# Gamma-Aminobutyric Acid in Peripheral Tissue, with Emphasis on the Endocrine Pancreas

## Presence in Two Species and Reduction by Streptozotocin

JOSEPH C. GERBER, III, AND THEODORE A. HARE

**SUMMARY**

**A screen of selected peripheral organs of the rat found that γ-aminobutyric acid (GABA) is generally present outside the central nervous system, and, of those organs examined, GABA was present at the highest concentration in the pancreas (~40 pmol/mg wet wt). Furthermore, this putative inhibitory neurotransmitter was found to be present at even higher levels in islets of Langerhans tissue isolated from rat pancreas (190 pmol/mg). Administration of streptozotocin, a selective β-cell toxin, decreased pancreatic GABA levels significantly, but had no or only small effects on the GABA content of other organs. Normal teleost (catfish) Brockmann body contains about the same level of GABA as normal rat islet tissue. DIABETES *28*:1073–1076, December 1979.**

Neurochemical studies of the mammalian central nervous system (CNS) have indicated that γ-aminobutyric acid (GABA) acts there as a neurotransmitter or modulator. In general, this compound acts to increase inward chloride-ion flux across excitable membranes, resulting in hyperpolarization and inhibition of neurotransmitter release.[1–3] GABA is present in mammalian brain at a level of 1–10 nmol/mg wet wt, depending on the species examined.[4] Until recently, analytic techniques for the measurement of GABA were not sufficiently sensitive or specific to reliably detect this compound outside the CNS, though recent attempts have been made that had varying degrees of agreement.[5–7] GABA has been reported to be present in the blood of various mammalian species at a concentration of about 850 pmol/ml,[8,9] and it appears that the idea that GABA is only present in the CNS of mammals is not tenable.

From the Department of Pharmacology, Thomas Jefferson University, Philadelphia, Pennsylvania.
Address reprint requests to T. A. Hare, Ph.D., Department of Pharmacology, Thomas Jefferson University, 1020 Locust Street, Philadelphia, Pennsylvania 19107.
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GABA was measured using the sensitive and specific, ion-exchange/fluorometric (I-E/F) high pressure liquid chromatographic (HPLC) technique[10] for GABA analysis, as recently improved.[11] The technique was originally used to detect the pmol/ml quantities of GABA in cerebrospinal fluid (CSF) from normal and neurologically diseased humans.[10,12,13] We report here the GABA concent of various peripheral organs of the rat and of the Brockmann body of the catfish (*Ictalurus nebulosis*).

### MATERIALS AND METHODS

**GABA analysis and full amino acid analysis.** GABA was analyzed by the I-E/F HPLC technique described elsewhere.[10,11] The method has been confirmed by amino acid analysis,[10] the radioreceptor assay,[14] and indirectly by gas chromatography/mass spectrographic analysis.[15,16] Because this is the first reported use of the technique in peripheral organs, the analytic results were compared with those of full amino acid analysis. This comparison was carried out in 11 samples of rat spleen, heart, liver, lung, testis, and pancreas.

GABA values by full amino acid analysis were obtained from a Perkin-Elmer amino acid analyzer, modified for HPLC and fluorescence detection using ophthalaldehyde.[17] A column (1 m × 2.0 mm) of HCB-X8 cation-exchange resin (Hamilton Instrument Company, Reno, Nevada) was used for separation of the various constituents in the samples. Elution buffers and buffer-change and temperature-change times are shown in Table 1.

**Animals and tissue.** Male Sprague-Dawley rats (180–220 g) were stabilized for at least 1 wk after delivery in communal cages with free access to rat chow (Ralston-Purina Company, St. Louis, Missouri) and water. After this period, they were killed by decapitation and the peritoneal and thoracic cavities were opened for rapid collection of organs. These were wrapped in foil and stored on ice until transferred to a −70°C freezer for storage. Within 1 wk, individual organs were thawed and about 500 mg of each organ was homogenized and deproteinized with 0.05 ml of 7.5 M perchloric

TABLE 1
Composition of lithium citrate buffers for amino acid analysis with temperature- and buffer-change times

| Buffer | 3M Citrate (ml/5 L) | 12 N LiCl (ml/5 L) | Thiodiglycol (ml/5 L) | Octanoate (ml/5 L) | 3N LiOH to pH |
|---|---|---|---|---|---|
| I | 160 | 45.3 | 25 | 0.5 | 2.95 |
| II | 160 | 45.3 | 25 | 0.5 | 3.80 |
| III | 175 | 349 | 25 | 0.5 | 4.60 |

| Occurrence | Time (min) |
|---|---|
| Temperature change (40°C → 60°C) | 60 |
| Buffer changes I → II | 100 |
| II → III | 200 |
| Shutdown | 370 |

acid and then rehomogenized after an additional 1.95 ml of deionized water was added. This suspension was spun at 4000 × *g* for 10 min at 0°C and the supernatant was decanted and stored at −70°C until analysis. Studies have shown GABA level to be stable under these conditions (unpublished).

In the streptozotocin studies, male Sprague-Dawley rats (180–220 g) were maintained in communal cages for 1 wk as described above and then transferred to individual metabolism cages, where they were afforded free access to food and water. After a control period, streptozotocin was administered at a level of 50 or 75 mg/kg i.p. No polydipsia, polyphagia, polyuria, or glycosuria was found during the control period, which lasted five days. These classic symptoms of diabetes began 48–72 h after administration of the drug, and all animals were killed 72 h after administration of the drug. Animals that did not become diabetic were excluded from the data analysis.

Catfish were maintained in a community tank and fed pelleted fish food (Shrimp-el-ettes, Hartz Mountain Corporation, Harrison, N.J.) daily. A 50% water exchange was performed weekly. The fish were killed by decapitation before any appreciable anoxia could occur and were opened along the ventral midline. The Brockmann body was visualized behind the gallbladder and removed carefully to avoid including any extraneous tissue. This was immediately frozen at −70°C until homogenization, deproteinization, and analysis as described above.

Rat islets of Langerhans tissue was isolated by the collagenase method[18] with modifications. Rather than using gradient centrifugation to harvest islet tissue, the islets were collected by hand from minced pancreases using a fine wire loop. In addition to minimizing non-islet tissue inclusions, this obviated the possibility that gradient centrifugation may have altered any membrane characteristics.

**Statistical analysis.** All values reported are arithmetic means ± standard deviation. Linear regression analysis was used to compare the two methods of GABA analysis and to assess the degree of correlation between plasma glucose and plasma or pancreatic GABA levels. Significance of differences was tested by Student's *t* test (two-tailed).

## RESULTS

Of the organs examined in this peripheral screen for GABA, the pancreas contained the highest concentration (41.2 ± 13.2 pmol/mg wet wt) (see Table 2). GABA was later found to be present at even higher levels in isolated islet tissue. Multiple analysis by the I-E/F technique of about 75 islets pooled from two normal rats yielded a GABA value of 190 pmol/mg wet wt. Analysis of 11 peripheral organs by both the I-E/F method and full amino acid analysis yielded a correlation coefficient of 0.95 by linear regression analysis, attesting to the suitability of the I-E/F method for measuring the GABA content of peripheral organs.

During the streptozotocin experiment, urine glucose and blood glucose were determined at the time of sacrifice to confirm the induction of diabetes in the experimental animals. Except for two animals, which were apparently not made diabetic, the experimental animals (n = 10) experienced a rise in blood glucose from 132 ± 30 mg/dl ($\bar{x}$ ± SD) to 357 ± 83 and were glycosuric. Streptozotocin caused a significant reduction in pancreatic GABA level, to about 34% of the control value (see Figure 1). A low degree of correlation was seen between plasma glucose level and pancreatic GABA level (r = 0.620). In addition, a similar correlation was seen in plasma between glucose and GABA concentrations (r = 0.664). The two noninduced animals

TABLE 2
GABA content of various peripheral organs from control and streptozotocin-diabetic rats

| Organ | GABA Content (pmol/mg wet wt) ($\bar{x}$ ± SD) | | | | |
|---|---|---|---|---|---|
| | Control | | | Streptozotocin treated | |
| | By full amino acid analysis | By I-E/F method | N | By I-E/F method | N |
| Pancreas | 48.3 | 41.2 ± 13.2 | 10 | 14.1 ± 8.92* | 10 |
| Thymus | — | 38.2 ± 6.12 | 3 | 65.8 ± 23.3 | 3 |
| Adrenal | — | 34.9 ± 15.8 | 3 | 47.2 ± 27.0 | 3 |
| Lesser curvature of the stomach | — | 27.7 ± 5.24 | 3 | 22.2 ± 6.55 | 3 |
| Liver | 19.3 | 21.8 ± 6.08 | 6 | 31.3 ± 3.23 | 3 |
| Spleen | 18.0 | 18.1 ± 6.18 | 6 | 26.8 ± 7.81 | 3 |
| Lung | 11.4 | 14.6 ± 9.16 | 6 | 13.6 ± 6.47 | 3 |
| Heart | 8.49 | 8.08 ± 2.51 | 5 | 11.0 ± 0.86 | 3 |
| Testes | 7.85 | 5.59 ± 2.7 | 3 | — | — |
| Plasma | — | 580 ± 111 (pmol/ml) | 3 | 658 ± 311 (pmol/ml) | 3 |

*$P < 0.001$.

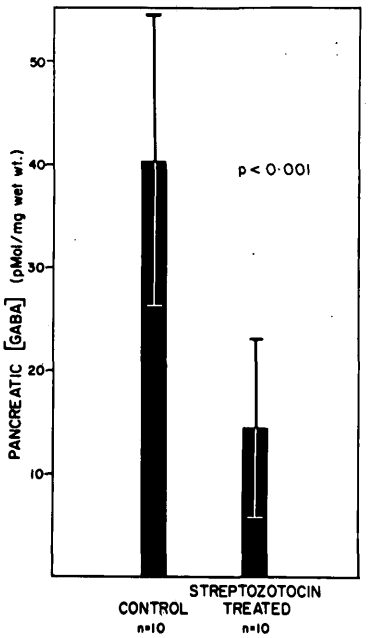


**FIGURE 1. Pancreatic GABA levels in control (N = 10) and streptozotocin-diabetic (N = 10) male Sprague-Dawley rats. Data are expressed as mean ± standard deviation.**

were normoglycemic and exhibited pancreatic GABA levels in the control range. These were excluded from data analysis.

The Brockmann body is a nodule of endocrine pancreatic tissue that is more or less anatomically separate from the exocrine pancreas in teleosts.[19] Analysis of three Brockmann bodies yielded values of 133, 103, and 128 pmol GABA/mg wet wt (mean ± SD = 121 ± 16). This compares favorably with results obtained in isolated rat islets of Langerhans tissue of 190 pmol GABA/mg wet wt.

## DISCUSSION

These results, which are consistent with our previous findings,[20,21] have shown that the presence of GABA is not restricted to the CNS, at least in the rat; but rather is ubiquitously present at various levels in the periphery. It is present at relatively high levels in the pancreas, thymus, and adrenal gland and at lower levels in lung, heart, and testes. Intermediate concentrations of GABA are found in the spleen, liver, and along the lesser curvature of the stomach. Other studies have shown that GABA is present at still lower levels in blood.[8,9] Rat blood has been shown to contain about 800 pmol GABA/ml,[6] a concentration roughly equal to 800 pmol/g or 0.8 pmol/mg. This could not contribute significantly to the 50 times higher levels of GABA in the pancreas or the, approximately, 230 times higher level in isolated islet tissue.

The presence of GABA in peripheral organs is not restricted to mammalian species, as evidenced by the fact that the Brockmann body of catfish contains a level similar to that of mammalian islet tissue. The somewhat lower level in Brockmann body tissue may be caused by the lower percentage of β-cells in this tissue or the fact that some exocrine tissue may be contained in the Brockmann body.[19] Electrophysiologic investigation of the teleost Brockmann body may be useful for determining the role of GABA in endocrine pancreatic function.

It is important to distinguish between a reduction in islet GABA as a consequence of the diabetic state and a reduction due to the loss of β-cells caused by streptozotocin. Because of the acute nature of these studies, it seems unlikely that the long-term consequences of the disease are noticeable. For this reason, and the fact that, in human insulinoma tissue, GABA levels are significantly increased,[6] it seems reasonable that the streptozotocin-induced loss of GABA from the pancreas is related to this selective toxin's ability to destroy β-cells. This is further reinforced by the data in Table 1, which show no significant change in GABA content after streptozotocin in any organ other than the pancreas. It is further reasonable to speculate, based on these findings, that GABA in the endocrine pancreas is associated with, or contained in, the β-cell of the islet and that loss of this cell population causes a significant loss of pancreatic GABA, since others[7] have shown no change in acinar GABA content after streptozotocin treatment.

The low degree of correlation between plasma glucose concentration and GABA concentration in plasma, as well as in the pancreas, is not necessarily surprising. In light of the fact that glucose is the major stimulus to insulin release, GABA may act, like somatostatin, as a local regulator in endocrine pancreatic function, and may not have major, direct effects on the level of blood glucose.

The quantitative data reported here parallel those of Okada and Taniguchi et al.,[6,7,22] but are consistently at least two to five times lower. For example, in 1975, they reported a value for GABA in pancreatic islet tissue of 19 mmol/kg (nmol/mg) wet wt. In 1976, that group reported a GABA value for whole pancreas corresponding to 0.6 mmol/mg wet wt. These values are about 24 times and 16 times greater, respectively, than those measured with the I-E/F method. In 1977 the same group[7] reported values 22 times greater than those determined by the I-E/F method for islets but only 2.4 times greater for whole pancreas. It is interesting to note that, for this last pancreas determination, enzymatic NADPH cycling was not used in the GABA assay.

Thus it seems that the enzymatic assay for GABA, especially when NADPH cycling is used, may be subject to interference and, therefore, may not be suitable for the measurement of GABA in peripheral tissue. In general, though, we agree that relatively high levels of GABA are present in the endocrine pancreas and that streptozotocin treatment reduces these levels. The concept of GABA's presence in the endocrine pancreas is consistent with the proposed neural crest origin of islet tissue and its inclusion by some investigators in the amine precursor uptake and decarboxylation (APUD) system.[23] The presence of glutamic acid decarboxylase has been reported in pancreas and islet tissue at sufficiently high activities to account for the GABA levels in these tissues.[7] Viewed in this light, it is not surprising to find relatively high GABA levels in the endocrine pancreas. The role of GABA in islet function is not known at this time. In addition to a purely metabolic role, the possibility exists that it may serve as a local modulator of endocrine pancreatic function.

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