitro by VIP (Fig. 4B).

This study is the first to evaluate pancreatic function in mice with a genetic ablation of secretin signaling. Although a reduction of pancreatic secretion has been seen, the Sctr−/− mice showed no signs of malabsorption, as indicated by normal feces. Thus, either pancreatic bicarbonate-rich fluid is not essential for normal digestion in mice or else other regulators such as acetylcholine or VIP are stimulating pancreatic secretion. While the action of secretin on ducts was not the aim of our study, further studies in this area using the Sctr−/− or Sctr−/− mice could provide valuable insights into the physiology of pancreas.

In conclusion, this study demonstrates that secretin is not involved in pancreas development and adaptive growth. Sctr−/− and Sctr−/− mice have not shown any modification in regard to the pancreas weight and the growth parameters analyzed. These results prove that secretin is not required for at least some pancreatic physiological mechanisms not involved in fluid secretion. Thus, CCK appears to be the primary relevant trophic hormone required for adaptive pancreatic growth.

ACKNOWLEDGMENTS
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REFERENCES


