Original Research

Pancreatic acinar cells utilize tyrosine to synthesize L-dihydroxyphenylalanine

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Impact statement

Dopamine, as a peripheral bioactive substance, plays a variety of regulatory roles in the pancreas, such as regulating hormones secretion, reducing inflammation, and affecting tumor growth. It has been known that pancreatic islet β cells can synthesize dopamine from L-DOPA, but the role of pancreatic acinar cells in the dopamine synthesis remains unclear. Our results indicate that pancreatic acinar cells could utilize tyrosine to synthesize L-DOPA, not DA, and that islet β cells used L-DOPA, not tyrosine, to synthesize DA. This study explores the synthesis process of L-DOPA and DA in pancreas, which is of great significance to reveal the normal physiological functions of DA system and the application of antipsychotic drugs.

Abstract

The pancreatic β cells can synthesize dopamine by taking L-dihydroxyphenylalanine, but whether pancreatic acinar cells synthesize dopamine has not been confirmed. By means of immunofluorescence, the tyrosine hydroxylase -immunoreactivity and aromatic amino acid decarboxylase (AADC)- immunoreactivity were respectively observed in pancreatic acinar cells and islet β cells. Treatment with L-dihydroxyphenylalanine, not tyrosine, caused the production of dopamine in the incubation of INS-1 cells (rat islet β cell line) and primary isolated islets, which was blocked by AADC inhibitor NSD-1015. However, only L-dihydroxyphenylalanine, but not dopamine, was detected when AR42J cells (rat pancreatic acinar cell line) were treated with tyrosine, which was blocked by tyrosine hydroxylase inhibitor AMPT. Dopamine was detected in the coculture of INS-1 cells with AR42J cells after treatment with tyrosine. In an *in vivo* study, pancreatic juice contained high levels of L-dihydroxyphenylalanine and dopamine. Both L-dihydroxyphenylalanine and dopamine accompanied with pancreatic enzymes and insulin in the pancreatic juice were all significantly increased after intraperitoneal injection of bethanechol chloride and their increases were all blocked by atropine. Inhibiting TH with AMPT blocked bethanechol chloride-induced increases in L-dihydroxyphenylalanine and dopamine, while inhibiting AADC with NSD-1015 only blocked the dopamine increase. Bilateral subdiaphragmatic vagotomy of rats leads to significant decreases of L-dihydroxyphenylalanine and dopamine in pancreatic juice. These results suggested that pancreatic acinar cells could utilize tyrosine to synthesize L-dihydroxyphenylalanine, not dopamine. Islet β cells only used L-dihydroxyphenylalanine, not tyrosine, to synthesize dopamine. Both L-dihydroxyphenylalanine and dopamine were respectively released into the pancreatic duct, which was regulated by the vagal cholinergic pathway. The present study provides important evidences for the source of L-dihydroxyphenylalanine and dopamine in the pancreas.

Keywords: Pancreatic acinar cells, tyrosine hydroxylase, L-DOPA, dopamine, pancreatic juice, physiology

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Introduction

Dopamine (DA) plays a variety of important regulatory roles in the pancreas. It has been reported that DA inhibits insulin secretion,[1,2] modulates pancreatic insulin-producing cell neogenesis in mice on embryonic day (12.5–13.5),[3] reduces inflammation in cholecystokinin-stimulated pancreatic acinar cells,[4] and is involved in

pancreatic tumor growth.[5] The pancreas is able to synthesize DA by itself,[1,6,7] and the contents of L-dihydroxyphenylalanine (L-DOPA) and DA in the pancreas increase with increasing tyrosine content in the diet.[8] Intraperitoneal injection of L-DOPA (50 mg/kg) can increase the content of DA in pancreatic tissue.[9] DA synthases,[4,10,11] dopamine-metabolizing enzymes catechol-O-methyltransferase (COMT),[12] monoamine oxidase (MAO),[13] dopamine transporter (DAT),[12,14] and L-type amino-acid transporters (LATs)[1] are all expressed in pancreatic tissue, which further suggests that the pancreas can synthesize DA. It is known that pancreatic islet β cells can synthesize DA by using L-DOPA,[1,7,9] but the role of pancreatic acinar cells in the synthetic process of pancreatic DA remains controversial.

Tyrosine is converted to L-DOPA by tyrosine hydroxylase (TH), followed by decarboxylation to DA by aromatic amino acid decarboxylase (AADC).[15] It has been reported that pancreatic acinar cells have the ability to synthesize DA based on the distribution of TH immunoreactivity (IR) in acinar cells and the detection of DA in the pancreas.[4,16] However, it is unclear whether DA is released from acinar cells or islet β cells and it is also unknown whether acinar cells express AADC *in situ*. Pancreatic islets are also considered to be potential sites for DA synthesis based on the localization of AADC-IR in islet β cells[10,11] and the detection of DA in the supernatant of primary cultured islets treated with L-DOPA.[1,9] However, it remains unclear whether islet β cells can utilize tyrosine to synthesize DA.

In the present study, we aimed to investigate the distribution of TH and AADC in pancreatic acinar cells and islet β cells, and the synthesis of L-DOPA and DA in pancreatic exocrine and endocrine by means of immunofluorescence, primary rat islet isolation, pancreatic juice drainage, and high-performance liquid chromatography (HPLC). This study provides new experimental and theoretical evidence for the synthesis of L-DOPA and DA in the pancreas, which may provide a new target for the application of DA-related drugs in the diagnosis and treatment of pancreatic diseases.

Materials and methods

Animals and tissue preparation

Adult Sprague-Dawley (SD) rats (220–250 g, 16–18 weeks old) were provided by the Laboratory Animal Services Center of Capital Medical University. All experimental operations conformed to the ethical guidelines established by the National Institutes of Health and were approved by the Animal Care and Use Committee of Capital Medical University, Beijing, China. All animals were housed under specific-pathogen-free conditions in individually ventilated cages (≤3 rats per cage) on a 12-h light/dark cycle and had free access to a standard rodent diet and water. All animals were euthanized by anesthesia with isoflurane (RWD Life Science Co., Ltd, Nanshan District, Shenzhen), after which the abdominal wall was opened, and the pancreatic tissue was quickly removed for pancreatic section preparation or determination of the L-DOPA and DA contents. During the experiments, we tried to minimize the pain of animals and used the minimum number of animals needed to produce reliable scientific data.

Immunofluorescence

For immunofluorescence (IF) staining, paraffin-embedded pancreatic sections (5 μM thickness) were torrefied, dewaxed, and then hydrated as the standard protocol. Prior to staining, microwave heat-induced antigen retrieval was required. Then, the sections were washed with phosphate buffer containing 0.3% Triton X-100 (PBST). After blocking with 5% donkey serum for 30 min, the sections were incubated with primary antibodies at 4°C overnight (Table 1). After washing with PBST (10 min × 3), the sections were incubated with secondary antibodies for 2 h at room temperature (Table 2). Then, the nuclei were stained with 4, 6-diamidino-2-phenylindole (DAPI) for 5 min. Finally, the sections were observed under a laser scanning confocal microscope (FV1000, Olympus, Tokyo, Japan). Colocalization studies were performed using double-labeling immunofluorescence with an antibody against TH or AADC and an antibody against insulin.

For IF staining of INS-1 and AR42J cell lines, cells were washed with phosphate buffer saline (PBS, 2 min × 3), digested, and inoculated on a round cover glass in the 24-well plate. The cells were cultured overnight in the incubator and IF staining was performed after the cells adhered stably. The cells were fixed with 4% paraformaldehyde solution (PFA) for 15 min, washed with PBST for 5 min × 3, blocked with 5% donkey serum for 30 min, and the staining process for primary and secondary antibodies was the

Table 1. Primary antibodies used in this study.

Primary antibody	Code	Company	Host species	Dilution
Anti-Insulin antibody	I2018-2ML	Sigma	Mouse	1:1000
Anti-TH antibody	2792S	Cell signaling Technology	Rabbit	1:100
Anti-AADC antibody	Sc-99203	Santa	Rabbit	1:100

Table 2. Secondary antibodies used in this study.

Secondary antibody	Code	Company	Dilution
Alexa Fluor® 488 donkey anti-mouse IgG (H + L)	A21202	Invitrogen	1:1000
Alexa Fluor® 594 donkey anti-mouse IgG (H + L)	A21203	Invitrogen	1:1000
Alexa Fluor® 488 donkey anti-rabbit IgG (H + L)	A21206	Invitrogen	1:1000
Alexa Fluor® 594 donkey anti-rabbit IgG (H + L)	A21207	Invitrogen	1:1000

same as pancreatic sections. After the cells were stained with DAPI, the cover glass in the 24-well plate was taken out, and the surface of the inoculated cells was attached to the slide with 50% glycerin and the cells were observed under the laser scanning confocal microscope. All co-staining experiments were repeated at least three times, observed and photographed with different magnifications.

Pancreatic juice drainage

Pancreatic juice drainage was performed according to a previous study.[17] Adult SD rats were anesthetized with an American Matrx-VMR small animal anesthesia machine to maintain isoflurane (RWD Life Science Co., Ltd, Nanshan District, Shenzhen) inhalation. A midline abdominal incision was made to expose the duodenum and pancreas. A PE10 polyethylene tube (internal diameter: 0.28 mm, external diameter: 0.64 mm) was inserted into the pancreatic duct at the major duodenal papilla. Pancreatic juice was drained out through the tube, collected for 30 min, and then stored at −80°C for subsequent tests.

The animals were randomly divided into five groups: the control group, bethanechol chloride (Bch) group, atropine-Bch group, TH inhibitor-Bch group, and AADC inhibitor-Bch group. After the rats were anesthetized with isoflurane inhalation, they were injected with the various drugs, and pancreatic juice was collected. In detail, in the Bch group, Bch was injected (i.p.) at $2\,mg{\cdot}kg^{-1}$ (Sigma, C5259, $1\,mg{\cdot}mL^{-1}$). In the atropine-Bch group, the TH inhibitor-Bch group, and the AADC inhibitor-Bch group, atropine (Tianjin Jinyao Pharmaceutical Co., Ltd, $1\,mg{\cdot}mL^{-1}$) at $2\,mg{\cdot}kg^{-1}$, a TH inhibitor (α-methyl-DL-tyrosine methyl ester hydrochloride, Sigma M3281, $100\,mg{\cdot}mL^{-1}$) at $200\,mg{\cdot}kg^{-1}$, or an AADC inhibitor (3-hydroxybenzylhydrazine dihydrochloride, Sigma 54880, $50\,mg{\cdot}mL^{-1}$) at $100\,mg{\cdot}kg^{-1}$ were injected (i.p.) 5 min before the injection of Bch, respectively. In the control group, saline ($2\,mL{\cdot}kg^{-1}$) was injected (i.p.). In all groups, pancreatic juice was collected for 30 min from the pancreatic duct. The doses of the inhibitors were chosen based on previous reports.[18,19] The collected pancreatic juices were stored at −80°C for subsequent determination of pancreatic enzymes, dopamine, L-DOPA, and insulin.

Bilateral subdiaphragmatic vagotomy

Bilateral subdiaphragmatic vagotomy was performed according to a published method.[20] Ten adult SD rats were randomly divided into two groups: a control group and a vagotomy group. Rats were anesthetized with an American Matrx-VMR small animal anesthesia machine to maintain isoflurane (RWD Life Science Co., Ltd, Nanshan District, Shenzhen) inhalation. A midline abdominal incision was made, and the esophagus was exposed by carefully cutting the fascia between the stomach and the liver. Then, the anterior and posterior branches of the vagal nerve were transected. Finally, the peritoneum, abdominal muscles, and skin were sutured. The control rats underwent a similar procedure without cutting off the vagus nerve. After vagotomy, the rats recovered for four weeks, and then the pancreatic juice and tissue were collected for testing.

Pancreatic islet isolation

The pancreatic islets were isolated as previously described.[21] Briefly, adult SD rats were anesthetized with American Matrx-VMR small animal anesthesia machine to maintain isoflurane inhalation. The midline abdominal wall of the rat was opened to expose the common pancreatic duct. A PE10 polyethylene tube was inserted into the common pancreatic duct near the liver. Collagenase V (8–10 mL, Sigma, C9263, $1\,mg{\cdot}mL^{-1}$) was injected into the pancreatic duct through a PE10 polyethylene tube. The pancreas was removed into a 50-mL centrifuge tube containing 5 mL of collagenase V (Sigma, C9263, $1\,mg{\cdot}mL^{-1}$) and digested in a water bath at 37°C for 25 min, and 20 mL of cold Hank's solution was added to end digestion. After being washed (2 min×3) and centrifuged (1000 r/min, 4°C, 1 min), the islets were selected after staining with dithizone (Sigma, D5130, $0.05\,mg{\cdot}mL^{-1}$) for 1 min and cultured in RPMI-1640 (Invitrogen, 11875085) medium. After overnight culture, islets (70 islets/well) were transferred and stabilized in 24-well plates containing glucose-free Krebs-ringer bicarbonate HEPES buffer (KRBH, pH = 7.4) at 37°C. The KRBH consisted of the following: 115 mM NaCl, 5.4 mM KCl, 2.38 mM $CaCl_2$, 0.8 mM $MgSO_4$, 1 mM Na_2HPO_4, 10 mM HEPES, and 0.1% bovine serum albumin. The specific incubation procedures were detailed in the following stimulation experiments with cell lines and primary isolated islets.

INS-1 and AR42J cell culture

INS-1 cells (rat islet β cell line, American Type Culture Collection, Rockville, MD, USA) were cultured in a humidified 95% O_2, 5% CO_2 atmosphere at 37°C in RPMI-1640 medium containing 11.1 mM glucose supplemented with 10% heat-inactivated fetal bovine serum (FBS), 1 mM sodium pyruvate, 10 mM HEPES, 2 mM glutamine, 50 μM β-mercaptoethanol, 100 $units{\cdot}mL^{-1}$ penicillin, and $100\,\mu g{\cdot}mL^{-1}$ streptomycin (all from Gibco). AR42J cells (rat pancreatic acinar cell line, American Type Culture Collection, Rockville, MD, USA) were grown in F-12K medium supplemented with 20% (v/v) FBS, $100\,U{\cdot}mL^{-1}$ penicillin, and $100\,\mu g{\cdot}mL^{-1}$ streptomycin at 37°C in a humidified atmosphere of 5% CO_2 and 95% O_2. Cells were seeded at a density of 2.5×10^5 cells per well in 24-well culture plates. For the coculture of INS-1 and AR42J cells, INS-1 cells at a density of 2.5×10^5 cells per well were placed in the upper well and cocultured with AR42J cells (2.5×10^5 cells per well, plated on the lower surface) in 24-well permeable membrane transwells (0.4-μm pore size, Corning) in INS-1 cell culture medium.

Cell lines and primary isolated islet stimulation

INS-1 cells and isolated islets were randomly divided into six groups: control group, 1 μM L-DOPA group, 10 μM L-DOPA group, 100 μM L-DOPA group, 10 μM L-DOPA and pretreatment with NSD-1015 group, and 10 μM

tyrosine group. The cells (2×10^5 cells/well) or islets (70 islets/well) were stabilized for 30 min in Krebs-ringer bicarbonate HEPES buffer in 24-well plates at 37°C. Subsequently, the medium was replaced with 1 mL of KRBH containing 1 μM L-DOPA, 10 μM L-DOPA, 100 μM L-DOPA, 10 μM AADC inhibitor +10 μM L-DOPA, 10 μM tyrosine or vehicle, and incubated for 60 min. The AR42J cells were randomly divided into six groups: control group, 1 μM tyrosine group, 10 μM tyrosine group, 100 μM tyrosine group, 10 μM tyrosine pretreatment with the TH inhibitor (AMPT) group, and 10 μM L-DOPA group. Cocultured INS-1 and AR42J cells were randomly divided into a control group and tyrosine groups at 1 μM, 10 μM, and 100 μM. The incubation protocol was similar to that for INS-1 cells and islets. The supernatants were collected for HPLC assays of L-DOPA and DA.

Pancreatic enzymes and insulin test

Pancreatic enzymes in the pancreatic juice were detected with a Pierce ™ BCA Protein Assay Kit (Thermo, 23225). Insulin concentrations were determined using a commercial radioimmunoassay kit (Beijing North Institute of Biological Technology, Beijing, P.R. China).

High-performance liquid chromatography analysis

The L-DOPA and DA contents in serum, pancreatic tissue, and juice were measured using high-performance liquid chromatography (HPLC) with fluorescence detector and electrochemical detection analysis as previously described.[22] Pancreatic tissue (50 mg) was homogenized in 500 μL of ice-cold methanol. The homogenates, serum, and pancreatic juice were centrifuged at 12,000 r/min for 20 min at 4°C, and then the supernatants were directly used for HPLC analysis or stored at −80°C and brought to room temperature before use. The calibration concentrations of L-DOPA were 10, 20, 40, 80, 160, 320, and 640 ng/mL, respectively. The calibration concentrations of DA were 5, 10, 25, 50, 100, 250, 500, and 1000 ng/mL, respectively. The detective standard curves of L-DOPA (Supplementary Figure 1) and DA (Supplementary Figure 2) contents were drawn and their correlation coefficient $R^2 > 0.99$ was determined for the analytes. All samples were injected into the HPLC system (Liquid chromatography-2695/2165, Waters, Central Laboratory of Capital Medical University). The contents of L-DOPA and DA in each sample were calculated according to its own peak area and corresponding standard curve equation.

Statistical analysis

Data were analyzed by unpaired *t*-tests between two groups and one-way ANOVA among more than two groups using GraphPad Prism 6.0 software. For ANOVA, *post hoc* tests were only applied when $P < 0.05$, and there was no significant variance in homogeneity. The results are expressed as the mean ± SEM, and $P < 0.05$ was considered significant.

Results

Contents of DA and L-DOPA in the pancreatic tissue and juice

By HPLC analysis, high levels of DA (41.15 ± 1.46 ng/g) and L-DOPA (63.47 ± 9.06 ng/g) were detected in the pancreatic tissue (Figure 1(a)). High levels of DA (31.21 ± 4.80 ng/mL) and L-DOPA (94.72 ± 5.46 ng/mL) were also detected in the pancreatic juice, which were significantly higher than those in serum (DA: 2.98 ± 0.39 ng/mL, $P = 0.0008$, $n = 7$; L-DOPA: 70.98 ± 3.56 ng/mL, $P = 0.0034$, $n = 7$) (Figure 1(b)).

Cellular localization of DA synthases in pancreas

The results of immunofluorescence staining showed that immunoreactivity (IR) of TH, which is the key enzyme in catecholamine synthesis, was not observed in insulin-positive islet β cells but was obviously observed in pancreatic acinar cells outside the islets. AADC-IR was observed in insulin-positive islet β cells in the rat pancreas (Figure 1 (c)). Similar results were observed in cultured cell lines: AADC-IR, but not TH-IR, was observed in INS-1 cells, while TH-IR but not AADC-IR was detected in AR42J cells (Figure 1(d)).

Islet β cells synthesize DA by taking L-DOPA, not tyrosine

The distribution of TH, not AADC, in pancreatic acinar cells and AADC, not TH, in islet β cells suggested that L-DOPA and DA might be synthesized in pancreatic acinar cells and islet β cells, respectively. Therefore, in the following study, INS-1 cells (rat islet β cell line), primary isolated islets, and AR42J cells (rat pancreatic acinar cell line) were employed to investigate the synthesis of L-DOPA and DA.

The results of incubation experiments showed that DA was not detected when INS-1 cells or primary isolated islets were supplied by tyrosine at 10 and 100 μM (Table 3). However, the contents of DA in incubation solution were 13.76 ± 1.01 ng/mL, 159.50 ± 32.93 ng/mL, and 613.50 ± 22.76 ng/mL when INS-1 cells were treated with L-DOPA at 1, 10, and 100 μM, respectively. DA was also detected at 81.52 ± 10.24 ng/mL and 287.10 ± 5.52 ng/mL when primary isolated islets were treated with 10 and 100 μM L-DOPA, respectively. The 10 μM L-DOPA-induced DA increase was significantly blocked by the AADC inhibitor NSD-1015 at 10 μM (Figure 2(a) and (b)). These results suggested that pancreatic islet cells could only use L-DOPA, not tyrosine, to synthesize DA.

Acinar cells synthesize L-DOPA, not DA, by taking tyrosine

The application of L-DOPA at 10 and 100 μM did not produce any detectable DA production in AR42J cells (Table 3). However, the addition of tyrosine caused a detectable production of L-DOPA in cultured AR42J cells, which was dose-dependent. The content of L-DOPA was 40.25 ± 1.06 ng/mL, 185.40 ± 1.11 ng/mL, and 387.70 ± 3.19 ng/mL

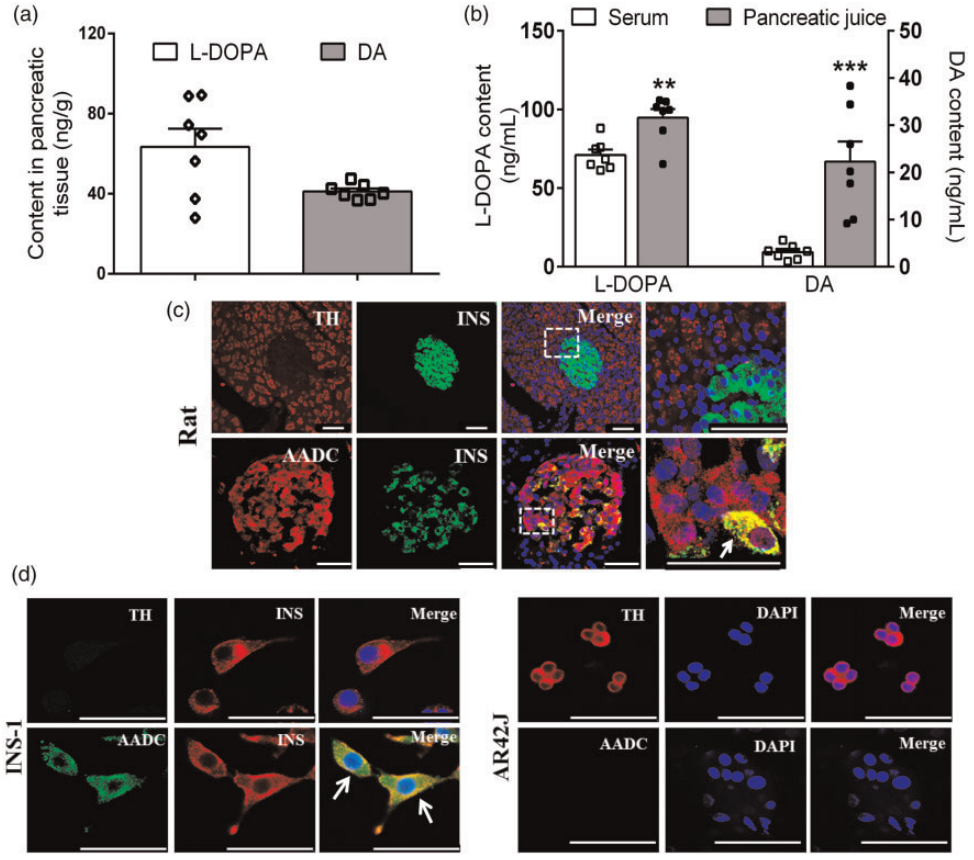

Figure 1. The contents of L-DOPA and DA in pancreatic tissue and juice, and the distribution of DA synthetases (TH and AADC) in the pancreas and cell lines. (a) L-DOPA and DA contents in rat pancreatic tissue ($n = 7$). (b) The content comparisons of L-DOPA and DA in the serum and pancreatic juice of rats ($n = 7$). $^{**}P < 0.01$; $^{***}P < 0.001$ * vs. contents in serum. (c) Cellular localizations of TH-IR (red), AADC-IR (red), and INS-IR (green) in rat pancreas. (d) Immunoreactivities of TH and AADC in INS-1 cells and AR42J cells. The arrows represented cells expressing both insulin and AADC. Scale bar: 50 μM. INS: insulin; DAPI (blue): 4'6-diamidino-2-phenylindole. (A color version of this figure is available in the online journal.)

Table 3. Contents of synthetic L-DOPA and dopamine in the culture supernatant of cell lines and islets.

	INS-1 cells		Islets		AR42J cells	
	L-DOPA(ng/mL)	DA(ng/mL)	L-DOPA(ng/mL)	DA(ng/mL)	L-DOPA(ng/mL)	DA(ng/mL)
Tyrosine (10 μM)	ND	ND	ND	ND	185.4 ± 1.1^a	ND
Tyrosine (100 μM)	ND	ND	ND	ND	387.7 ± 3.2^a	ND
L-DOPA (10 μM)	/	159.5 ± 32.9^a	/	81.5 ± 10.2^a	/	ND
L-DOPA (100 μM)	/	613.5 ± 22.8^a	/	287.1 ± 5.5^a	/	ND

[a]Refers to the mean ± SEM of numerical data in Figure 2 when L-DOPA and DA were detectable;/, not checked.
ND: not detected.

when tyrosine was added at 1, 10, and 100 μM, respectively. In addition, the tyrosine (10 μM)-induced increase in L-DOPA content in AR42J cells was significantly blocked by the TH inhibitor AMPT at 10 μM (Figure 2(c)). Furthermore, administration of tyrosine at 1, 10, and 100 μM caused dose-dependent DA production at 1.54 ± 0.37 ng/mL, 3.23 ± 0.60 ng/mL, and 4.68 ± 1.04 ng/mL, respectively, when AR42J cells and INS-1 cells were cocultured (Figure 2(d)). These results suggested that pancreatic acinar cells could utilize tyrosine to synthesize L-DOPA but not DA.

Vagal cholinergic stimulation promotes the synthesis of L-DOPA and DA *in vivo*

To demonstrate the synthesis of L-DOPA by acinar cells and DA by islet β cells, we further observed the cholinergic effect on the contents of L-DOPA and DA in the pancreatic

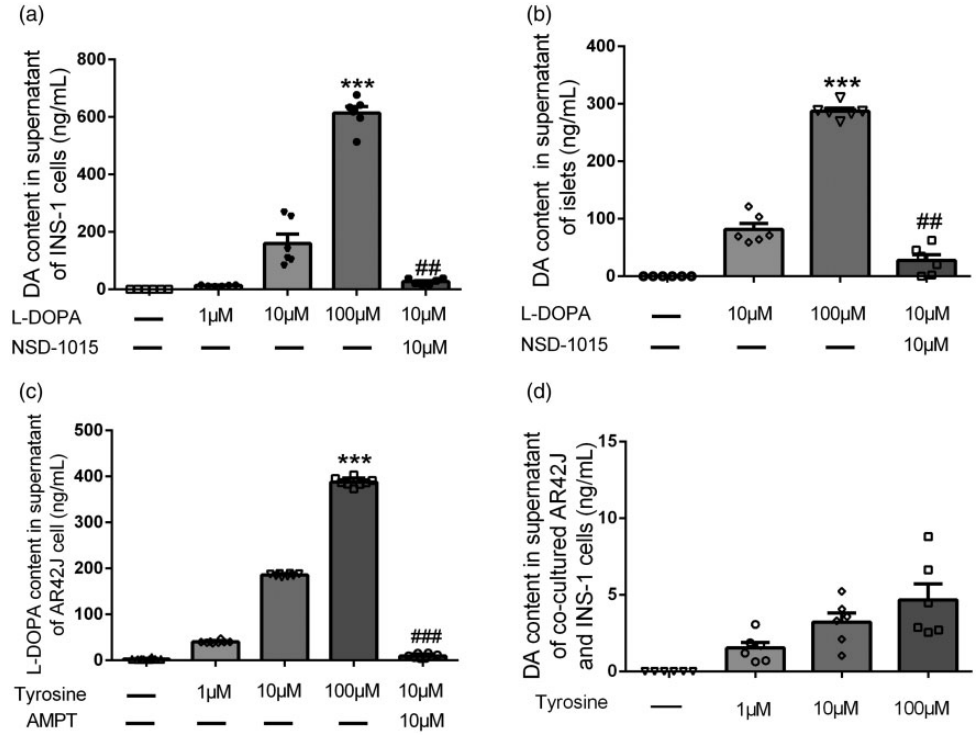

Figure 2. The synthetic contents of L-DOPA and DA in INS-1 cells, AR42J cells, and primary isolated islets. (a and b) DA contents in the supernatant of INS-1 cells (a) and primary isolated islets (b) incubated with L-DOPA and pretreated with NSD-1015 in the 10 μM L-DOPA group ($n = 6$). (c) L-DOPA content in the supernatant of AR42J cells incubated with L-tyrosine and pretreated with 10 μM AMPT in the 10 μM L-tyrosine group ($n = 8$). (d) DA content in the supernatant of cocultured AR42J cells and INS-1 cells incubated with L-tyrosine ($n = 6$). NSD-1015: AADC inhibitor, 3-hydroxybenzylhydrazine dihydrochloride; AMPT: TH inhibitor, α-methyl-DL-tyrosine methyl ester hydrochloride. ***/###$P < 0.001$ */# vs. 10 μM L-DOPA or L-tyrosine group.

juice since vagal cholinergic stimulation promotes the secretion of pancreatic enzymes from acinar cells and insulin from islet β cells. Intraperitoneal injection of the cholinergic drug bethanechol chloride (Bch), an M-cholinergic receptor agonist, significantly increased not only the contents of pancreatic enzymes in the pancreatic juice from 3.57 ± 0.35 mg/mL to 18.58 ± 1.37 mg/mL but also the levels of L-DOPA from 83.98 ± 7.11 ng/mL to 160.7 ± 19.75 ng/mL (Figure 3(a)). Similarly, Bch also significantly increased the contents of insulin from 277.40 ± 41.30 μIU/mL to 1145.00 ± 59.39 μIU/mL) and DA from 30.67 ± 2.64 ng/mL to 61.88 ± 2.40 ng/mL in the pancreatic juice (Figure 3(b)). Moreover, Bch-induced the secretion of pancreatic enzymes, L-DOPA, insulin and DA was blocked by atropine, an antagonist of the M-cholinergic receptor (Figure 3(a) and (b)). In addition, pretreatment with the TH inhibitor AMPT significantly inhibited the increase in L-DOPA (138.5 ± 11.00 to 101.70 ± 8.78 ng/mL) and DA (61.88 ± 2.40 to 25.32 ± 2.83 ng/mL) in pancreatic juice induced by Bch (Figure 3(c)). However, pretreatment with the AADC inhibitor only blocked the increase in DA (61.88 ± 2.40 to 5.89 ± 3.44 ng/mL), and the content of L-DOPA was not inhibited but significantly increased from 138.5 ± 11.00 to 175.90 ± 6.12 ng/mL (Figure 3(c)).

To further investigate the regulation of vagal cholinergic nerves on the secretion of pancreatic L-DOPA and DA and their contents in pancreatic juice, bilateral subdiaphragmatic vagotomy was performed. The results showed that the rats with vagotomy had significant reductions in L-DOPA from 79.36 ± 7.22 to 50.91 ± 6.72 ng/mL ($n = 5$, $P = 0.0204$), and DA from 38.09 ± 1.86 to 23.58 ± 2.11 ng/mL ($n = 5$, $P = 0.0009$), in pancreatic juice (Figure 3(d)).

These results suggested that pancreatic acinar cells and islet β cells could synthesize L-DOPA and DA, respectively, and both were released into pancreatic juice accompanied by the secretion of pancreatic enzymes and insulin, respectively.

Discussion

The present study demonstrates that pancreatic acinar cells cannot synthesize DA, and they can only convert tyrosine into L-DOPA through self-expressed TH. By comparison, islet β cells can only utilize L-DOPA, not tyrosine, to synthesize DA since they express AADC instead of TH. High contents of L-DOPA and DA are present in pancreatic juice, and the changes in L-DOPA and DA in pancreatic juice are the same as the trend of pancreatic enzymes and insulin secretion, which are all regulated by the cholinergic pathway.

Mezey *et al.* concluded that pancreatic acinar cells synthesize DA based on the distribution of TH in pancreatic acinar cells and the detection of DA in pancreatic tissue,[16] but the study did not determine whether AADC is present in acinar cells. Han *et al.* also reported that DA was produced by pancreatic acinar cells based on the detection of TH and AADC protein in pancreatic acinar cells of Balb/C mice by Western blotting.[4] But the distribution of DA

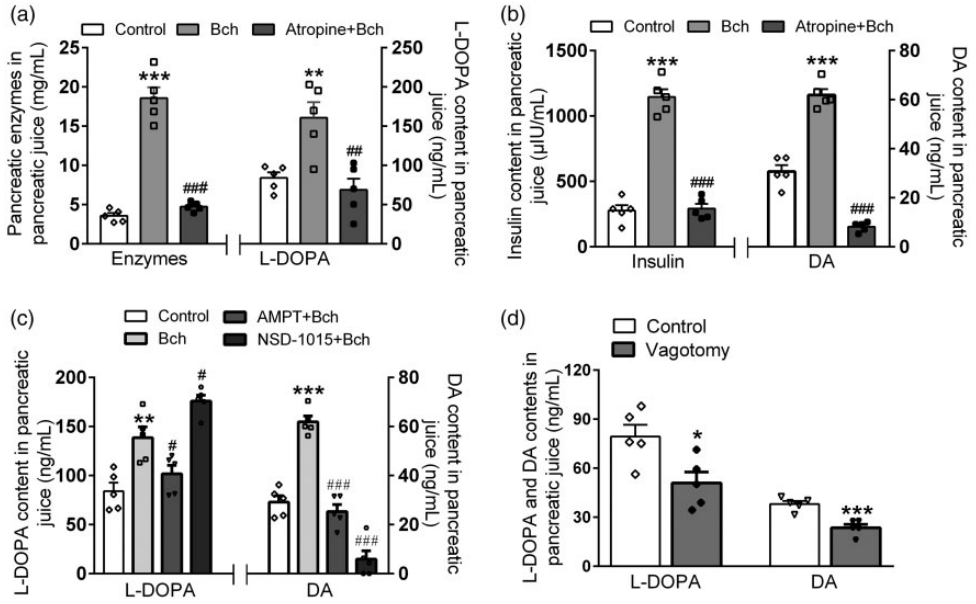

Figure 3. Effects of cholinergic drugs on the contents of L-DOPA and DA in pancreatic juice. (a) The content alterations of pancreatic enzymes and L-DOPA in pancreatic juice after intraperitoneal injection of bethanechol chloride (Bch) with or without atropine ($n = 5$). (b) Changes in insulin and DA contents in pancreatic juice after peritoneal injection of Bch with or without atropine ($n = 5$). (c) The effects of AMPT and NSD-1015 on Bch-induced alterations in the content of L-DOPA and DA in pancreatic juice ($n = 5$). (d) The effect of vagotomy on the contents of L-DOPA and DA in rat pancreatic juice ($n = 5$). Bch: Bethanechol chloride; AMPT: TH inhibitor, α-methyl-DL-tyrosine methyl ester hydrochloride; NSD-1015: AADC inhibitor, 3-hydroxybenzylhydrazine dihydrochloride *vs. control group, #vs. Bethanechol Chloride group. */#$P < 0.05$, **/##$P < 0.01$, ***/###$P < 0.001$.

synthases might be different in the pancreas of different species.[7] Mitok *et al.* declared that TH was present in islet β cells of PWK/PhJ and CAST/EiJ mice but not in C57BL/6J mice[7] and AADC has been reported in the INS-1E cell line and isolated islets of mice, rats, and humans.[10,11] Our present study demonstrates the predominant distribution of AADC in endocrine islet β cells rather than acinar cells in the rat pancreas. Our study also demonstrates that TH is mainly located in rat pancreatic acinar cells, not islet β cells. And the absence of TH activity may be unique to rats, which may not be applicable to other species, including some mouse strains and humans.[7] Although the mRNA of TH and AADC were all expressed in primary isolated rat pancreatic tissue without islets, isolated rat islets, AR42J cells, and INS-1 cells (Supplementary Figure 3), the translation of mRNA to protein might be different in different parts of pancreas. Furthermore, AR42J cells are able to convert tyrosine to L-DOPA, which can be blocked by a TH inhibitor. In addition, the AR42J cells could not use L-DOPA to synthesize DA. A high level of L-DOPA was detected in the pancreatic juice, and the Bch-induced increase in L-DOPA secretion was reduced by the TH inhibitor. All of these findings suggest that acinar cells can synthesize L-DOPA by using tyrosine and cannot synthesize DA by using tyrosine or L-DOPA in rat pancreas.

Some previous studies have reported that AADC is distributed in islet cells, and islet β cells can use L-DOPA to synthesize DA.[14,15,23] Farino *et al.* also demonstrated DA synthesis in islets by measuring L-DOPA with an [^{3}H] L-DOPA uptake assay.[1] It is generally believed that islet β cells can draw L-DOPA from circulating blood to synthesize DA, and L-type amino acid transporters (LATs) may be responsible for L-DOPA transport.[1] Recently, Aslanoglou *et al.* found the human and mouse pancreatic α cells expressed the catecholamine biosynthetic and catabolic machinery by analyzing an RNA sequencing data set, and αTC1–6 cells, a glucagon-secreting mouse α-cell line, could synthesize L-DOPA and DA.[24] At the same time, TH-positive neurons exist in both human[25] and mouse[26] pancreas, which may also provide L-DOPA for the synthesis of pancreatic DA. In fact, pancreatic DA might have several sources originated from nerves,[25,26] peripheral circulating blood,[27] pancreatic α[24] and β cells,[1,14,15] but the process of dopamine synthesis in pancreas might be diverse and different in different species.[7] Similar to the results of these reports, our study also confirmed that islet β cells could use L-DOPA to synthesize DA. In addition, our study further demonstrates that rat islet β cells cannot use tyrosine to synthesize L-DOPA or DA, which was confirmed by the expression of AADC instead of TH in islet β cells and our functional tests. INS-1 cells and primary isolated islets of rats could only use L-DOPA, not tyrosine, to synthesize DA. In the present study, we could not provide direct evidence for islet β cells synthesizing DA by taking up L-DOPA from pancreatic juice. Previous studies have reported that topographical associations between islet cells and pancreatic duct cells are frequent in adult rat [28] and human [29] pancreas. L-DOPA secreted by acinar cells into pancreatic juice might be utilized by islet cells through the special structure connection between pancreatic ducts and islet cells. In the *in vivo* study, the content of synthetic DA in pancreatic juice increased with increasing L-DOPA content. The pretreatment with the TH inhibitor blocked the increase of both L-DOPA and DA. The pretreatment with the AADC inhibitor only blocked the increase in DA and the content of L-DOPA was even significantly increased, which suggested

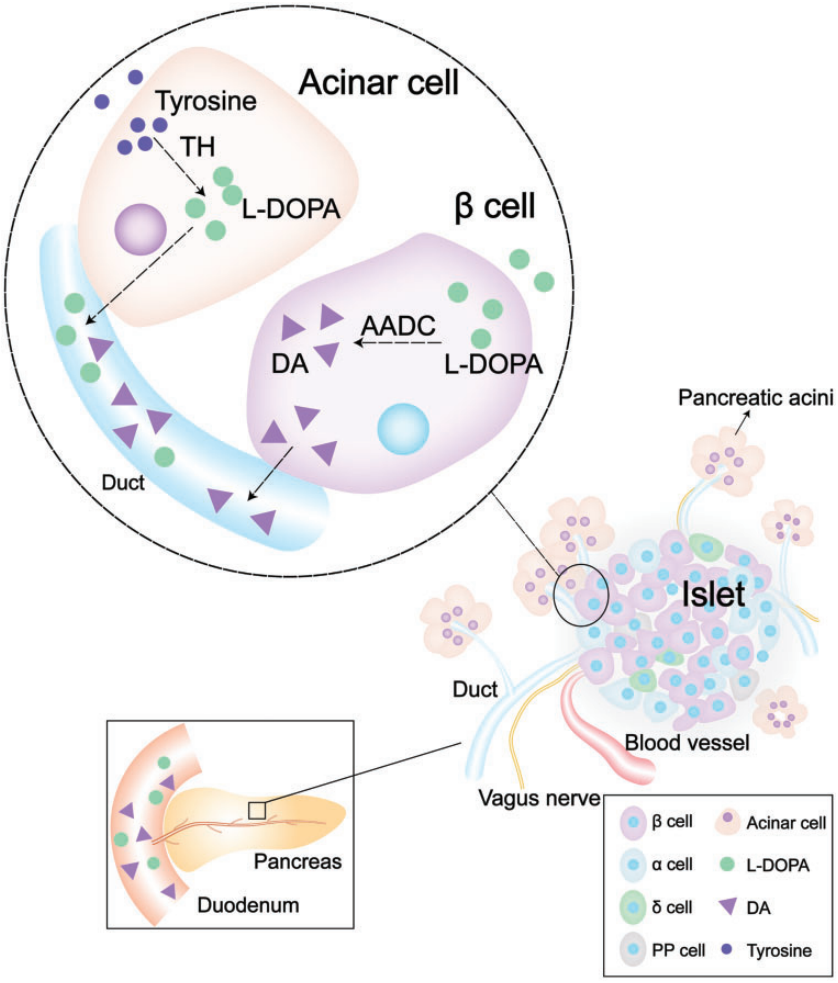

Figure 4. Schematic of L-DOPA and DA synthetic process in the pancreas. Pancreatic acinar cells synthesize L-DOPA using tyrosine via TH, and islet β cells synthesize DA by L-DOPA via AADC. The synthesis of L-DOPA and DA in the pancreas is regulated by the cholinergic pathway. TH: tyrosine hydroxylase; AADC: aromatic amino acid decarboxylase; PP cell: pancreatic polypeptide cell. (A color version of this figure is available in the online journal.)

that the AADC inhibitor blocked the islets from using L-DOPA to synthesize DA from pancreatic juice. The process of DA synthesis from tyrosine can be completed only when AR42J cells and INS-1 cells are coclutured. All these results demonstrate that L-DOPA produced by acinar cells may be utilized by islets. Besides, L-DOPA in pancreatic juice can also be transported to the intestine and utilized by the enteric nervous system[30] or intestinal bacteria[31] to synthesize DA, where DA plays a variety of regulatory roles.[32–34] The discovery of L-DOPA synthesis in pancreatic acinar cells may be more conducive to providing the precursor for pancreatic and intestinal DA synthesis.

It is well known that vagal cholinergic stimulation can promote pancreatic enzymes and insulin secretion.[35,36] In the present study, by means of vagotomy and cholinergic stimulation, we demonstrate that the activation of cholinergic M receptors could not only stimulate the secretion of pancreatic enzymes and L-DOPA from acinar cells, but also promote the secretion of insulin and DA from islets. Vagotomy and a TH inhibitor (AMPT) can reduce the contents of L-DOPA and DA, and the AADC inhibitor NSD-1015 can only block the synthesis of DA from islet β cells. These results further confirmed that L-DOPA was synthesized in pancreatic acinar cells and that DA was finally synthesized in islet β cells.

In summary, pancreatic acinar cells express TH and utilize tyrosine to synthesize L-DOPA, not DA, while islet β cells express AADC and utilize L-DOPA, not tyrosine, to synthesize DA in rats (Figure 4). These findings provide the important expermental evidence for the differential roles of acinar cells and islet β cells in pancreatic L-DOPA and DA synthesis.

AUTHORS' CONTRIBUTIONS

J-XZ designed the research project; G-WL, FH, JL, HC, and YC performed the experiments; G-WL, FH, and X-YF analyzed the data; YZ provided technical support; J-HL produced the pattern diagram (Figure 4); G-WL and FH wrote the manuscript; J-XZ and FH modified the manuscript. All authors approved the final version of the article for publication.

DECLARATION OF CONFLICTING INTERESTS

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SUPPLEMENTAL MATERIAL

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