Guinea pig pancreatic neurons: morphology, neurochemistry, electrical properties, and response to 5-HT

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Liu, Min-Tsai, and Annette L. Kirchgessner. Guinea pig pancreatic neurons: morphology, neurochemistry, electrical properties, and response to 5-HT. Am. J. Physiol. 273 (Gastrointest. Liver Physiol. 36): G1273–G1289, 1997.—The morphology, neurochemistry, and electrical properties of guinea pig pancreatic neurons were determined. The majority of neurons expressed choline acetyltransferase (ChAT) immunoreactivity; however, ChAT-negative neurons were also found. Both cholinergic and noncholinergic neurons expressed nitric oxide synthase (NOS) immunoreactivity. Three types of pancreatic neurons were distinguished. Phasic neurons fired action potentials (APs) at the onset of depolarizing current pulse, tonic neurons spiked throughout the duration of a suprathreshold depolarizing pulse, and APs could not be generated in nonspiking neurons, even though they did receive synaptic input. APs were tetrodotoxin sensitive, and all types of neurons received fast and slow excitatory postsynaptic potentials (EPSPs). Fast EPSPs had cholinergic and noncholinergic components. The majority of pancreatic neurons appeared to innervate the acini. NOS- and/or neuropeptide Y-immunoreactive phasic and tonic neurons were found. Microejection of 5-hydroxytryptamine (5-HT) caused a slow depolarization that was inhibited by the 5-HT<sub>T</sub> antagonist N-acetyl-5-hydroxytryptophyl-5-hydroxytryptophan amide and mimicked by the 5-HT<sub>T</sub> agonist 6-hydroxyindalpine. A pancreatic 5-HT transporter was located, and inhibition of 5-HT uptake by fluoxetine blocked slow EPSPs in 5-HT-responsive neurons by receptor desensitization.

choline acetyltransferase; nitric oxide synthase; 5-hydroxytryptamine receptors; 5-hydroxytryptamine transporter

CIRCULATING HORMONES play an important role in the regulation of pancreatic endocrine and exocrine secretion (2, 10). Nevertheless, despite the fact that the pancreas responds to endocrine factors, ganglia are also present in the organ. Pancreatic ganglia are found in the interlobular connective tissue, often in proximity to islets. Terminal axons appear to supply both the endocrine and the exocrine portions of the organ, which suggests that pancreatic ganglia play a role in the neural control of the secretory activity of the pancreas.

Until recently, pancreatic ganglia were considered to function as relay centers for incoming signals from the parasympathetic and sympathetic nervous system. However, it is now known that pancreatic ganglia share some of the characteristics of ganglia of the enteric nervous system (ENS) or intrinsic innervation of the bowel. These include the linking of pancreatic ganglia into a network by neural connectives (21); they also contain monoamine oxidase type B, as do intrinsic enteric neurons, nitric oxide synthase (NOS; Ref. 17), and many of the neuropeptides known to be present in enteric neurons (for review see Ref. 35). In addition, 5-hydroxytryptamine (5-HT)-immunoreactive nerves are present in pancreatic ganglia (14, 22). The serotonergic neural phenotype is a marker for the ENS and is rare in extraenteric peripheral ganglia. Pancreatic ganglia are innervated by neurons located in the myenteric plexus of the stomach and duodenum (14). Some of the enteropancreatic neurons are cholinergic. These cells give rise to excitatory nicotinic synapses in pancreatic ganglia. Another subset of enteropancreatic neurons is serotonergic. Because pancreatic ganglia contain no serotonergic nerve cell bodies and enteric serotonergic neurons are labeled after the injection of retrograde tracers into the pancreas (14), it seems likely that all intrapancreatic serotonergic fibers are of enteric origin.

The axons of serotonergic enteropancreatic neurons appear to form inhibitory a xo-axonic synapses in the pancreas (15). 5-HT inhibits nerve-mediated amylase secretion. Therefore, the targets of the serotonergic enteropancreatic innervation are likely to include the cholinergic axons that innervate acinar cells. 5-HT-mediated inhibition of amylase secretion is mimicked by the 5-HT<sub>T</sub> agonist 5-hydroxyindalpine (5-OHIP). Moreover, the effects of 5-HT and those of 5-OHIP are abolished by the highly selective 5-HT<sub>T</sub> antagonist N-acetyl-5-hydroxytryptophyl-5-hydroxytryptophan amide (5-HTP-5HTP). These data are consistent with the hypothesis that the inhibitory receptor is a 5-HT<sub>T</sub> site. 5-HT<sub>T</sub> binding sites have been demonstrated in the pancreas by rapid filtration of pancreatic membranes, by radioautography, and by immunocytochemistry (18, 20). The 5-HT<sub>T</sub> receptor has not been detected in the brain and has not yet been cloned. It can be identified operationally by its pharmacology. 5-HT<sub>T</sub>-mediated responses are not blocked by antagonists known to be effective at other subtypes of 5-HT receptor; however, they are specifically antagonized by 5-HTP-5-HTP and a substituted benzamide, renzapride (BRL 24924) (27, 28, 36). Hydroxylated indalpines (27) are agonists at 5-HT<sub>T</sub> receptors. An anti-idiotypic antibody (α-id) that recognizes 5-HT<sub>T</sub> receptors has revealed that these receptors are located on nerve fibers and ganglia in the pancreas (20).

The current study was undertaken to characterize guinea pig pancreatic neurons and determine their response to 5-HT. Intracellular recordings were made from pancreatic neurons to determine their active and passive membrane properties. In addition, the response to fiber tract stimulation was examined to establish their synaptic inputs. Electrophysiologically characterized neurons were injected with Neurobiotin to establish their morphology and neurochemically coded by immunocytochemistry. The electrophysiolog-
cal and morphological characteristics of guinea pig pancreatic neurons were compared with enteric and parasympathetic neurons in general. Finally, the effects of 5-HT on pancreatic neurons were determined, the receptor(s) involved characterized, and a pancreatic 5-HT transporter (5-HTT) was located.

MATERIALS AND METHODS

Tissue Preparation

Male guinea pigs (250–350 g) were stunned by a blow to the head and then exsanguinated. This procedure has been approved by the Animal Care and Use Committee of Columbia University. The pancreas was rapidly removed along with the attached segment of duodenum and placed in a beaker of Krebs solution, which was kept on ice. The pancreas was then separated from the adjacent duodenum and pinned flat under recirculating iced Krebs solution in a dish lined with Sylgard (Dow Corning, Midland, MI). Pancreatic ganglia were located under a dissecting microscope (Olympus) in the interlobular connective tissue (Fig. 1A) and then isolated from the pancreatic parenchyma with their attached nerve trunks. A 1.0 × 0.7 cm segment of the preparation was then transferred and pinned to Sylgard resin at the bottom of a 1.5-ml recording tissue chamber. The chamber was placed on the stage of an inverted microscope (Zeiss, Axiovert 35) for electrophysiological recording.

Electrophysiological Recording

For dissecting purposes the pancreas was divided into three parts: 1) the head of the pancreas was defined as the part attached to the duodenum, 2) the body, as the area attached to the greater curvature of the stomach, and 3) the tail, as the part attached to the spleen. In the present study were obtained from neurons located in the body and tail regions of the pancreas, because it was difficult to obtain a flat preparation of the head region that was suitable for intracellular recording. For physiological recording the preparations were continuously perfused at 3 ml/min with a modified Krebs solution that contained (in mM) 121 NaCl, 5.9 KCl, 2.5 CaCl2, 1.2 MgCl2, 14.3 NaHCO3, 1.3 NaH2PO4, and 12.7 glucose (gassed with 95% O2-5% CO2, 37°C). Preparations were allowed to equilibrate for 30 min before neurons were impaled. Individual pancreatic neurons were visualized with Hoffman modulation contrast optics.

Intracellular recordings were made from pancreatic neurons using conventional intracellular microelectrodes filled with 2% Neurobiotin (Vector, Burlingame, CA) in 1.0 M KCl (tip resistance 90–150 MΩ). Neurobiotin has no effect on the properties of intracellular recording electrodes. An amplifier (Axoclamp 2B) with bridge circuitry for injecting positive and negative current pulses was used to record membrane potentials. Rectangular electrical current pulses with a duration of 40–400 ms were injected through the microelectrode and were driven by Grass S88 stimulators (Grass Instruments,
Satisfactory impalements resulted in a stable resting membrane potential (RMP) of ~35 mV or more. The input resistance of the impaled cell was determined from the amplitude of electrotonic potentials produced by current pulses (100-200 ms duration) passed through the recording microelectrode at 1- to 5-s intervals. Membrane potentials and intracellular current injections were displayed on a digital storage oscilloscope (DSO450, Gould, Cleveland, OH), and permanent records were made on a thermal array chart recorder (TA240, Gould). The action potentials (APs) and synaptic potential parameters were measured using the digital oscilloscope.

Synaptic activation of neurons was elicited by direct stimuli applied to nerve trunks attached to a pancreatic ganglion with monopolar extracellular electrodes made from Teflon-insulated platinum wire (25-μm diam). To evoke fast excitatory postsynaptic potentials (EPSPs), nerve fibers were stimulated with single stimuli of 0.5-ms duration applied at a rate of 0.2 Hz. When studying fast EPSPs, four individual responses were averaged. Slow EPSPs were evoked by trains of stimuli (20 Hz for 500 ms to 5 s, 0.5-ms pulse duration, 4–20 V). Krebs solution with an elevated concentration of Mg2+ (15 mM) and deficient in Ca2+ (0.1 mM) was used to block synaptic transmission.

Agonists were ejected from micropipettes (tip diam ~20 μm) with bursts of N2 (10 psi) by using a Picospritzer (General Valve Inc.), 5-HT and 6-hydroxydopamine (6-OHIPP) were dissolved in normal Krebs solution to a final concentration of 5–20 mM for pressure ejection. Antagonists or the 5-HT uptake inhibitor fluoxetine were applied by superfusion in a known concentration by addition to the superfusing Krebs solution. Antagonists were applied for 4–12 min before measuring the amplitude of the evoked response. All antagonists, except 5-HTP-DP, were dissolved in Krebs solution. 5-HTP-DP was dissolved in a small volume of 10% ethanol and then diluted with Krebs solution to the appropriate concentrations (final ethanol concentrations were found not to alter neuronal responses).

5-HTP-DP was obtained from the New York State Psychiatric Institute (New York, NY). 6-OHIPP was provided by Dr. Michael Gershon (Columbia University, New York). Other compounds included tropisetron (donated by Sandoz Pharmaceutical, Basel, Switzerland), spiperone and ketanserin (Janssen Pharmaceuticals), fluoxetine (Research Biochemicals International, Natick, MA), 5-HT, hexamethonium bromide, and tetrodotoxin (TTX) (all from Sigma Chemical).

Statistics

Data are expressed as means ± SE. Student’s t-test was used to test for significance of differences between paired groups of data, and analysis of variance was used to test for significance of difference between unpaired groups of data. Differences between means were considered significant if the probability that they were random was <0.05.

Intracellular Labeling With Neurobiotin

After the impaled neuron was characterized electrophysiologically, a depolarizing current was passed through the microelectrode (0.6 nA, 200 ms for 45 min) to inject the Neurobiotin (Vector). After dye injection the preparation was fixed in 4% paraformaldehyde overnight (at 4°C), followed by several washes in 0.1 M phosphate-buffered saline (PBS). Tissues were permeabilized with Triton X-100 (1%) and then incubated with fluorescein isothiocyanate (FITC) or Texas red conjugated to avidin (Vector, 1:200), diluted in PBS (0.1 M) at room temperature for 1 h. After washing in PBS the tissues were mounted in Vectashield (Vector) and coverslipped. FITC fluorescence was visualized by vertical fluorescence microscopy using a Chroma Optical filter set (excitation 480 ± 15 nm, dichroic 505 nm, emission 535 ± 20 nm). Texas red fluorescence was visualized using a Chroma Optical filter set (excitation 540 ± 12.5 nm, dichroic 565 nm, emission 605 ± 27.5 nm).

Confocal Microscopy

The morphology and projections of Neurobiotin-filled pancreatic neurons were examined with an LSM 410 laser scanning confocal microscope (Zeiss, Thornwood, NY), equipped with a krypton-argon laser, and attached to a Zeiss Axiovert 100 TV microscope. Usually, 20 optical sections were taken at 0.5-μm intervals. Images of 512 × 512 pixels were obtained, processed using Adobe Photoshop 3.0 (Adobe Systems, Mountain View, CA), and printed using a Tektronix Phaser 440 printer.

Immunocytochemistry

Cholinergic neurons were identified with antisera raised against choline acetyltransferase (ChAT; Ref. 30). Frozen sections and whole mounts of pancreas were incubated with 4% (vol-vol) normal horse serum in PBS containing 1% Triton X-100 for 30 min. The preparations were then exposed overnight (at 4°C) to goat antiserum to ChAT (diluted 1:100; Chemicon International, Temecula, CA). After being washed in PBS, the preparations were incubated with donkey anti-goat secondary antibodies coupled to indocarbocyanine (Cy3; Jackson Immunoresearch Labs, West Grove, PA) and diluted 1:2,000 for 3 h. The preparations were washed again with PBS, and then the tissues were coverslipped with Vectashield (Vector). In every experiment parallel control sections were included that were incubated with normal goat serum instead of primary antibodies. Cy3 fluorescence was visualized by vertical fluorescence microscopy using a Chroma Optical filter set (excitation 540 ± 12.5 nm, dichroic 565 nm, emission 605 ± 27.5 nm). Double-label immunocytochemistry was used to determine the total number of cells that were ChAT positive and to examine the coexistence of ChAT immunoreactivity with other neuronal markers. Double labeling was made possible by using primary antibodies raised in different species in conjunction with species-specific secondary antibodies coupled to different fluorophores (Cy3: donkey anti-rabbit, Jackson; diluted 1:200) coupled to contrasting fluorophores (FITC). Reagents used to locate antigens simultaneously with ChAT included rabbit antibodies to neuron specific enolase (NSE; Polyscience, Warrington, PA; diluted 1:2,000), NOS (Euro-Diagnostica; diluted 1:500), and neuropeptide Y (NPY; Peninsula Laboratories, Belmont, CA; diluted 1:1,000).

The 5-HTT was located in frozen sections and whole mounts of pancreas obtained from rats. Immunoreactivity was demonstrated by incubating the fixed and permeabilized tissues (as above) with rabbit polyclonal antibodies (48 h, 4°C) directed against the final 34 amino acids (597–630) of the intracellular carboxyl terminus of the rat brain 5-HTT (29). Tissues were rinsed in buffer and then incubated with donkey anti-rabbit secondary antibodies coupled to FITC (Jackson; diluted 1:200). Demonstration of the 5-HTT in sections of rat small intestine served as a positive control (37). Double-label immunocytochemistry was used to identify the islet cells that express 5-HTT immunoreactivity. Guinea pig antibodies to glucagon (Linco, St. Charles, MO) were applied for 24 h at a dilution of 1:2,000. After being rinsed, the preparations were incubated with goat anti-guinea pig secondary antibodies coupled to tetramethylrhodamine isothiocyanate (Kirkegaard and Perry, Gaithersburg, MD), diluted 1:100 for 3 h.
RESULTS

ChAT-Immunoreactive Pancreatic Neurons

ChAT-immunoreactive cell bodies were found in ganglia located at the intersection of interganglionic connectives (Fig. 1B) and alongside and within nerve bundles (Fig. 1C). Most pancreatic neurons were ChAT immunoreactive; however, ChAT-negative neurons were also found (Fig. 2C). The mean number of ChAT-immunoreactive cells per ganglion was 9.3 ± 1.2 (n = 50 ganglia from 3 animals). To get an estimate of the proportion of ChAT-immunoreactive cells per ganglion, preparations were double labeled with antibodies to ChAT and antibodies to NSE. Although most of the NSE-stained neurons were also ChAT positive, NSE-immunoreactive cells that were ChAT negative did occur (data not shown). Total cell numbers were counted in preparations stained for NSE. The mean number of NSE-immunoreactive cells per ganglion was 10.8 ± 1.5 (n = 50 ganglia from 3 animals). This indicates that ~86% of all pancreatic neurons are ChAT positive.

In addition to nerve cell bodies, ChAT-immunoreactive nerve fibers were also observed. Numerous ChAT-immunoreactive fibers were present in all interganglionic connectives, and individual nerve fibers were found among the acini (Fig. 1E) and in a subset of islets (Fig. 1F). ChAT-immunoreactive nerve fibers were varicose within ganglia, among the acini, and within islets. In contrast, ChAT-immunoreactive fibers were smooth within nerve bundles (Fig. 1, C and D). ChAT immunoreactivity was also found to be expressed by a subset of islet cells (Fig. 1F).

Preparations immunostained with antibodies to ChAT were double labeled with antibodies to NPY or NOS to examine the coexistence of ChAT with other neuronal markers. All ChAT-immunoreactive pancreatic neurons expressed NPY immunoreactivity, and all NPY-immunoreactive neurons expressed ChAT (data not shown). In preparations that were costained for ChAT and NOS, 72.5 ± 2.5% of ChAT-immunoreactive neurons expressed NOS immunoreactivity (n = 500 neurons, Fig. 2, A and B). Neurons that expressed NOS
immunoreactivity alone were also observed (Fig. 2, C and D) and represented ~5.0% of the NOS-immunoreactive cells. These findings indicate that pancreatic ganglia contain both cholinergic and noncholinergic neurons. As was observed for ChAT, numerous NOS-immunoreactive fibers were present in interganglionic connectives and were found in ganglia, among the acini, and in a subset of islets (data not shown). NOS-immunoreactive nerves were also observed adjacent to blood vessels (Fig. 2E); however, these fibers were ChAT negative (Fig. 2F).

Electrical Properties of Pancreatic Neurons

General classification. Intracellular recordings were made from 209 neurons (1 cell/ganglion) in 154 in vitro preparations. APs were evoked by a brief cathodal current pulse (0.1–1 nA for 4–400 ms) through a recording electrode. Neurons were generally classified into one of three types of cells (phasic, tonic, or nonspiking) based on their passive and active membrane properties. Phasic neurons spiked only one to six times at the onset of a depolarizing current pulse. In contrast, tonic neurons spiked throughout a suprathreshold depolarizing current pulse. Nonspiking neurons did not spike at all in response to a depolarizing pulse yet received synaptic input. The majority of pancreatic neurons were phasic (79.4%), with a smaller percentage of cells being tonic (9.1%) or nonspiking (11.5%). Cells that are characterized by a prolonged afterhyperpolarization (AH cells) were not observed in the guinea pig pancreas, although this type of neuron is abundant in the adjacent duodenum (6).

Phasic neurons. The mean RMP and mean input resistance of phasic cells were $-51.9 \pm 0.6$ mV and $84.0 \pm 2.3$ MΩ, respectively (Table 1). Phasic cells spiked (1–6 times) only at the onset of a suprathreshold current pulse, regardless of the amplitude (0.1–1 nA) or duration (100–800 ms) of the current pulse (Fig. 3A). The mean amplitude of spikes was $53.2 \pm 1.0$ mV (Table 1). Phasic cells typically exhibited anodal break excitation at the offset of intraneuronally injected hyperpolarizing current pulses (data not shown), and APs were blocked by TTX (0.3–0.5 µM) in seven of seven phasic cells tested (Fig. 3A). Current-voltage

![Fig. 3. Voltage recordings from phasic and tonic pancreatic neurons. A: phasic cells generate a single action potential (AP) at the onset of a depolarizing current pulse. APs are tetrodotoxin (TTX) sensitive, and the effect of TTX is reversible (Wash). B: tonic neurons fire continuously for the duration of each suprathreshold current clamp. APs of tonic cells are TTX sensitive, and the effect of TTX is reversible. Traces were obtained from a single neuron, with a resting membrane potential (RMP) of $-50$ mV (A) and $-45$ mV (B).](http://ajpgi.physiology.org/)
plots were always linear around RMP; however, rectification was observed during depolarizing current steps (Fig. 4A). The after-spike hyperpolarization (ASH) of the phasic cells was 168.6 ± 23.6 ms in duration and 12.6 ± 1.1 mV in amplitude (see Table 1). The amplitude of the ASH decreased when the membrane was hyperpolarized and as the $K^+$ concentration of the circulating Krebs solution was increased (Fig. 4B). When Krebs solution was replaced with Ca$^{2+}$-free Krebs solution, both the amplitude and duration of the ASH were decreased (Fig. 4C). These results suggest that the ASH in phasic cells involves the activation of voltage-dependent $K^+$ conductances. The $K^+$ conductances that are involved are probably Ca$^{2+}$ dependent because a decrease in both the amplitude and the duration of the ASH was seen in Ca$^{2+}$-free Krebs solution.

Tonic neurons. Tonic firing was exhibited by 9.1% of pancreatic neurons. An example is shown in Fig. 3B. The pattern took the form of continuous firing of APs for the duration (100–800 ms) of each suprathreshold current clamp. The frequency of repetitive discharge increased in direct proportion to the size of depolarizing current pulses (data not shown). Mean RMP and mean input resistance for tonic cells were $246.5 \pm 1.5$ mV and $82.1 \pm 5.2$ MΩ, respectively. The mean RMP of tonic cells was significantly lower than the mean RMP of the other cell types (see Table 1). Threshold did not differ between the two spiking neurons. Therefore, the amount of depolarizing current required to bring tonic cells to threshold for continuous firing was lower than the current threshold of phasic neurons. Similar to phasic neurons the current-voltage relationship in tonic neurons was linear in the range of RMP ± 10 mV (Fig. 4A). In addition, tonic neurons exhibited anodal break excitation at the offset of a hyperpolarizing current pulse, and APs were eliminated by TTX in seven of seven tonic cells tested (Fig. 3B). The ASH of tonic neurons was $39.3 \pm 9.7$ ms in duration and the amplitude was $10.9 \pm 1.9$ mV (see Table 1); therefore, tonic neurons had a significantly ($P < 0.05$) shorter ASH than phasic neurons.

Nonspiking neurons. Nonspiking neurons did not generate APs in response to depolarizing current pulses, regardless of stimulus, amplitude or duration. However, nonspiking neurons did receive fast and/or slow synaptic input (see below) and often became excitable after exposure to certain excitatory modulators (23). Moreover, nonspiking neurons that received synaptic input were identified morphologically as neurons (see below). Nonspiking neurons had a mean RMP and mean input resistance of $-53.5 \pm 1.6$ mV and $84.6 \pm 5.4$ MΩ, respectively (Table 1).

Glial cells. A subset of cells that did not generate APs in response to depolarizing current pulses did not receive synaptic input. These cells had a mean RMP of $-72.0 \pm 1.4$ mV (n = 10), a value significantly higher than the RMP of nonspiking pancreatic neurons. These cells were identified morphologically as glial cells (see Fig. 10B) and were not studied in greater detail.
Synaptic Behavior of Pancreatic Neurons

Synaptic behavior was studied in 80 neurons (1 neuron/ganglion) in preparations from 60 guinea pigs. Neurons were classified as phasic, tonic, or nonspiking. Fast EPSPs. Fast EPSPs were evoked by fiber tract stimulation (FTS) in the majority of pancreatic neurons (Fig. 5, A and B; see Table 1). Responses were identified as fast EPSPs, rather than antidromic APs, if their duration was relatively long (>5 ms) and if their amplitude increased when the membrane potential was hyperpolarized. The duration of fast EPSPs ranged from 7.5 to 21 ms, and the amplitude ranged from 6 to 14 mV. The mean amplitude and duration of the fast EPSP were similar for the three types of pancreatic neurons; therefore, the mean amplitude of the fast EPSP in guinea pig pancreatic neurons was 8.6 ± 0.4 mV, and the mean duration was 14.0 ± 0.6 ms (n = 36). These properties are very similar to those reported for neurons in the cat pancreas (33). Increasing the strength of the stimulus beyond threshold gave rise to additional responses brought about by the recruitment of additional fibers in the nerve bundle to the neuron from which the recording was made (Fig. 5C). Occasionally, focal stimulation of interganglionic connectives with single pulses evoked antidromic spikes, indicating that the impaled neuron projected a process into the same fiber tract from which it received synaptic input (Fig. 5C). Antidromic spikes, in contrast to fast EPSPs, did not increase in amplitude when the membrane potential was hyperpolarized and were unaffected by hexamethonium (not shown).

Spontaneously occurring fast EPSPs were observed in 16.3% of phasic neurons, 26.3% of tonic neurons, and 12.5% of nonspiking neurons (Fig. 5D; Table 1). EPSPs were termed “spontaneous” when they occurred in the absence of stimulus application by the experimenter. Spontaneously occurring fast EPSPs sometimes reached the threshold for spike discharge.

Both spontaneously occurring and stimulus-evoked fast EPSPs were reversibly blocked or significantly attenuated by superfusion with TTX (0.3–0.5 µM) or elevated Mg²⁺ and reduced Ca²⁺ in the superfusion medium (data not shown). Hexamethonium (100–200 µM) reversibly abolished the stimulus-evoked fast EPSPs in 23 of 36 neurons, including each of the three cell types (Fig. 5A). These fast EPSPs were considered to be cholinergic.

In 36.1% of pancreatic neurons, hexamethonium did not completely block the fast EPSP (Fig. 5B). The amplitude of the fast EPSPs was reduced to 68.4 ± 5.3% of the control response. A higher concentration of hexamethonium (300 µM) did not further reduce the fast EPSP; therefore, these data suggest that a subset of fast EPSPs in pancreatic ganglia a mixture of hexamethonium-sensitive and -insensitive synaptic events.

Slow EPSPs. Repetitive orthodromic stimulation of fiber tracts was performed during the impalement of 42 cells. Repetitive stimulation consisted of a train (0.5 to 5 s) of electrical shocks (0.5-ms duration) at frequencies between 5 and 20 Hz. Slowly developing depolarizing potentials were evoked by repetitive stimulation in 21 of 42 cells (Fig. 6). These slow depolarizations were eliminated by exposure to Ca²⁺-free Krebs solution and are therefore presumed to be slow EPSPs (Fig. 6A). The percentage of the total number of cells of each type that exhibited slow EPSPs was the following: 38.5% phasic neurons, 50% tonic neurons, and 50% nonspiking neurons. Slow EPSPs, which outlasted the duration of the trains of stimulus pulses, had the following characteristics: peak amplitude of the depolarizing potential (2.1–14.0 mV, n = 21) and duration of the depolarizing

![Figure 5](http://ajpgi.physiology.org/). Fast excitatory postsynaptic potentials (EPSPs) in pancreatic neurons. A and B: fast EPSPs are observed in phasic cells in response to single stimuli of pancreatic nerve trunks. Hexamethonium-sensitive and -insensitive fast EPSPs are observed. A: the fast EPSP was completely blocked by hexamethonium (100 µM). B: recording from a different phasic pancreatic neuron with a fast EPSP that was partly reduced in amplitude by hexamethonium (100 µM). C: effect of increasing stimulus intensity on synaptic responses observed in a phasic neuron. Nerve trunks were stimulated with 0.5-ms pulses at increasing voltage. With increasing stimulus strength, additional synaptic responses were observed. D: ongoing synaptic activity recorded in a phasic neuron. Ongoing activity consisted of subthreshold fast EPSPs and APs. A portion of the left trace is expanded in the right trace to illustrate better the nature of individual synaptic events. RMP was −54 and −52 mV for A and B, respectively, −51 mV for C, and −52 mV for D. APs in this and in subsequent figures are truncated because of the limited frequency response of the recorder.
low-Ca²⁺ (0.1 mM), high-Mg²⁺ (15 mM)-containing solution blocks the slow EPSP recorded in a phasic neuron. B: a slow EPSP accompanied by fast EPSPs and AP. The slow EPSP is unaffected by superfusion of hexamethonium (100 µM); however, the fast EPSPs and AP are blocked. RMP, −50 and −49 mV for A and B, respectively.

Morphology of Pancreatic Cells

Phasic neurons. Thirty-four phasic neurons were sufficiently well filled with Neurobiotin to be placed into morphological categories. All cells were unipolar in that a single long process arose from the cell body. For further description it was assumed that this long process represented the axon of the cell. Twenty-five of the phasic neurons had generally smooth cell bodies that gave rise to short, filamentous processes that emanated from the cell body near the origin of the axon. Confocal microscopic reconstructions of these cells revealed that short spines arose from the axon near the cell body (Figs. 7, A and B, insets). Nine of the phasic neurons had “triangular-shaped” cell bodies with numerous, short processes emanating from all sides of the soma. The axon of all phasic cells traveled in a nerve bundle emanating from the ganglion in which the cell was found. Typically (20 of 34 cells), the axon of phasic cells could be followed into the acinar tissue (Fig. 7C), where it broke into several branches (Fig. 7, D and E) and ended in varicose structures (Fig. 7E). In preparations that contained several ganglia, processes of four of four labeled phasic neurons could be followed to an adjacent ganglion, where each process appeared to innervate a subset of the neurons (Fig. 7F). Processes of 14 labeled cells terminated as expansion bulbs, indicating that they may have been severed during the dissection.

Phasic neurons expressed NOS (8 of 8 cells; Fig. 8, A and B) and NPY (6 of 6 cells; Fig. 8, C–F) immunoreactivity. NPY-immunoreactive fibers are found in ganglia (Fig. 8D) among the acini (Fig. 8D), the islets (Fig. 8F), and around blood vessels (Figs. 8, D and F). The presence of NPY-immunoreactive neurons in the pancreas suggests that at least part of this innervation is derived from intrinsic pancreatic neurons. Phasic NPY-immunoreactive neurons appeared to innervate the acini (Fig. 8, C and D). Projections to blood vessels and islets were not observed (Fig. 8, C–F); therefore, perivascular- and islet-innervating NPY-immunoreactive fibers are more likely to be derived from extrinsic sources.

Tonic neurons. Seven tonic cells were successfully injected with Neurobiotin. Like phasic neurons, all tonic cells were unipolar in that a single long process (the axon) arose from the cell body. Tonic cells had a morphology that was indistinguishable from that of the phasic cells. Four of the seven cells were triangular in shape, with numerous short processes (Fig. 9A). Confocal microscopic reconstructions of these cells revealed that the short processes possessed spines, and the long process of these triangular-shaped tonic cells frequently contained “knob-like” swellings (data not shown) and varicose “tufts” (Fig. 9C). Three of the seven tonic cells were round in shape and possessed several processes that appeared to be concentrated near the axon hillock (Fig. 9B). The axon of tonic cells typically branched (Fig. 9C) and, similar to phasic neurons, could be followed into the acinar tissue (data not shown). Tonic cells did not appear to innervate other ganglia (Fig. 9D); the axon of tonic cells passed ganglia by traveling along interganglionic connectives. Four of four tonic cells expressed NOS immunoreactivity. Two of two tonic cells were NPY negative (data not shown).

Nonspiking neurons. Two nonspiking neurons that received fast EPSPs were successfully injected with Neurobiotin. One nonspiking cell was triangular in shape (Fig. 10A), and the other cell possessed a generally smooth soma and fewer processes. Axons could be followed into the acinar tissue where they appeared to terminate. We did not determine the neurochemical content of these cells.

Glial cells. Three nonspiking cells that did not receive fast EPSPs were injected with Neurobiotin. When a single nonspiking cell was injected, multiple cells in
the ganglion were stained, presumably because of dye-coupling of the cells (Fig. 10B). These cells were identified as glial cells and correspond to type II cells found in the cat pancreas (11, 33).

Response to 5-HT

Intracellular recordings were made from 69 neurons in 38 in vitro preparations. Recordings were accepted for analysis if the initial RMP was more negative than −35 mV, if APs demonstrated a positive overshoot, and if the neurons exhibited no visible signs of swelling throughout the duration of the impalement. Impalements were maintained for 1–6 h. In 14 neurons (20.3% of total tested) pressure microejection of 5-HT (10⁻² M) did not cause a change in RMP, input resistance, or excitability. In 55 pancreatic neurons (79.7%), microejected 5-HT evoked a single type of response, a slowly developing membrane depolarization, similar to the "slow response" observed in enteric neurons (40), and neurons in the cat pancreas (25, 33; Fig. 11). Of the total number of cells that responded to 5-HT, 67.3% were phasic neurons, 3.7% were tonic neurons, and 29.1% were nonspiking neurons. The slow response was dependent on the duration of the microejection and desensitized with repeated application of the amine (<5-min intervals). 5-HT-evoked depolarizations averaged 8.8 ± 0.4 mV in amplitude and 73.5 ± 3.7 s in duration (n = 55). On occasion, 5-HT-induced depolarizations were associated with spontaneous spike discharge (Fig. 11A). Control applications of normal Krebs solution, using the same duration and ejection pressure used to eject 5-HT, had no effect on membrane potential or membrane input resistance (data not shown). Interrupting synaptic transmission either by TTX (0.5 µM) or a low Ca²⁺-high Mg²⁺ solution did not inhibit the slow response to 5-HT, suggesting a direct action of 5-HT on the postsynaptic membrane (n = 4; data not shown).

Changes in membrane input resistance associated with 5-HT-evoked depolarizations were examined by intracellular injection of hyperpolarizing current pulses and by measuring the amplitude of the resulting electrotonic potentials during application of 5-HT. The change in membrane input resistance that was associated with the 5-HT-evoked depolarizing response was variable. In 7 of 25 neurons tested an increase (+11.6 ± 2.6% of control, P < 0.05) was observed. In 13 neurons a decrease (−12.1 ± 1.5% of control, P < 0.05) was observed. In 5 neurons there was no change. A variable change in membrane input resistance associated with
Fig. 8. Phasic pancreatic neurons that express NOS and/or neuropeptide Y (NPY) immunoreactivity project to the acini. A: a Neurobiotin-filled phasic pancreatic neuron. B: NOS immunoreactivity demonstrated simultaneously in the same pancreatic ganglion and visualized with tetramethylrhodamine isothiocyanate (TRITC). C: a Neurobiotin-filled phasic pancreatic neuron. D: NPY immunoreactivity demonstrated simultaneously in the same pancreatic ganglion and visualized with TRITC. The Neurobiotin-filled neuron is NPY immunoreactive (arrowhead) and projects to the acini (arrow, C). E: a Neurobiotin-filled phasic pancreatic neuron. F: NPY immunoreactivity demonstrated simultaneously in the same pancreatic ganglion and visualized with TRITC. The Neurobiotin-filled neuron is NPY immunoreactive (arrow). It does not project to the blood vessel (BV) or islet (I); however, these structures are innervated by NPY-immunoreactive nerve fibers. Markers, 30 µm.

Fig. 9. Morphology of tonic pancreatic neurons. A and B: photomicrographs of Neurobiotin-filled tonic cells. Insets: confocal microscopic reconstructions. C and D: the long process of a tonic cell branched (C) and contained tufts of varicosities (C, inset). It bypassed adjacent ganglia (D) and ended among the acini. Markers, 30 µm.
5-HT-evoked depolarizing responses have been observed in cat pancreatic neurons and appears to be due to changes in both Na⁺ and K⁺ conductance (25).

**Effect of Selective 5-HT Receptor Antagonists and 5-HT₁₃ Agonists**

Ketanserin, spiperone, and tropisetron antagonize 5-HT responses mediated by 5-HT₂, 5-HT₁₃, and 5-HT₃ receptors, respectively (7). We tested the effect of these antagonists on the 5-HT-evoked slow response. Superfusion of pancreatic ganglia with ketanserin, spiperone, or tropisetron, in concentrations of up to 10 µM (n = 2–5 for each drug) for 20 min, had no significant effect on the slow depolarizing response to 5-HT (Fig. 11B). In the bowel and cat pancreas, the slow depolarizing response to 5-HT is mediated by 5-HT₁₃ receptors (25, 27, 28, 33). 5-HT₁₃ receptors are present in the guinea pig pancreas (19, 20); therefore, we examined the actions of 5-HT₁₃-specific drugs. In six 5-HT-responsive neurons (1 phasic, 1 tonic, and 4 nonspiking), the slow depolarizing response to 5-HT was significantly inhibited by the 5-HT₁₃ antagonist 5-HTP-DP (300 µM, Fig. 12A). 5-HTP-DP induced a 50.4 ± 2.6% decrease in the amplitude and a 48.2 ± 5.6% decrease in the duration of the 5-HT response. Recovery of the slow response to 5-HT was obtained after 30–60 min of washout of 5-HTP-DP (Fig. 12A). Microprojection of the 5-HT₁₃ agonist 6-OHIP mimicked the slow depolarizing response to 5-HT (n = 3 phasic and 3 nonspiking neurons, Fig. 12B). 6-OHIP-evoked depolarizations averaged 6.8 ± 0.6 mV in amplitude and 61.0 ± 6.1 s in duration and were associated with an increase in input resistance (+9.6 ± 2.1% of control, P < 0.05). Moreover, the effect of the 5-HT₁₃ agonist was significantly reduced by 5-HTP-DP (Fig. 12B). 5-HTP-DP induced an 83.7 ± 2.3% decrease in the amplitude and a 61.3 ± 9.8% decrease in the duration of the 6-OHIP response (n = 6). These results support the idea that the depolarizing response to 5-HT is mediated, at least in part, by the 5-HT₁₃ receptor.

**Localization of Pancreatic 5-HTT and Effects of 5-HT Uptake Inhibitor**

If 5-HT is an excitatory neurotransmitter in the pancreas, as the reported observations imply, then an appropriate inactivating mechanism should be present to terminate its effects and to prevent desensitization of receptors. Inactivation of 5-HT at synapses in the gut is achieved by a 5-HTT that is expressed by serotonergic neurons (37). Inhibition of 5-HT uptake by fluoxetine causes enteric 5-HT₁₃ receptors to desensitize (37). Antibodies to the rat brain 5-HTT (29) were therefore used to test immunocytochemically the hypothesis that this molecule is present in the pancreas and to determine its location. In addition, the ability of the 5-HT uptake inhibitor fluoxetine to inhibit the response to 5-HT and slow EPSPs in pancreatic neurons was assessed. Slow EPSPs were examined because in enteric neurons (36) and neurons in the cat pancreas (33) at least a subset of slow EPSPs is mediated by 5-HT₁₃ receptors. Slow responses to 5-HT (n = 7; data not shown) and slow EPSPs in pancreatic neurons (n = 4, Fig. 13A) were each inhibited by superfusion of fluoxetine (10 µM). Fluoxetine significantly decreased the amplitude of the slow depolarizing response to 5-HT from 8 ± 0.5 to 2 ± 0.5 mV (P < 0.05) and significantly reduced the amplitude of the slow EPSP to 23.1 ± 5.4% of control (P < 0.05). The effects of fluoxetine were reversible after washout with normal Krebs solution for 10–20 min. Fluoxetine had no effect on RMP or membrane input resistance (n = 7).

5-HTT-immunoreactive fibers were abundant in the pancreas (Fig. 13B). However, no 5-HTT-immunoreactive nerve cell bodies were found, although they were present in the adjacent duodenum (Fig. 13C). The pattern of fibers labeled by the antibodies to the 5-HTT was comparable to sites labeled with antibodies to 5-HT (14). Both varicose and nonvaricose (Fig. 13B) axons were labeled. In addition, the 5-HTT was expressed by glucagon-immunoreactive islet cells (Fig. 13, D and E). No labeling was seen when the primary antibodies were omitted.
DISCUSSION

The first goal of the present study was to acquire information regarding the morphology, neurochemical content, and electrical properties of neurons in the guinea pig pancreas. Previous studies have demonstrated that guinea pig pancreatic ganglia contain NPY-, vasoactive intestinal peptide-, NOS-, and substance P-immunoreactive neurons (17, 21). Most, if not all, guinea pig pancreatic neurons also contain acetylcholinesterase reaction product (14); however, no morphological evidence for acetylcholine (ACh) synthesis by pancreatic neurons has been presented until now. Data from the present experiments indicate that the majority, (86%), of guinea pig pancreatic neurons express ChAT immunoreactivity and are therefore likely to synthesize ACh. These findings indicate that guinea pig pancreatic neurons express ACh as well as additional neuroactive compounds that have been described here and elsewhere. For example, in addition to ChAT, guinea pig pancreatic neurons expressed NPY and/or NOS immunoreactivity. All ChAT positive neurons expressed NPY; therefore, NPY immunoreactivity can be used as a marker of cholinergic pancreatic neurons. In contrast, only a subset of ChAT-immunoreactive neurons expressed NOS. These findings indicate that guinea pig pancreatic neurons express ACh as well as additional neuroactive compounds that have been described here and elsewhere. For example, in addition to ChAT, guinea pig pancreatic neurons expressed NPY and/or NOS immunoreactivity. All ChAT positive neurons expressed NPY; therefore, NPY immunoreactivity can be used as a marker of cholinergic pancreatic neurons.

In addition to nerve cell bodies, ChAT-immunoreactive nerve fibers were abundant in the pancreas. Putative targets of ChAT-immunoreactive axons were identified as structures innervated by varicose terminal axons. Such potential targets included acini, ganglia, and a subset of islets. The majority of ChAT-immunoreactive fibers appeared to express NOS. Fibers positive for NOS and negative for ChAT were observed around blood vessels; therefore, a potential target of noncholinergic pancreatic neurons may be pancreatic blood vessels.
Intracellular recordings revealed two types of spiking neurons in pancreatic ganglia: 1) phasic and 2) tonic cells. Phasic cells, which were the most common neurons encountered, could be distinguished from tonic cells by differences in RMP and on the basis of their pattern of discharge in response to depolarizing current. Phasic cells had a significantly higher RMP (−52 vs. −46 mV) than the tonic cells and generated only one to six APs at the onset of a depolarizing current pulse, regardless of stimulus, amplitude, or duration. In contrast, tonic cells spiked repetitively throughout a depolarizing current pulse. Phasic cells also had a higher threshold value for spike discharge (−30 vs. −34 mV) than tonic cells. Both phasic and tonic pancreatic neurons displayed anodal-break excitation at the offset of a hyperpolarizing current pulse, and their APs were completely blocked by TTX; therefore, the depolarizing phase of the AP of both cell types is primarily the result of inward Na$^+$ currents.

As is characteristic of autonomic neurons in general, an ASH followed the AP of both pancreatic cell types; however, the amplitude was larger in phasic neurons than in tonic cells and its duration was significantly longer. The longer duration of the ASH in phasic cells probably restricts the firing rate of these cells. The ASH of pancreatic neurons is most likely the result of an activation of K$^+$ conductances. This idea is supported by the K$^+$ concentration-dependent changes that were observed in the amplitudes of ASH in these cells. In neurons of the cat pancreas (11) and guinea pig gallbladder (26), the duration of the ASH has been attributed to the activation of a Ca$^{2+}$-dependent K$^+$ conductance, because it can be blocked when extracellular Ca$^{2+}$ concentration is removed. In the present study a decrease in both the amplitude and duration of the ASH was seen in Ca$^{2+}$-free, high-Mg$^{2+}$ solutions; therefore, the K$^+$ conductances that are involved are also probably Ca$^{2+}$ dependent.

Phasic and tonic firing neurons have previously been described in the cat pancreas (type I; Refs. 11, 25, 33). Phasic neurons were the most common neurons encountered in the cat pancreas. However, in contrast to the guinea pig pancreas, fewer than 2% (vs. 9%) of the impaled neurons in the cat pancreas exhibited a tonic firing pattern. The higher occurrence of tonic-firing neurons in the guinea pig pancreas may be species dependent. The proportions of phasic and tonic discharging neurons have been found to differ within the same ganglion between species (3, 9). The difference in proportions of the two subtypes of neuron may also be attributed to differences in the regions of the pancreas studied. All recordings in this study were made from neurons

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located in the body and tail regions of the pancreas. In contrast, recordings in the cat pancreas were primarily made from neurons in the head region, located near the choleduodenal junction. These two areas are derived from different primordia and represent distinct islet cell populations (10). Therefore, it is not unreasonable to propose that regional differences in the distribution of tonic neurons may be present in the pancreas. Phasic neurons in both species displayed spontaneous activity; however, in the cat pancreas, the APs of phasic cells were not blocked by TTX. Phasic neurons in the guinea pig pancreas are therefore more similar to sympathetic (12) and other parasympathetic (26) ganglion cells in possessing TTX-sensitive Na⁺ channels for the generation of APs.

The other cell type encountered in guinea pig pancreatic ganglia was nonspiking. These cells did not generate APs in response to direct depolarizing current applied across the cell membrane; however, they did receive synaptic input and exhibited a slow depolarization in response to 5-HT. The RMP and input resistance of nonspiking cells were not different from phasic neurons. Moreover, injection of Neurobiotin into nonspiking cells revealed that they were neurons. This is in accordance with the morphological demonstration by Erde et al. (4) that some electrophysiologically identified nonspiking cells in the gut are neurons. The role of nonspiking neurons is difficult to predict. Alternatively, it is possible that nonspiking neurons are cells that were damaged during the tissue dissection. If this were the case, then a nonspiking neuron may actually be a phasic or tonic cell.

Gliaal cells could be distinguished from neurons by their higher RMP (~70 mV) and inexcitability. This type of cell is also found in the cat pancreas (type II; Refs. 11, 33) and has been described in the ENS (4). When Neurobiotin was injected into a single glial cell, additional glia also became labeled by the dye. Networks of enteric glial cells have been reported to become labeled after the injection of the dye into a single cell, presumably because of dye coupling between adjacent glial cells (5).

All three types of guinea pig pancreatic neuron received synaptic input that was blocked by a low Ca²⁺-high Mg²⁺ solution. As found in the cat pancreas (11, 33), fast EPSPs were the primary synaptic event in the ganglia of the guinea pig pancreas. The majority of fast EPSPs were blocked by hexamethonium, suggesting that fast synaptic transmission in pancreatic ganglia is primarily mediated by ACh acting on nicotinic receptors. This was expected because pancreatic ganglia are innervated by vagal efferent fibers and cholinergic myenteric (14) and intrinsic pancreatic (21) neurons. Spontaneous fast EPSPs were common in pancreatic neurons and presumably resulted from ongoing spike activity in other neurons located within the neural network (33). Spontaneous synaptic activity was blocked by hexamethonium, supporting the idea that it resulted, at least in part, from the activity of cholinergic pancreatic neurons.

In a subset of pancreatic neurons fast EPSPs were only partially blocked by hexamethonium, suggesting that ACh does not entirely mediate fast synaptic transmission. Noncholinergic fast EPSPs have recently been reported to be present in the bowel (20, 24). In the gut at least a subset of the noncholinergic fast EPSPs appears to be mediated by purinergic and/or glutamatergic receptors. Further studies will be needed to determine if ATP, glutamate, or some other transmitter mediates noncholinergic fast EPSPs in pancreatic neurons.

FTS evoked slow EPSPs in all three subtypes of pancreatic neuron. However, in contrast to fast EPSPs, slow EPSPs were found in only a subset of the neurons. Experimentally evoked slow EPSPs have been reported in the gut (38) and cat pancreas (11, 25, 32). In the ENS slow EPSPs are observed mainly in AH-type 2 neurons. This type of neuron does not appear to be present in the pancreas and may explain the paucity of slow EPSPs in this organ. Alternatively, slow EPSPs may occur more frequently in neurons located in ganglia in the head, rather than in the body and tail regions of the guinea pig pancreas. Supporting this idea, slow EPSPs are frequently observed in neurons located in ganglia in the head region of the cat pancreas (33). The neurotransmitters involved in the generation of slow EPSPs in guinea pig pancreatic neurons are unknown. 5-HT mediates slow EPSPs in enteric neurons (36) and neurons in the cat pancreas (33); therefore, 5-HT appears to be a possible mediator of slow EPSPs in the guinea pig pancreas (see below). Consistent with this idea, pancreatic ganglia are innervated by nerves immunoreactive for the amine (14, 22).

Pancreatic ganglia are colonized by the same set of precursor cells that give rise to the neurons of the ENS (13). Therefore, it is reasonable to propose that some of the electrical properties of pancreatic neurons would be similar to the neurons of the gut. Depending on the region of the bowel where they are found, myenteric neurons can be classified into four types based on their electrical properties (see Ref. 38 for review). Phasic pancreatic neurons resemble enteric AH-type 2 in that they fire only one or six APs. However, they exhibit anodal-break excitation, lack a long-duration ASH, and are affected by TTX. Consequently, phasic pancreatic neurons are more like the type of enteric neuron found only in the stomach (31) and colon (38). Tonic pancreatic neurons display all of the electrical properties of enteric S-type 1 neurons. Pancreatic nonspiking neurons resemble the fourth type of enteric neuron and, like those found in the bowel, are converted to spiking by the application of forskolin (unpublished observations). Although there are several similarities between the electrical and morphological properties of pancreatic and enteric neurons, it appears that the most characteristic neuron of the bowel, the AH-type 2, is not present in the pancreas. Most enteric AH-type 2 neurons are calbindin immunoreactive (6). Therefore, it is not surprising that calbindin-immunoreactive neurons are not found in pancreatic ganglia. The observation that this type of neuron does not exist in the pancreas is
consistent with its proposed role in the bowel. Several studies suggest that AH-type 2 neurons are the intrinsic primary afferent neurons of the gut (6). The pancreas does not appear to contain intrinsic primary afferent neurons. Rather, pancreatic neurons appear to consist primarily of neurons that respond to intrinsic and extrinsic neural and hormonal cues and thereby modulate pancreatic exocrine and endocrine function.

Microejection of 5-HT evoked a slow depolarizing response in the majority of guinea pig pancreatic neurons. The response to 5-HT was due to a direct effect on the postsynaptic membrane because it was not affected by TTX or by a low Ca\(^{2+}\)-high Mg\(^{2+}\) solution. The response of guinea pig pancreatic neurons to 5-HT was similar to that observed in enteric neurons and neurons in the cat pancreas. 5-HT did not evoke a fast depolarization or a membrane hyperpolarization, actions that are mediated by 5-HT\(_{3}\) and 5-HT\(_{1A}\) receptors, respectively. Thus the body and tail regions of the guinea pig pancreas do not contain postsynaptic gangliionic 5-HT\(_{3}\) or 5-HT\(_{1A}\) receptors. The slow response to 5-HT was insensitive to the 5-HT\(_{2}\) antagonist t ropiserrone. It was also unaffected by a number of other serotoninergic receptor antagonists, suggesting that it does not belong to one of the classic 5-HT\(_{1a-d}\), 5-HT\(_{2}\), or 5-HT\(_{3}\) receptor subtypes (see Ref. 7 for review). Tropiserrone in micromolar concentrations also failed to inhibit this response, suggesting that it does not belong to the 5-HT\(_{3}\) receptor subtype (7). Similar to previous studies on enteric neurons and neurons in the cat pancreas, the slow response to 5-HT was only inhibited by the 5-HT\(_{1P}\) receptor antagonist 5-HTP-DP (25, 27, 33). In addition, the slow response to 5-HT was mimicked by the 5-HT\(_{1P}\) agonist 6-OH1P.

In cat pancreatic neurons, a subset of slow EPSPs appears to be mediated by 5-HT\(_{1P}\) receptors (33). As discussed previously stimulus-evoked slow EPSPs were observed in a subset of guinea pig pancreatic neurons. Moreover, they could be evoked in neurons that exhibited a slow depolarizing response to 5-HT that appeared to be mediated by 5-HT\(_{1P}\) receptors. 5-HT-immunoreactive fibers are present in the pancreas and are derived from neurons located in the myenteric plexus of the stomach and duodenum (14). Thus one source of slow synaptic input to the pancreas appears to be enteric ganglia. The physiological significance of slow synaptic excitation in the nervous system of the pancreas is unknown. In the gut slow EPSPs are abundant and are hypothesized to be a mechanism for long-lasting activation or inhibition of gastrointestinal effector systems (see Ref. 38). In the pancreas the slow depolarization produced by 5-HT would be expected to amplify central and peripheral synaptic inputs arriving in pancreatic ganglia, increasing the likelihood for summation of fast EPSPs to reach threshold for spike generation. In the present study, evoked slow EPSPs were associated with fast EPSPs and spike activity. Thus prolonged secretory responses in exocrine and/or endocrine cells may be related to an increased probability of spike discharge in the postsynaptic neuron during slow EPSPs.

The mechanism of pancreatic 5-HT inactivation is not known. If 5-HT is a transmitter of enteropancreatic nerves then pancreatic 5-HT will have to be removed rapidly to terminate responses to it and prevent receptor desensitization. We thus tested the hypothesis that the same plasmaemal 5-HTT that is expressed by enteric serotoninergic neurons and accounts for the inactivation of 5-HT at synapses in the ENS is expressed in the...
pancreas. Because the pancreas contains no serotoninergic neuronal cell bodies (14), this transporter would have to be expressed by enteropancreatic serotonergic nerve fibers. A functional 5-HTT was indeed found to be expressed in the pancreas. 5-HTT-immunoreactive nerve fibers were observed to be present in nerve bundles and within pancreatic ganglia. The pattern of fibers labeled by the antibodies to the 5-HTT was comparable to those of fibers that take up radioautographically detectable [3H]5-HT (22) or that are labeled by antibodies to 5-HT (14). 5-HTT immunoreactivity was also found to be expressed by glucagon-immunoreactive islet cells. These cells are also labeled by antibodies to 5-HT (14). Therefore, similar to its expression in the ENS, fibers and cells that contain 5-HT in the pancreas are the structures that also express the 5-HTT.

Evidence that 5-HT inactivation is important in pancreatic physiology was derived from studies examining the actions of fluoxetine, a selective inhibitor of 5-HT uptake, on slow synaptic events in pancreatic ganglia. A high concentration of fluoxetine blocked slow EPSPs in pancreatic neurons. In addition, the slow response to 5-HT was also reduced by superfusion of fluoxetine, supporting the idea that inhibition of 5-HT uptake causes 5-HT receptors to desensitize, inhibiting 5-HT-mediated slow synaptic events and responses to exogenous 5-HT. Interference with serotonergic transmission would be expected to affect exocrine and/or endocrine function.

In conclusion, although their role in the physiology of exocrine and endocrine secretion is as yet unknown, pancreatic ganglia should not be regarded as simply relay ganglia intercalated between the vagus and effectors. They are much more complex, and because of this complexity, the pancreas displays a degree of independence when cut off from the brain, spinal cord, or gut (34). Observations of spontaneous activity within connected pancreatic ganglia support the idea of an endogenous neural network regulating pancreatic function. Further studies are needed to clarify the role of pancreatic ganglia in exocrine and endocrine secretion.

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