Electrophysiological effects of GABA on cat pancreatic neurons

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Sha, L., S. M. Miller, and J. H. Szurszewski. Electrophysiological effects of GABA on cat pancreatic neurons. Am J Physiol Gastrointest Liver Physiol 280: G324–G331, 2001.—In mammalian peripheral sympathetic ganglia GABA acts presynaptically to facilitate cholinergic transmission and postsynaptically to depolarize membrane potential. The GABA effect on parasympathetic pancreatic ganglia is unknown. We aimed to determine the effect of locally applied GABA on cat pancreatic ganglion neurons. Ganglia with attached nerve trunks were isolated from cat pancreata. Conventional intracellular recording techniques were used to record electrical responses from ganglion neurons. GABA pressure microejection depolarized membrane potential with an amplitude of 17.4 ± 0.7 mV. Electrically evoked fast excitatory postsynaptic potentials were significantly inhibited (5.4 ± 0.3 vs. 2.9 ± 0.2 mV) after GABA application. GABA-evoked depolarizations were mimicked by the GABA_α receptor agonist muscimol and abolished by the GABA_α receptor antagonist bocuculline and the Cl^- channel blocker picrotoxin. GABA was taken up and stored in ganglia during preincubation with 1 mM GABA; β-aminobutyric acid application after GABA loading significantly (P < 0.05) increased depolarizing response to GABA (15.6 ± 1.0 vs. 7.8 ± 0.8 mV without GABA preincubation). Immunolabeling with antibodies to GABA, glial cell fibrillary acidic protein, protein gene product 9.5, and glutamic acid decarboxylase (GAD) immunoreactivity showed that GABA was present in glial cells, but not in neurons, and that glial cells did not contain GAD, whereas islet cells did. The data suggest that endogenous GABA released from ganglionic glial cells acts on pancreatic ganglion neurons through GABA_α receptors.

γ-Aminobutyric acid A receptors; glial cells; γ-aminobutyric acid release; electrophysiology; immunohistochemistry

GABA was discovered over 40 years ago as a key inhibitory neurotransmitter in the brain (5, 25). Since then, evidence has accumulated that this amino acid may function as a neurotransmitter not only in the central nervous system but also in the peripheral nervous system, including the myenteric plexus (3, 17, 42), major pelvic ganglia (2), and sympathetic ganglia, encompassing the rat superior cervical ganglion (7, 20, 45) and abdominal prevertebral ganglia (18, 33, 41).

The mammalian pancreas, like the gut wall, has an intrinsic nervous system consisting of ganglia, interconnecting intrinsic nerve fibers, and extrinsic parasympathetic and sympathetic nerves (6, 22, 23, 27–30, 38). Histological studies suggest that pancreatic ganglia resemble enteric ganglia anatomically and are considered to be a subclass of parasympathetic ganglia (40). Pancreatic ganglion neurons receive input from cholinergic (4), adrenergic (26), serotonergic (23), peptidergic (12, 26), and nitricergic nerves (24, 37).

GABA is present in pancreatic islets in concentrations comparable to that found in the brain (10, 19, 31, 32), and GABA is cosecreted with insulin from β-cells (11, 34). Although there is as yet no evidence for the existence of GABA-containing nerves in the pancreas (15, 43), there is nevertheless evidence that GABA and GABA_α receptors modulate endocrine function (14, 21, 35, 43). There is no information on whether GABA receptors are present on pancreatic ganglion neurons or on the effect of exogenous GABA on pancreatic ganglion neuron excitability. Thus the present study was designed to determine in vitro the effect of GABA and GABA receptor-modulating drugs locally applied to pancreatic ganglion neurons.

MATERIALS AND METHODS

Adult cats of either sex were anesthetized with an intramuscular injection of ketamine hydrochloride (100 mg; Bristol Laboratories) and then killed by an intraperitoneal injection of pentobarbital sodium (325 mg; Fort Dodge Laboratories). The use of cats and the method of euthanasia used in these studies were approved by the Mayo Institutional Animal Care and Use Committee. The cats used in this study also provided tissues for experiments conducted by other investigators. Through a midline abdominal incision, the pancreas was rapidly removed and a section of pancreas (1.5 × 1.8 cm) from the head or body region was removed and pinned to the Sylgard-coated floor of a recording chamber. The chamber was superfused with normal Krebs solution equilibrated with 97% O_2-3% CO_2 at 34–36°C. The composition of the solution was (in mM) 137.4 Na^+, 5.9 K^+, 2.5 Ca^{2+}, 1.2 Mg^{2+}, 124 Cl^-, 15.5 HCO_3^-, 1.2 H_2PO_4^-, and 11.5 glucose.

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Pancr...inue the surrounding pancreatic parenchyma. Nerve trunks were designated as being either central or peripheral in origin, as described previously (22).

Glass microelectrodes filled with 3 M KCl or 3 M KAc (resistance: 50–80 MΩ) were used to record intracellularly from ganglion neurons. The membrane potentials and intracellular current injections were displayed on an oscilloscope (Tektronix 513), and permanent records were made on a chart recorder (Gould Brush 220) and an FM tape recorder (Hewlett Packard 3968A). Satisfactory impalements resulted in a stable rest membrane potential equal to or more negative than −40 mV.

Nerve trunks were stimulated using bipolar platinum-wire electrodes (0.5 mm between the 2 poles) connected to a stimulator (Grass S88) and stimulus isolation unit (Grass SIU5). Stimuli of 5–40 V with pulse durations of 0.5 ms were used to evoke fast excitatory synaptic potentials. Stimuli of 100 V with pulse duration of 0.5 ms at 2 Hz and train duration of 3 s were used to evoke slow excitatory synaptic potentials.

The following drugs dissolved in Krebs solution were applied by pressure microjection (2 × 10−3 Torr, 5–20 ms) from a glass micropipette placed close to the ganglia: GABA (5 mM), ACh (20 mM), muscimol (10 mM), baclofen (10 mM), and β-aminobutyric acid (BABA; 30 mM). Glass micropipettes filled with drug solution were brought as close as possible to the site of the recording microelectrode. Bicuculline (10 μM), picrotoxin (20 μM), TTX (10 μM), hexamethonium bromide (10 μM), atropine sulfate (5 μM), and nipeptic acid (0.5 mM) were dissolved in Krebs solution and applied by bath perfusion. In some experiments, GABA in concentrations of 0.1, 0.5, or 1.0 mM was applied by bath perfusion. All drugs were obtained from Sigma Chemical. A low-Ca2+, high-Mg2+ solution was made by decreasing the Ca2+ concentration to 1 mM and increasing the Mg2+ concentration to 15 mM.

Immunohistochemical staining for GABA was performed on ten ganglia from two pancreata. The ganglia were fixed overnight at 4°C in 4% paraformaldehyde. The tissues were then rinsed in 0.1 M PBS (pH 7.4), incubated overnight in PBS containing 30% sucrose, and frozen in isopentane at −40°C to −50°C. Cryostat sections (12 μm thick) were cut, mounted on glass slides, and blocked for 60 min in PBS containing 10% normal donkey serum (NDS) and 0.3% Triton X-100. Tissue sections were incubated overnight in GABA antiserum (rabbit polyclonal; Sigma Chemical) diluted 1:200 in PBS containing 5% NDS and 0.3% Triton X-100 at room temperature. After being rinsed for 15 min in PBS, sections were incubated in fluorescein- or Cy3-conjugated donkey anti-rabbit secondary antiserum (Chemicon) diluted 1:100 in PBS containing 2.5% NDS and 0.3% Triton X-100 for 90 min at room temperature. Some sections were double immunostained for GABA and protein gene product 9.5 (PGP 9.5; mouse monoclonal; Boehringer Mannheim) to label glial neurons or glial fibrillary acidic protein (GFAP; mouse monoclonal; Boehringer Mannheim) to label glial cells. The secondary antibodies for double labeling were mixtures of donkey anti-rabbit fluorescein and donkey anti-mouse Cy3 or donkey anti-rabbit Cy3 and donkey anti-mouse fluorescein. The presence of glutamic acid decarboxylase (GAD), the biosynthetic enzyme for GABA, in glial cells and islets was examined using a rabbit polyclonal GAD antibody (Chemicon) at 1:500–1:1,000 dilution. Islets were identified using a mouse monoclonal synaptophysin antibody (16) at 1:20 dilution (Dako, Carpenteria, CA). Tissues were examined by epifluorescence and by laser scanning confocal microscopy. Control tissues, omitting primary antibodies or using mismatched secondary antibodies, showed no immunoreactivity for GABA, PGP 9.5, GFAP, or GAD.

All data are expressed as means ± SE and compared using Student’s t-test. P < 0.05 was considered significant, and n represents the number of neurons tested.

RESULTS

Effects of exogenous GABA. Intracellular recordings were made from 84 neurons in pancreatic ganglia of 51 cats. These neurons had a mean resting membrane potential of −49.1 ± 0.7 mV and a mean input resistance of 61.2 ± 3.4 MΩ. All 84 neurons responded with a depolarization of the membrane potential on microjection (5 mM at 2 × 10−3 Torr for 5–20 ms) of GABA. The GABA-evoked response consisted of a fast rising depolarization followed by a slow repolarization. During the depolarizing response, action potentials were evoked in eight (~10%) neurons. In 61 neurons, the recording microelectrodes were filled with 3 M KCl. The mean amplitude of the GABA-evoked depolarization was 17.4 ± 0.7 mV, and the duration was 47.3 ± 4.0 s. The membrane input resistance during the GABA-evoked response (n = 25) significantly (P < 0.05) decreased to 36.8 ± 3.8 MΩ compared with 63.2 ± 4.8 MΩ observed in the same neurons before GABA application. In 23 other neurons, when the recording microelectrode was filled with 3 M KAc, the GABA-evoked depolarization was 7.7 ± 0.5 mV in amplitude and 35.7 ± 4.3 s in duration. These values are significantly (P < 0.05) less compared with the corresponding values obtained when KCl-filled microelectrodes were used. During application of GABA, the membrane input resistance significantly decreased to 43.7 ± 4.1 from 59.5 ± 4.8 MΩ observed in the same neurons before applying GABA.

The amplitude of GABA-evoked depolarizations was dependent on the membrane potential. The more positive the membrane potential, the smaller the amplitude of the GABA-evoked depolarization. The depolarization to GABA was abolished when the membrane potential was −30.2 ± 0.3 mV (n = 6), and a hyperpolarization was evoked by GABA when the membrane potential was held at −15.7 ± 0.2 mV (n = 6; Fig. 1).

The concentration dependence of GABA-evoked depolarizations was tested in four experiments. Bath superfusion with 0.1 mM GABA depolarized the membrane potential by 1.7 ± 0.2 mV. The depolarization persisted for the duration of bath superfusion (15–25 min). An additional depolarizing response was evoked when GABA was applied by pressure ejection (5 mM for 20 ms). When 0.5 mM GABA was superfused, the membrane potential depolarized by 13.2 ± 0.6 mV in the first minute. During continuous superfusion with 0.5 mM GABA, the membrane potential repolarized. After 15 to 20 min, it remained depolarized by 3.1 ± 0.3 mV above the control resting membrane potential. During this steady-state depolarization, pressure ejection of GABA (5 mM for 20 ms) could not evoke further
depolarization. When the bath was superfused with 1.0 mM GABA, the membrane potential depolarized by 21.1 ± 1.3 mV in the first minute. During continuous superfusion, the membrane potential partially repolarized but remained depolarized by 7.7 ± 0.5 mV above the control resting membrane potential for the duration of GABA application. Further depolarization could not be evoked by pressure ejection of GABA until GABA was washed out.

To eliminate the possible involvement of cholinergic nicotinic and muscarinic receptors in the GABA response, the nicotinic receptor antagonist hexamethonium and the muscarinic receptor antagonist atropine were used to block cholinergic transmission. Superfusing ganglia with hexamethonium (10 μM; n = 8 neurons) for 30 min inhibited ACh-evoked fast nicotinic responses but did not affect GABA-evoked depolarizations (Fig. 2B). Superfusing ganglia with atropine (5 μM; n = 6 neurons) for 30 min abolished ACh-evoked slow muscarinic depolarization but did not affect GABA-evoked depolarizations (Fig. 2D). These results indicate that neither cholinergic nicotinic receptors nor cholinergic muscarinic receptors were involved in GABA-evoked depolarizations.

To determine whether an increase in membrane permeability to sodium was involved in the GABA-evoked depolarization, TTX, a sodium channel blocker, was used. Superfusing ganglia with TTX (5 μM) for 30 min did not affect GABA-evoked depolarizations (Fig. 3). The mean amplitude of GABA-evoked depolarizations was 19.6 ± 0.8 mV in normal Krebs solution (n = 2) and 20.1 ± 0.7 mV when TTX was present. These values were not significantly different (P > 0.05). These data suggest that GABA-evoked depolarizations in pancreatic neurons did not involve an increase in membrane permeability to sodium.

To determine whether GABA acted postsynaptically to evoke depolarization, low-Ca²⁺, high-Mg²⁺ solution was used to block synaptic transmission. The mean amplitude of GABA-evoked depolarizations in normal Krebs solution and in the low-Ca²⁺, high-Mg²⁺ solution was similar (18.1 ± 0.9 vs. 17.8 ± 1.0 mV; P > 0.05; n = 6 neurons). An example of the GABA-evoked depolarization is shown in Fig. 2C.

Fig. 1. Effect of conditioning depolarization and hyperpolarization on GABA-evoked depolarization in a pancreatic neuron. The resting membrane potential (RMP) was −42 mV. Note that the reversal potential for the GABA-evoked depolarization was −16 mV.

Fig. 2. Lack of effect of cholinergic receptor antagonists on GABA-evoked depolarizations. A–D, upper traces show the response to pressure application to ACh; lower traces show the response to pressure application of GABA. Note that in normal Krebs solution, the response to ACh consisted of a fast depolarization followed by a lower amplitude slow depolarization (A and C). Hexamethonium blocked the fast depolarization to ACh (B) but had no effect on the GABA response. Atropine blocked the slow depolarization, but had no affect on the depolarizing response to GABA. Recordings in A and B were from the same neuron; recordings in C and D were from another neuron. Downward deflections in traces in A and B are membrane potential changes in response to intracellular injection of hyperpolarizing current (i).
depolarization under the two test conditions is shown in Fig. 4.

Twenty-two neurons were tested to determine whether either fast nicotinic or slow synaptic transmission could be affected by application of GABA. In 15 neurons tested, the amplitude of the fast excitatory postsynaptic potential (fEPSP) in normal Krebs solution was 5.4 ± 0.3 mV; the duration was 26.8 ± 1.8 ms. After pressure ejection of GABA, the amplitude of fEPSP decreased significantly to 2.9 ± 0.2 mV (P < 0.01). This inhibitory effect of GABA on the nicotinic fEPSP lasted from 1 to 3 min in the different experiments. In seven other experiments, ganglia were preincubated with 1 mM GABA for 1 h and the slow excitatory postsynaptic potentials (sEPSP) were evoked by repetitive nerve stimulation (20 Hz for 3 s). Under these conditions, the amplitude of the sEPSP was 5.5 ± 0.4 mV. In the same experiments, bicuculline was added to determine whether endogenously released GABA acting through GABA<sub>A</sub> receptors affected the amplitude of the sEPSP. After superfusion of bicuculline (10 µM) for 15 min, the amplitude of the sEPSP was reduced to 4.8 ± 0.4 mV, but this was not statistically significant (P > 0.05) compared with control measurements.

Effect of GABA receptor agonist and antagonist. Pressure microejection (5–20 ms) of baclofen (10 mM), a GABA<sub>B</sub> receptor agonist, had no significant effect on resting membrane potential (62.5 ± 4.0 vs. 63.8 ± 4.5 mV; P > 0.05; n = 6). In contrast, pressure microejection (5–30 ms) of muscimol (10 mM), a GABA<sub>A</sub> receptor agonist, evoked a membrane depolarization in 15 of 15 neurons tested (Fig. 5). Muscimol-evoked depolarizations had a mean amplitude of 17.1 ± 1.9 mV and a mean duration of 44.3 ± 5.7 s, similar to GABA-evoked depolarizations. Muscimol also significantly decreased membrane input resistance (66.3 ± 4.9 mΩ before application vs. 37.0 ± 5.4 mΩ in presence of muscimol; P < 0.05; n = 5).

Bicuculline was used to determine whether GABA<sub>A</sub> receptors mediated GABA-evoked depolarizations. After recordings were made in normal Krebs solution, the ganglia were superfused with Krebs solution containing bicuculline (10 µM) for 10–15 min, followed by
washout of bicuculline with normal Krebs solution. During superfusion of bicuculline and washing out, GABA was applied by pressure microejection every 4–5 min. In 10 of 10 neurons tested, after 10–15 min of superfusion with bicuculline, GABA-evoked depolarizations were completely blocked. GABA-evoked depolarizations partially recovered 2 min after washout of bicuculline and completely recovered after 10 min (Fig. 6).

To determine whether GABA-evoked depolarizations were dependent on Cl⁻, the effect of picrotoxin, a Cl⁻ channel blocker, was tested. In 8 of 8 neurons studied, superfusion of picrotoxin (20 μM) almost completely inhibited the depolarizing response to GABA (Fig. 7).

**GABA uptake and release.** To determine whether GABA was taken up by and released from pancreatic ganglia, the effect of BABA was tested before and after prolonged superfusion with a GABA-containing Krebs solution. BABA, a weak GABA receptor agonist, is a substrate for the GABA carrier in glial cells; BABA stimulates efflux of GABA from glial cells (9). In 12 neurons tested, microinjection of BABA (30 mM for 5–6 ms) evoked a depolarization that had an amplitude of 7.8 ± 0.8 mV and a duration of 16.7 ± 1.2 s. The membrane input resistance decreased from 60.4 ± 4.7 to 45.2 ± 4.1 MΩ during the GABA-evoked depolarization, a reduction similar to that recorded during GABA-evoked depolarizations. In 11 other neurons, the ganglia (n = 9) were pretreated by superfusing Krebs solution containing 1 mM GABA for 1 h, as required to potentiate the action of BABA (9). After 30 min washout of the GABA-containing solution, neurons were impaled and tested for their response to BABA. In these neurons, BABA (30 mM for 5–6 ms) evoked a depolarization that was 15.6 ± 1.0 mV in amplitude and 35.5 ± 2.3 s in duration. These values are significantly (P < 0.01) greater compared with values recorded without GABA preincubation. An example of the response to BABA with and without GABA preincubation is shown in Fig. 8.

In another series of experiments (n = 9), the GABA uptake inhibitor nipecotic acid (0.5 mM) was added to Krebs solution containing 1 mM GABA. The ganglia were superfused for 1 h with the solution containing nipecotic acid and GABA. After a 30-min washout, neurons were impaled and tested for their response to BABA. The amplitude of the BABA-evoked depolarization was 4.7 ± 0.6 mV (n = 9), a value significantly lower (P < 0.05) compared with the amplitude of the BABA-evoked depolarization seen in the neurons pre-treated with only GABA.

**Fig. 6.** Effect of bicuculline, a GABA_A receptor antagonist, on GABA-evoked depolarizations. The antagonist blocked GABA-evoked depolarizations (B). The effect was reversible on washout of the antagonist (C and D). Downward deflections in A–D are membrane potential changes in response to intracellular injection of hyperpolarizing current. Recordings were made from the same neuron.

**Fig. 7.** Effect of picrotoxin, a Cl⁻ channel blocker, on GABA-evoked depolarizations. Picrotoxin blocked GABA-evoked depolarizations (B); picrotoxin had no effect on the resting membrane potential. Downward deflections in A and B are membrane potential responses to intracellular injection of hyperpolarizing current. The resting membrane potential of −52 mV applies to both A and B.

**Fig. 8.** The effect of preincubation with GABA on β-aminobutyric acid (BABA)-evoked depolarizations. BABA evoked a lower amplitude depolarization compared with the GABA-evoked depolarization in the same neuron (A). In a ganglion pretreated with 1 mM GABA, the BABA-evoked depolarization was of higher amplitude and similar in amplitude to the GABA-evoked depolarization (B). Downward deflections in A and B are membrane potential responses to intracellular injection of hyperpolarizing current. All recordings were made from the same neuron.
Immunohistochemistry. GABA immunoreactivity was present in pancreatic ganglia (Fig. 9). Double immunolabeling for GABA and the glial cell marker GFAP and double labeling for GABA and the nerve cell marker PGP 9.5 showed that GABA immunoreactivity was colocalized with GFAP but not with PGP 9.5. An example of colocalization of GABA and GFAP is shown in Fig. 9. These results indicate that GABA was present in glial cells but absent from ganglion neurons. However, immunoreactivity for GAD, the biosynthetic enzyme for GABA, was absent in pancreatic ganglia (Fig. 10). Pancreatic islets, identified with synaptophysin antibody (44), contained immunoreactivity for GAD (Fig. 10), suggesting that islets but not glial cells synthesize GABA in cat pancreas.

Fig. 9. Micrograph, obtained using a laser scanning confocal microscope, of a section of cat pancreatic ganglion that was double immunostained for GABA and glial fibrillary acidic protein (GFAP). GABA immunoreactivity was colocalized with GFAP immunoreactive glial cells (arrowheads). Principal ganglion neurons (*) were not immunoreactive for GABA. Scale bar = 100 μm.

Fig. 10. Glutamic acid decarboxylase (GAD)-like immunoreactivity in cat pancreas. Tissue was double-immunolabeled with antibodies to GAD and synaptophysin. A and B: pancreatic islet. GAD-like immunoreactivity was present in synaptophysin-positive cells at the periphery of the islet. C and D: pancreatic ganglion. Synaptophysin immunoreactive varicosities but no GAD immunoreactivity was detected (*). Micrograph was obtained using a laser scanning confocal microscope. Scale bars: A and B = 100 μm; C and D = 50 μm.
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DISCUSSION

The two key observations made in the present study were that 1) GABA was found by immunohistochemistry in glial cells in pancreatic ganglia and 2) GABA\textsubscript{A} receptors were located on cell bodies of pancreatic ganglion neurons. The presence of GABA in glial cells and the absence of GABA immunoreactivity in ganglion neurons and nerve fibers and endings suggest that GABA in pancreatic ganglia functions as a paracrine messenger molecule rather than as a neurotransmitter substance. The absence of GABA in intraparacrine nerve structures is in agreement with previous studies (15, 43). It has been shown that GABA is present in pancreatic islets (10, 19, 31, 32) and that GABA is cosecreted with insulin (11, 34). Our study also shows that GAD immunoreactivity was not present in glial cells but was found in pancreatic islets. Based on these observations and the ones made in the present study, it can be suggested that release of GABA from islet cells could act directly on ganglion nerve cell bodies or be stored for later release from glial cells.

It is known that GABA is transported in both directions across the glial cell membrane (9) and that glial cells in superior cervical ganglia take up GABA at concentrations as low as 1 \( \mu \text{M} \) (8, 46). Normally, the outward transport rate of GABA from glial cells is submaximal. However, when BABA is present on the outside surface of the cell membrane, the inward transport of BABA increases the turnover rate of the carrier (8) especially when glial cells are loaded with GABA by preincubation with exogenous GABA. The present study showed that BABA, a substrate with high affinity for the GABA carrier located in glial cells (9), evoked a membrane depolarization that was significantly potentiated when ganglia were preincubated with GABA. These data suggest that the increased turnover rate of the carrier by BABA accelerated the efflux of GABA, thereby evoking a larger amplitude GABA-evoked depolarization. If BABA acted via release of GABA through a transport process in glial cells, then pretreatment with nipeptic acid should block the effect of BABA. The results obtained show that nipeptic acid significantly reduced the effect of BABA. The remaining BABA-evoked depolarization most likely was due to BABA acting on GABA\textsubscript{A} receptors because BABA is a weak GABA\textsubscript{A} receptor agonist (9).

The GABA-evoked depolarization observed in pancreatic ganglia in the present study was mediated by postsynaptic GABA receptors because the GABA response was well maintained in the presence of a low-Ca\textsuperscript{2+}, high-Mg\textsuperscript{2+} solution to block synaptic vesicle release. Similarly, in the major pelvic ganglion, there is no evidence for presynaptically located GABA receptors (2). The absence of any presynaptic effect of GABA in pancreatic ganglia and in the pelvic ganglia (a mixed sympathetic, parasympathetic ganglia) is in sharp contrast to prevertebral sympathetic ganglia in which GABA acts on presynaptic GABA\textsubscript{A} receptors (33, 41) as well as on postsynaptic GABA\textsubscript{A} receptors. There was no evidence for the existence of GABA\textsubscript{B} receptors on pancreatic ganglion neurons, because the GABA\textsubscript{B} receptor agonist baclofen was without effect.

The present results suggest that GABA\textsubscript{A} receptors mediated the GABA depolarizing response, because muscimol, a GABA\textsubscript{A} receptor agonist, mimicked the GABA response and bicuculline, a GABA\textsubscript{A} receptor antagonist, blocked it. Recently (2), it was shown in the major pelvic ganglion that GABA can evoke an early and late depolarizing response mediated through GABA\textsubscript{A} and GABA\textsubscript{C} receptors, respectively, and that the latter response is bicuculline insensitive. In the present study, the GABA-evoked depolarization was completely blocked by bicuculline. These data indicate that GABA\textsubscript{C} receptors are not present in pancreatic ganglia.

Although GABA and nicotine are similar in structure (36), GABA did not interact with nicotinic receptors because the response to exogenously applied GABA was not affected by nicotinic blockade with hexamethonium. Furthermore, the GABA response was also not affected by muscarinic receptor blockade with atropine, ruling out the possibility that GABA may have released ACh, which in turn acted postsynaptically on muscarinic receptors.

Previous studies (1, 13, 18, 41) on sympathetic ganglion neurons show that exogenously applied GABA acts on GABA\textsubscript{A} receptors to increase Cl\textsuperscript{−} conductance, thereby evoking membrane depolarization. The present results suggest that GABA increases Cl\textsuperscript{−} conductance also in pancreatic ganglion neurons because the Cl\textsuperscript{−} blocker picrotoxin inhibited the GABA depolarizing response. This conclusion is supported by the observation in the present study that the reversal potential for the GABA-evoked depolarization was approximately \( -30 \text{ mV} \), a value close to the Cl\textsuperscript{−} equilibrium potential in autonomic ganglion neurons (18).

Although we observed a statistically significant inhibitory effect of GABA on the fEPSP, it was not so clear whether the depolarizing response to GABA also inhibited the sEPSP.

In conclusion, the present study shows the following: pancreatic ganglion neurons contain GABA\textsubscript{A} receptors; exogenously added GABA acts through GABA\textsubscript{A} receptors to cause depolarization, inhibiting fEPSPs; and ganglionic glial cells store and can release endogenous GABA under the experimental conditions used in this study. It remains to be determined whether in vivo GABA originating from glial cells or islet cells alters synaptic transmission in pancreatic ganglia.

Some of the results of this study were published previously in abstract form (39).

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