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COMMENTARY

GABA IN PANCREATIC ISLETS: METABOLISM AND FUNCTION

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γ-Aminobutyric acid (GABA) was discovered over 40 years ago in brain [1–3], where it was later identified as the key inhibitory neurotransmitter [4, 5]. Since that time, evidence has accumulated that this amino acid may be of functional importance not only in the central nervous system but also in peripheral tissues, such as gut, urinary bladder, heart, lung, ovary and pancreas [6–9]. The concentration of GABA in mammalian organs varies considerably; it is particularly high in brain, with an average value of 2–3 μmol/g wet weight (20–30 μmol/g protein) and a regional distribution of 10–30 μmol/g protein [10, 11]. By contrast, in most peripheral tissues GABA content is low, about 1% of that in brain [6–9]. An exception is the female genital tract [12, 13] and pancreatic islets [14, 15] where considerably larger amounts have been found. Several recent reviews have evaluated the possible role of GABA outside the mammalian brain [16–20], but none dealt specifically with its metabolism and physiological function in either the pancreas or the islets of Langerhans. The object of this article is to correct this oversight and to provide a critical review of the literature on the subject, to identify areas of obscurity and/or controversy, and to suggest directions for future investigations.

Islet GABA concentration

The concentration of GABA† in the pancreas and its subfractions has been measured in a number of laboratories (Table 1). A comparison of the values obtained is difficult, however, because of the diversity of ways in which the results are presented. The basis for expression includes: wet weight, dry weight, DNA and protein, where the latter had been measured with a variety of techniques. To attempt a meaningful analysis of the data we have recalculated the original figures to the same common unit, using conversion factors specified in the legend. Inspection of these latter results shows that in spite of disappointingly large differences among laboratories, certain conclusions can be drawn because trends toward patterns of behavior seem relatively consistant. First, there appear to be variations in the levels of pancreatic GABA in different animal species; the values reported are low in mouse and rabbit and high in rat. There is also some evidence, which is not documented in the table, that age may be important, since the GABA content is almost 5-fold higher in organs from immature animals [31]. Second, pancreatic islets are enriched in GABA, irrespective of the species. Third, human insulinomas (presumably of β-cell in origin) contain large amounts of the amino acid. Fourth, acinar cells, i.e. those of the exocrine pancreas, also contain GABA albeit at a level 1/10 that in islets.

The discrepancies in GABA concentration in the pancreas and its components measured in different laboratories deserve a comment. In rat pancreatic islet for example, the difference between the data of Gerber and Hare [8, 24] and those of Okada and associates [19, 21, 29] is 25-fold, which is surprising because both groups used apparently very similar techniques (HPLC vs amino acid analyzer). It is, therefore, difficult to decide which value is correct. If, however, one assumes that the figure given by Gerber and Hare [8] of about 190 pmol/mg wet weight corresponds to the true intracellular content, one can calculate assuming a water content of wet weight of about 60%, that this translates into a concentration of less than 0.5 mM. This is by at least an order of magnitude less than in brain and low for a tissue where GABA is supposed to play an important physiologic function. Our own measurements (Table 1), made with an HPLC [32], yielded numbers very close to those of Okada and coworkers and were in the range of 3–5 mM. The latter value is close to that in brain and much more typical of tissues which use GABA as a transmitter of information.

The apportionment of GABA within pancreatic islets has been evaluated using enzymatic and immunological techniques. Both give consistent results in that they show a non-homogenous distribution among cells as well as within the structure of an individual cell. Enzymatic determinations have indicated that GABA is present in the inner as well as the outer part of the islet, although in the former its concentration is considerably higher [29]. By contrast, immunological studies have revealed that GABA-reactive cells are present exclusively in the central core of islets [33, 34], which is believed to be composed mainly of β-cells [35]. Comparing the localization of GABA with that of insulin, glucagon and somatostatin [33, 34], the amino acid was detected only in the insulin-containing β-cells.

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† Abbreviations: GABA, γ-aminobutyric acid; GAD, L-glutamate decarboxylase: and GABA-T, GABA-α-ketoglutarate transaminase.

Table 1. Comparison of GABA concentrations in different regions of the pancreas (the last column represents data from original papers recalculated to the same unit)

Tissue	Reference	Original units	GABA	$GABA_{mmol/kg\ wet\ wt}$
Rat				
Pancreas	[8]	pmol/mg wet wt	48.3	0.048
	[21]	mmol/kg dry wt	2.51	0.632
	[22]	nmol/g wet wt	54	0.054
	[23]	mmol/kg wet wt	0.0286	0.029
	[23]	mmol/kg wet wt	0.0083*	0.008
	[19]	mmol/kg wet wt	0.125	0.125
	[24]	pmol/mg wet wt	41	0.041
	Nonpublished	mmol/kg wet wt	0.060	0.060
Pancreas (tail)	[7]	mmol/kg wet wt	0.153	0.153
Acini	[21]	mmol/kg dry wt	1.97	0.496†
Islets of Langerhans				
	[8]	pmol/mg wet wt	190	0.190
	[21]	mmol/kg dry wt	18.9	5.04‡
	[23]	mmol/kg wet wt	0.193	0.193
	Nonpublished	mmol/kg dry wt	14.9	3.97‡
	[19]	mmol/kg dry wt	15.3	4.13‡
Rabbit				
Pancreas (tail)	[24]	pmol/mg wet wt	14.0	0.014
Pancreas (body)	[24]	pmol/mg wet wt	3.0	0.003
Dog				
Pancreas	[23]	mmol/kg wet	0.43	0.43
Cat				
Pancreas	[27]	mg/100 g wet wt	0.7	0.68
Mouse				
Pancreas	[28]	μmol/g wet wt	0.004	0.004
Islets of Langerhans	[29]	mmol/kg dry wt	3.0	0.800‡
	[30]	mmol/kg dry wt	3.1§	0.827‡
	[15]	mmol/kg dry wt	2.1§	0.560‡
Human insulinoma				
Nontumor region	[21]	mmol/kg dry wt	2.81	0.708‖
Tumor region	[21]	mmol/kg dry wt	25.47	6.42‖

* Streptozotocin diabetic rats.

† Since sufficient data are not available, for acini we use water content of 74.8% reported for whole pancreas [25].

‡ Calculated from original data using the average rat islet dry weight of 0.8 μg, average protein content of 800 ng, and wet weight of 3 μg per islet [26]. The same values were used for mouse islet, since it seems to be similar in protein content and dry weight.

§ Islets isolated from ob/ob mouse.

‖ For insulinoma we used water content of 74.8% reported for whole pancreas [25].

Moreover, administration of streptozotocin, a selective β-cell toxin, decreased pancreatic levels of GABA significantly [8] as well as reduced the number of GABA-immunoreactive cells [33]. All these results indicate that within islets, the insulin-producing β-cell is the main repository of GABA and, if the amino acid is also contained in either α or δ cells, it must be present at very low concentrations.

There are some reports on intracellular distribution of GABA within the β-cell. Using immunostaining, Garry *et al.* [36] demonstrated the amino acid in the nuclear, mitochondrial and cytoplasmic compartments but not in the secretory granules. In percentages, the distribution values were 11, 5.4, 80 and 2.8, respectively, for the four compartments. Later studies established that in a β-cell derived line, βTC3, the immunoreactivity for GABA was particularly high in neurite-like processes that stained strongly for synaptophysin [37]. It was postulated, therefore, that the amino acid is concentrated in regions which are enriched in "synaptic-like microvesicles" (SLMVs), a population of vesicles present in β-cells [37] and distinct from the secretory granules [38]. Thus, although it appears that GABA is present in the cytoplasm of the β-cell, it may also be sequestered within specific vesicular structures which resemble granules that undergo exocytosis.

The presence of GABA in exocrine pancreas has been established by enzymatic measurements of the amino acid content [21] and later confirmed with immunocytochemical techniques [39]. Quantitative analyses of the distribution of the GABA immunogold particles within the acinar cell revealed that the reactivity was localized predominantly over the zymogen granules [39].

Metabolism of GABA

Localization of GABA-producing and -metabolizing enzymes. The major pathway that synthesizes

γ-aminobutyric acid is decarboxylation of glutamate catalyzed by L-glutamate decarboxylase (GAD, L-glutamate-1-carboxylyase; EC 4.1.1.15). The activity of this enzyme has been described in both β-cells of the islets and acinar cells of exocrine pancreas at a ratio of about 13:1 [21]. Immunological studies have confirmed the exclusive location of GAD in β-cells [39, 40] but did not yield consistent data on its existence in the exocrine part of the organ. Using an antibody against the enzyme from mouse brain, Vincent *et al.* [40] could not detect GAD in this tissue whereas Garry *et al.* [39] reported a positive result when the presence of the protein was probed with an antibody directed against the rat brain antigen. The reason for the discrepancy between the two studies is not clear. Although the simplest explanation is that there may be species differences in the antigenic properties of GAD, this seems to us an unlikely solution.

Detailed studies on β-cells have identified two antigenic forms of GAD, a M_r 65,000 hydrophilic and soluble form and a M_r 64,000 component of a rather complex structure [41]. A major portion of GAD_{64} is hydrophobic and firmly bound to the membrane so that it can only be extracted from membrane fractions by the use of detergents. A second portion is also hydrophobic but soluble while a third, minor component is soluble and hydrophilic. All the GAD_{64} forms can be resolved into two isoforms, α and β which differ by about 1 kDa in mobility on sodium dodecyl sulfate–polyacrylamide gel electrophoresis. Both forms of islets GAD, i.e. GAD_{65} and GAD_{64}, are identical to the larger and small forms of the brain enzyme [41]. Studies with light and electron microscopy immunocytochemistry co-localized GAD, to a large extent, with synaptophysin in the same cell; this observation served as the basis for a suggestion that the enzyme was bound to the synaptic-like microvesicles [37]. However, GAD could not be recovered in either synaptic vesicles or the synaptic-like microvesicles purified by immunoisolation [37] which is surprising in view of the findings mentioned above that a portion of the enzyme is so tightly bound to cellular membranes that detergents are required for its extraction [41]. Hence, the identity of the cellular membrane to which the hydrophobic GAD_{64} attaches remains an open question. A low level of GAD immunolabeling was also observed throughout the cytoplasm [37] which is consistent with the existence of the hydrophilic form of the enzyme.

Interestingly, GAD was not detected in RINm5F cells [37] which were originally derived from β-cells and are often used as their model. Whether or not this lack is due to the fact that RINm5F cells also exhibit some features typical of α-cells [42], which do not contain GAD [40], is not yet clear.

GABA degradation is catalyzed by a transaminase (GABA-α-ketoglutarate transaminase, GABA-T, EC 2.6.1.19). Using immunohistochemical techniques, GABA-transaminase has been detected exclusively in the β-cells of the pancreas [40, 43], where its presence is restricted to the mitochondrial compartment [43]. GABA-transaminase could not be found in cytoplasm, mitochondria or zymogen granules of the acinar cells [39].

Production of GABA. Both β-cells and acinar cells can synthesize GABA from glutamate [21]. The amount of GABA produced by islets is 66.7 mmol/kg dry weight per 60 min, over 20-times the amount synthesized by whole pancreas and 10-fold that by acini ([12], Table 2). The most important sources that provide glutamate, the substrate for GAD, are glucose and glutamine. The former has to be metabolized to α-ketoglutarate, which undergoes reductive amination to glutamate. The latter is hydrolyzed through the action of glutaminase. Experiments with radioactive glucose [30] showed that in pancreatic islets microdissected from obese-hyperglycemic mice, glucose can serve as an efficient precursor of GABA. After incubation with 5 mM D-[U-^{14}C]glucose, most of the radioactivity was found in aspartic acid, glutamic acid and GABA. The specific incorporation into GABA was much higher than into glutamic acid, what suggests that there may be intracellular compartmentation of GABA-generating pathways.

Production of radioactive γ-aminobutyric acid from L-[U-^{14}C]glutamine in rat islets has also been demonstrated [44]. In the presence of antimycin A (an inhibitor of mitochondrial respiration which also blocks GABA catabolism by preventing oxidation of succinic acid semialdehyde), utilization of L-[U-^{14}C]glutamine occurs at a rate of 7.8 pmol/islet per 120 min or 0.02 μmol/min per g wet weight if average islet wet weight is 3 μg [26]. If one assumes that under such conditions the predominant reaction which consumes glutamine is glutamate decarboxylation and that glutaminase activity is high enough not to be limiting, the value calculated above corresponds to the rate of GAD activity. Consistent with the supposition that glutaminase is not the rate-limiting enzyme are recent studies on islet homogenates which show that the velocity of glutamine breakdown is high particularly in the presence of orthophosphate [45]. At physiologic phosphate concentration (4–10 mM) the amount of glutamate produced from glutamine is 20–32 nmol/mg protein per min [45] which is at least one order of magnitude greater than the rate of glutamate decarboxylation (66 mmol/kg dry weight per hr [21] or 1.2 nmol/mg protein per min as calculated using the conversion factors presented in the legend to Table 1).

The rate of GABA synthesis in intact islets can also be calculated from measurements of the increase in amino acid content when its degradation is blocked. Taniguchi *et al.* [46] showed that in the presence of inhibitors of GABA-transaminase, γ-vinyl GABA (100 μM) and aminooxyacetate (10 μM but not at higher concentrations), the islet content of the amino acid (16.6 mol/kg dry weight) rose to 177 and 212% of control, respectively, after 2 hr of incubation. Assuming a conversion factor of 4 from the dry to wet weight, the synthesis of 12.8 to 18.6 mmol/kg of dry weight per 120 min corresponds to a rate of about 0.025 to 0.04 μmol/g wet weight per min, which is not much different from the value calculated above from the results of Malaisse and coworkers [44].

Glutamate decarboxylation, however, is not the sole pathway for GABA formation in either brain

Table 2. GAD activities in pancreas

Source	GAD activity ($mmol_{GABA}$/kg dry wt per hr)	Reference
Rat		
Whole pancreas	2.58	[21]
	1.3*	[22]
Islets	66.7	[21]
Acini	4.67	[21]
Human insulinoma		
Nontumor region	2.01	[21]
Tumor region	138.2	[21]

* mmol/kg wet wt per hr.

[47] or peripheral tissues [6]. An alternative route is via oxidative deamination of putrescine (1,4-diaminobutane), which in islets in contrast to brain involves diamine oxidase and not monoamine oxidase [22]. Evidence, however, has been presented that inhibitors of either mono- or diamine oxidases do not change significantly GABA level in the pancreas. Thus, it seems that the putrescine pathway does not contribute to any significant extent to pancreatic GABA production [22].

Catabolism of GABA. There is no quantitative information on the rate of GABA catabolism in islets, although the localization of the transaminase to β-cells indicates that the latter are responsible for the amino acid breakdown. Perusal of the literature shows that activities of GABA-T in the peripheral tissues are, in general, lower than in the central nervous system [9]. Since there is no straight forward correlation between GABA level or GAD activity and the velocity of GABA-transaminase [9], even approximate estimates of the latter are impossible.

GABA uptake. The presence of GABA in a cell means that either the amino acid is produced there or that it is taken up from outside. For this reason, transport can be considered a component of metabolism.

Uptake of GABA has been investigated in both pieces of pancreas [24] and isolated islets [48]. In fragments of rabbit pancreas, no increase in the tissue content was observed at either 0.1 of 10 μM external amino acid levels, but a 16-fold rise was detected at a concentration of 1 mM [24]. This may suggest that there is no high-affinity transport for GABA, i.e. one that occurs with a micromolar K_m value. However, in a heterogeneous system, such as pancreas, even a large accumulation can pass unnoticed, if it involves only a very small population of cells. Moreover, in thick (mm^3) tissue fragments incubated with micromolar GABA concentrations, the actual level seen by cells situated further below the surface or deep inside may be considerably smaller than in the bulk medium because of large diffusional distances; this could limit transport activity. A different situation might occur at high external amino acid concentrations when its "trapping" in the interstitial space could result in spuriously elevated tissue levels. For these reasons, experiments quoted above cannot be used as evidence either for or against the existence of GABA uptake in pancreas.

Another approach to evaluate GABA uptake is by following accumulation of a radiolabeled analogue [31, 48–50]. Autoradiographic examinations of [^{3}H]-GABA transport in the tissue from adult rats showed that the exocrine part (acini) of the pancreas contained higher levels of radioactivity than the endocrine part [48]. Within islets only a small proportion of heavily labeled cells was present in the outer region which was tentatively identified as belonging to the location of δ-cells. Moreover, a comparison of the accumulation of radioactive GABA and leucine revealed that in islets, in contrast to brain, the ratio of radioactivity in the tissue to that in the medium (a so-called uptake ratio which is an index of the capability of a cell to concentrate the amino acid) was lower for GABA than for leucine, even after a 50-min incubation [48]. Since the GABA concentration used was very low, 0.5 μM, this result seems to support our earlier contention that the high-affinity uptake system may be absent from islets.

Analyses of [^{3}H]GABA uptake by pancreata from animals at various stages of development indicated that in the early stages, transport is much more prominent [31]. In organs from both the fetal and perinatal period radioactivity could be detected in acini (and centroacinar cells) as well as in the endocrine pancreas. Within the latter, some δ-cells and immature β-cells were labeled. Uptake of GABA by acinar cells was found to be inhibited by β-alanine whereas that by cells of the endocrine pancreas was not; this might be because different types of transporters are involved. In pancreata from adult animals, no labeling of exocrine pancreas was observed in this series of experiments [31] which is in contrast to the earlier work of Taniguchi *et al.* [48]. However, in both studies there was no accumulation of radioactivity by β-cells of adult rats and substantial accumulation by a small proportion of cells identified as δ-cells by the presence of somatostatin-containing granules [50].

On the basis of the accumulation of radioactivity from a medium containing 0.5 μM GABA, Gilon and coworkers [31, 50] concluded that δ-cells contain a high-affinity transporter for this amino acid. However, such a postulate is not justified because

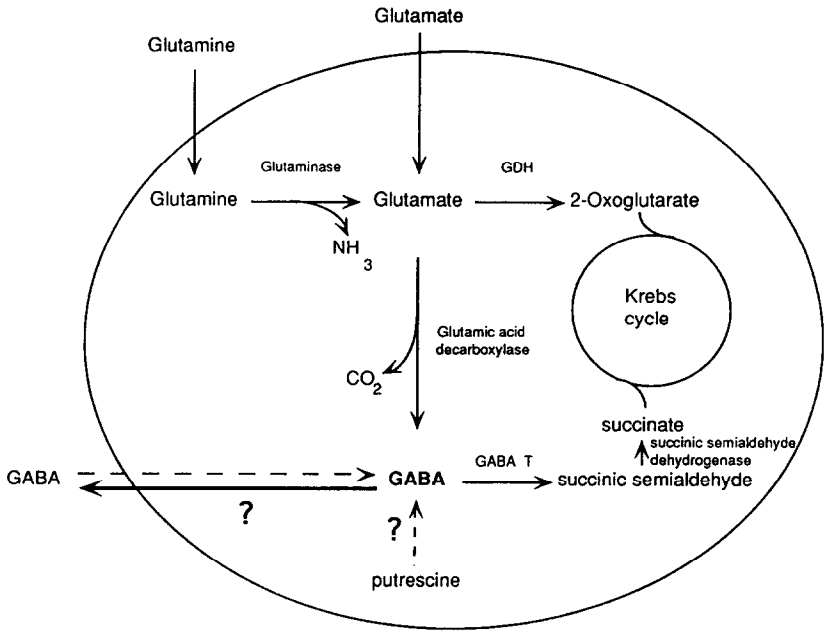

Fig. 1. Metabolic pathways of GABA in pancreatic β-cells. See text for details. Abbreviations used: GDH, glutamate dehydrogenase; and GABA-T γ-aminobutyric acid transaminase.

identification of the nature of transport requires knowledge of the K_m value for substrate. Accumulation of radioactivity from a highly labeled source, after long incubation times (30–50 min in different experiments), may also occur via a low-affinity process which functions even at a low (μM) concentration of the amino acid, albeit very slowly. Notwithstanding the mechanism of uptake, the actual amount of GABA which accumulates inside has to be very small because immunological methods failed even to detect its presence in the δ-cells (see above).

It is important to note that plasma [GABA] is less than 1 μM [9] and extracellular concentration is not likely to be much higher. This indicates that if GABA uptake is of any importance to cellular function in either exo- or endocrine pancreas, it has to occur via a high-affinity system.

Relationship among GABA levels, GABA transporter and GABA metabolism. To understand the role of GABA in pancreas it is necessary to have a clear picture of the relation between GABA level on the one hand, and its uptake and metabolism on the other (Fig. 1). Table 3 summarizes, in a qualitative way, the results from the literature which provide information on the distribution of the amino acid and the appropriate enzymes in various constituent cells of the organ. Inspection of the data shows that β-cells exhibit a pattern which is easy to explain. These cells possess a GABA-producing enzyme, GAD, as well as the degrading enzyme, GABA-transaminase. Both enzymatic reactions can maintain a steady-state level of the amino acid without the necessity for uptake from outside. Of the two remaining types of islet cells, α-cells seem neither to contain nor metabolize GABA whereas the situation in δ-cells appears to be more complex. There is reasonably good evidence that the latter accumulate GABA from outside [50]. On the other hand, they do not possess enzymes involved in the metabolism of the amino acid. Since the steady-state level of GABA in δ-cells is very low (see discussion above), it could be maintained by a slow and inefficient transporter which mediates exit as well as entry. This mechanism would prevent excessive loading of cells with the amino acid.

A somewhat similar situation exists in acinar cells which synthesize ([21] but see [40] to the contrary) and take up [48] GABA, but have no GABA-transaminase activity [39]. The existence of GAD could allow larger accumulation of the amino acid than that which occurs through a rather inefficient uptake system alone. An obvious conclusion is that exocrine pancreas must also either release GABA continuously or metabolize it via an alternate, as yet undefined, route in order to prevent excessive loading of the acinar cells.

Physiological functions of GABA

The physiologic role of GABA can be related to one of the following functions or a combination thereof: (i) GABA either produced intracellularly or taken up from outside serves as a fuel; (ii) GABA serves as a regulator of some β-cell function(s), or (iii) GABA is released from one cell and acts either on the same, or another cell by binding to specific sites on the membrane.

GABA as a fuel. Metabolism of GABA occurs via the GABA-transaminase reaction which produces succinic acid semialdehyde and enzyme-bound pyridoxamine. The former is converted to succinate and oxidized by the mitochondrial respiratory chain, whereas the amino group is transferred onto α-

Table 3. Cellular and subcellular localization of GABA, GABA-transaminase and GAD in rat pancreatic islets and acini

Cell type	GABA	GABA uptake	GABA-T	GAD	Reference
α-Cells	−		−		[34, 43]
	+*				[29]
β-Cells		+†			[31]
Membrane fraction				+++	[41]
Cytoplasm	+++			+++	[36]
"Small vesicles"	+++				[37]
Mitochondria	++		+++		[36, 43]
Secretory granules	−				[36]
RINm5F cells				−	[37]
δ-Cells	−	+	−		[31, 34, 43, 48]
	†*				[29]
Acinar cells	+	+‡		+	[7, 21, 31, 48]
Zymogen granules	+++		−	+	[39]
Cytoplasm	+		−	+++	[39]

* Measurement was done in the outer part of the pancreatic islet consistent with α- and δ-cells.
† Immature β-cells only.
‡ In fetal and perinatal period; controversial results were obtained in adult animals [31, 48].

ketoglutarate, thus regenerating glutamate. The only pancreatic cells that possess GABA-T are β-cells of the islets; hence, only in these could GABA serve as a fuel [40]. In the so-called GABA-shunt, i.e. the cycle of reactions which include GAD and GABA-T, the latter enzyme is usually not rate limiting and the velocity of the shunt depends, to a large extent, on the velocity of GAD. As calculated above, under conditions which exist in islets this latter amounts to 0.02 to 0.04 μmol/g wet weight per min. This can be compared with the figure of 0.03 μmol/min per g of tissue in brain [51]. it is evident that the values are rather similar and, assuming that the crude calculation of flux in islets is correct, it indicates that metabolism of GABA by β-cells is substantial and thus the amino acid could serve as a potential source of fuel. An interesting additional similarity between brain and β-cells is that in both the glutaminase reaction may be the key source of glutamate that is then converted to GABA.

Intracellular functions of GABA. The only indication in the literature that intracellular GABA may exert a regulatory function is the finding of Taniguchi *et al.* [7] who reported that elevation of the intracellular concentration of this amino acid by treatment with γ-vinyl-GABA stimulates incorporation of [^{3}H]leucine into insulin, but not into other proteins. Since there was neither an increase of cellular insulin content nor of its release [46], the observation indicates that increased synthesis of the protein must have been accompanied by an equally enhanced degradation. Variations in exogenous [GABA] did not influence leucine incorporation, which argues against the involvement of plasma membrane receptors. The physiological relevance of this phenomenon is not clear for the following reason. Administration of a high concentration of glucose (which causes insulin release) is accompanied by a fall in islet [GABA] but results in stimulation of insulin synthesis [52]. On the basis of the postulate above one would expect an opposite effect, if there was a causal relation between GABA content and production of the hormone.

GABA as an intercellular messenger. For any molecule to be accepted as a mediator of signal transfer certain minimum criteria must be fulfilled: (i) existence of a specific receptor has to be demonstrated (ii) evidence for a release has to be provided; and (iii) a specific physiologic effect has to be identified. With respect to the first, there is some evidence in the literature (although this is by no means very firm; see Refs. 16 and 20 for review) that peripheral tissues contain GABA receptors which resemble those in the central nervous system. They fall into two categories: the classic $GABA_A$ postynaptic receptor (which also includes in its structure the chloride channel and the benzodiazepine binding site), which is activated by muscimol and inhibited by bicuculline and picrotoxin, and the $GABA_B$ receptor where baclofen is an agonist. There has been no attempt to categorize and quantify GABA receptors in pancreas (or its subfractions) using binding assays. This is surprising in view of the fact that several specific radiolabeled agonists and antagonists have been developed over the years and used extensively in investigations on the nervous system. The only direct evidence for the existence of pancreatic GABA receptors comes from the work of Rorsman *et al.* [53]. Using an antibody originally produced against β-subunit of the brain $GABA_A$ receptor [54, 55], the latter authors were able to detect a positive immunostaining reaction in glucagon-containing α_2 cells (δ-cells) and somatostatin-containing α_1 cells (α-cells). This result is consistent with the presence of $GABA_A$ receptors on the surface of these two types of islet cells.

Evidence for GABA release from any pancreatic structures is also very scarce. The only relevant observation seems to have been made by Gerber and Hare [8] who reported that upon transfer of pieces of pancreas from a medium containing 2.8 mM to one with 27.8 mM glucose, the external

concentration of GABA doubled in 15 min. The source of the released GABA was not established. The synaptic-like microvesicles of β-cells are an obvious candidate since they should be able to undergo exocytosis. Pancreatic nerve cells and fibers are unlikely to secrete GABA because there is no evidence that these structures contain the amino acid [40]. In other systems, such as neurons, amino acids can also be released, under some conditions, in a calcium-independent manner [56], most likely from the cytoplasm. However, there is no information on the possible existence of such a mechanism in the pancreas. In conclusion, work is needed to identify a source of GABA and a mechanism of its secretion.

With respect to the third criterion, there is reasonably good evidence from at least two different systems that application of either GABA or its agonists influences release of other pharmacologically active substances. The first is the effect on somatostatin secretion. Addition of 1 μM muscimol to perfused rat pancreas decreases by 38% the release of this hormone triggered by high glucose [57]. Similarly, inclusion of 100 μM GABA in the buffer used to perifuse isolated islets reduces by 30–40% secretion of somatostatin which occurs in the presence of 16.7 mM glucose [58]. The effect is prevented by simultaneous addition of bicuculline. Consistent with the acute experiments, culturing islets for 3 days with either GABA (0.1 or 1 mM) or muscimol (1 μM) results in an increased somatostatin content of the tissue, in the presence of low as well as high glucose concentration. Sensitivity of the changes to bicuculline suggests that this effect may be mediated by $GABA_A$ receptors. In apparent disagreement with the above studies on rats are experiments on perfused dog pancreas in which secretion of somatostatin was elicited by infusion of 10 mM arginine [59]. In this system GABA (1, 10 and 100 μM) induced a small, transient increase in hormone release. A surprising observation of this same study was that 50 μM bicuculline on its own exerted effects similar to those of GABA, i.e. agonist-like; this latter finding makes the interpretation of the observation of Kawai and Unger rather difficult.

The second system concerns the interaction between GABA and the release of glucagon. In an elegant and well-controlled study, Rorsman *et al.* [53] showed that in perifused guinea pig islets stimulated with 10 mM arginine, the enhanced secretion of glucagon was blocked almost 70% by 100 μM GABA. Bicuculline (100 μM) prevented this inhibition to a great extent. In isolated glucagon-containing cells, application of either 100 μM GABA or muscimol (at an unspecified concentration) caused an increase in chloride conductance and hyperpolarization of the plasma membrane. Both changes were prevented by bicuculline and picrotoxin. On the basis of their results the authors proposed that activation by agonists of the $GABA_A$ receptor–chloride channel complex hyperpolarizes the cell which in turn lowers intracellular free calcium concentration and thus reduces glucagon secretion. It was also postulated that *in vivo* the β-cells serve as the source of GABA that inhibits glucacon release. Although the existence of the amino acid in synaptic-like vesicles of these cells [37] and the direction of blood flow in islets [60] support this hypothesis, the postulate requires a direct demonstration that GABA is released from β-cells by high glucose concentration. As discussed above, the evidence for this is rather weak [24]. Moreover, not all data in the literature are consistent with this view. For example, in perfused dog pancreas stimulated with high arginine, GABA up to 100 μM had no effect on glucagon secretion [59], whereas in humans a single oral dose of GABA (5 or 10 g) resulted in an increase in the plasma level of the hormone [61]. How specific the effect was in the latter system is not clear because it could not be duplicated by muscimol.

The effects of GABA on insulin secretion have also been explored; in this case the results are, yet again, conflicting [46, 57, 61, 62]. GABA and its agonists influenced neither insulin release caused by addition of a high glucose concentration to isolated perifused islets [7] nor altered insulin content of islets cultured for 3 days [46, 63]. However, in pieces of pancreas, 0.1 and 10 μM GABA induced a statistically significant albeit small, 20–25%, rise in release of this hormone which, nevertheless, was not observed with 1 mM GABA [24]. An increase in the plasma level of insulin was noted in humans after a high single oral dose of GABA while muscimol was ineffective [61, 62]. By contrast, in perfused dog pancreas, high-arginine triggered insulin release was inhibited by GABA (1, 10 and 100 μM) in a concentration-dependent manner [59]. It is self-evident that no conclusion can be drawn on the basis of these results. All these considerations indicate that more work is needed to ascertain the relationship between GABA and insulin secretion.

There is no evidence that the pancreas contains $GABA_B$ receptors. Administration of baclofen to human subjects results in changes in the levels of some pancreatic hormones but it is not clear if the effect of the drug is direct or indirect [62]. Baclofen penetrates the blood–brain barrier and may have a central action to that on pancreas. Moreover, there was no response to baclofen in diabetics [64].

Concluding remarks

The pancreas, and in particular the islets of Langerhans, contain γ-aminobutyric acid at relatively high concentrations. This fact along with the presence of GAD, GABA-T and glutaminase in β-cells indicate that in the latter structures the amino acid can serve as a fuel generated by a pathway alternative to glycolysis. However, there are many areas where our knowledge of the physiological function(s) of GABA remains insufficient, controversial or nonexistent.

The first issue is the possible regulatory role of GABA within the β-cell. Although an elevation of the intracellular concentration of the amino acid seems to stimulate insulin synthesis, neither the hormone content nor its secretion are changed. Moreover, since alterations in the internal level of GABA and physiologically relevant enhancement of insulin production occur in opposite directions, a causal relationship between the two events is thus far questionable.

The second issue is the possible role of GABA in modification of secretory events in pancreatic islets. Several specific questions await answers: (1) is GABA secreted from β-cells and by what mechanism? (2) what is the effect of GABA on secretion of the various pancreatic hormones *in vitro* and *in vivo*? (3) what is the nature of the GABA transport and identity of cells which take it up? and (4) what is the fate of GABA in cells such as δ-cells, which accumulate the amino acid, but do not possess enzymes of its metabolism?

The final issue which remains to be solved is the role of GABA in acinar cells.

In conclusion, elucidation of the role of GABA in the physiology of pancreas and islets of Langerhans awaits further studies.

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Note added in proof: When this review was accepted for publication it came to our attention that some of the same issues were also discussed in a recent paper by Sorenson RL, Garry DG and Brelje TC, Structural and functional considerations of GABA in islets of Langerhans. β-Cells and nerves. *Diabetes* **40**: 1365–1374, 1991.

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