An osmotic-sensitive taurine pool is localized in rat pancreatic islet cells containing glucagon and somatostatin

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Bustamante, J., M. V. T. Lobo, F. J. Alonso, N.-T. A. Mukala, E. Giné, J. M. Solís, J. Tamarit-Rodríguez, and R. Martín Del Río. An osmotic-sensitive taurine pool is localized in rat pancreatic islet cells containing glucagon and somatostatin. Am J Physiol Endocrinol Metab 281: E1275–E1285, 2001.—Previous reports have dealt with the hypoglycemic properties of taurine and its effects on insulin secretion by adult and fetal islets. We have studied the presence and cellular distribution of taurine in rat islets, the conditions to evoke its release, and its possible modulatory action on insulin secretion. We localized taurine by techniques of double immunolabeling in most glucagon-positive cells and in some somatostatin-positive cells, whereas insulin-positive cells were not labeled with the taurine antibody. Although high-glucose stimulation did not evoke any taurine release, a hyposmotic solution (17% osmolarity reduction) induced a specific phasic release of taurine and GABA (34 and 52% increase on their basal release rate). On the other hand, taurine (10 mmol/l) application slightly reduced the second phase of insulin secretion induced by glucose stimulation. In conclusion, taurine is highly concentrated in glucagon-containing cells of the islet periphery. It is not liberated by glucose stimulation but is strongly released under hyposmotic conditions. All of these data suggest that taurine plays an osmoregulatory role in α-cells.

Taurine distribution; γ-aminobutyric acid release; amino acid content; insulin secretion; immunocytochemistry

Taurine (2-aminoethanesulfonic acid) is one of the most abundant free amino acids in mammalian tissues, where it exhibits a profusion of biological actions (see Ref. 30 for review). Taurine is required for the synthesis of bile salts in the liver. In the brain, taurine has neuroinhibitory effects, acting as a weak GABA receptor agonist (16, 47), and it also induces a long-lasting potentiation of synaptic transmission in the hippocampus (17, 19). In addition, this amino acid has been shown to be fundamental during brain development (58). In a number of tissues, taurine seems to participate in cell volume regulation by acting as an osmolyte (45, 49, 57), and it has also been involved in the modulation of calcium movements through plasmatic (54) and mitochondrial (48) membranes. In the pancreas, taurine appears to be specifically concentrated in the islets (9, 10); however, there are very few studies addressing its physiological role in this organ.

No systematic clinical studies relating taurine with pancreatic pathophysiology appear in the literature, although it is known that taurine plasma levels are low in diabetic patients and that its supplementation reduces the increased tendency to platelet aggregation in this illness (18). On the basis of experimental evidence, some authors have proposed that taurine is involved in the regulation of carbohydrate metabolism at systemic levels (32, 34) and in the function of pancreatic islets (13, 14, 62). A hypoglycemic and antidiabetic action of taurine has been explained by an increased glucose accumulation in different tissues caused by a direct action of taurine on the insulin receptor (32, 39). However, the studies addressing the taurine action on the glucose-stimulated insulin release have yielded conflicting results; thus a suppression of insulin release in acutely isolated mouse islets has been reported (62). On the other hand, a potentiation of this response has been found in cultured rat fetal islets (13, 14). It has also been described that taurine could ameliorate some of the alterations found in diabetic rats, such as returning plasma triglycerides to normal levels (25) or protecting islet β-cells against destruction and insulin depletion in streptozotocin-induced diabetic rats (61).

All of these data suggest that taurine might play some physiological role(s) in the pancreas. In the present work, we have determined the concentration of taurine and its cellular distribution in rat islets. In addition, we have investigated the conditions that evoke taurine release and its possible modulatory action on insulin secretion.

METHODS

Chemicals and Antisera

Collagenase P and fatty acid-free BSA were obtained from Boehringer Mannheim (Indianapolis, IN). Taurine, D-glucose, 5-sulfosalicylic acid, Mowiol, and pig insulin were obtained from Sigma Chemical (St. Louis, MO). Rat insulin was...
obtained from Linco Research (St. Louis, MO). Organic solvents (gradient grade) and inorganic substances and salts (analytical grade) were acquired from Merck (Darmstadt, Germany). Eukitt was purchased from O. Kindler (Freiburg, Germany).

The polyclonal rabbit anti-taurine antibody was purchased from Chemicon International (Temecula, CA). Mouse monoclonal anti-insulin, anti-glucagon, and anti-somatostatin (prediluted, ready-to-use solution) antibodies were purchased from ICN (Aurora, OH), Sigma Chemical, and Bio-Genesis (Poole, UK), respectively. FITC-conjugated goat anti-mouse IgG and rhodamine-conjugated goat anti-rabbit IgG were purchased from Boehringer Mannheim. The biotinylated goat anti-rabbit IgG and the streptavidin-peroxidase complex were purchased from Zymed Laboratories (San Francisco, CA). Goat anti-rabbit IgG coupled to colloidal gold particles (15 nm diameter) was purchased from British Bio-cell International (Cardiff, UK).

Animals

All experiments were performed with male Wistar albino rats (250–300 g).

Immunocytochemistry

Three rats were anesthetized with ketamine (90 mg/kg ip), xylazine (10 mg/kg ip), and atropine (0.05 mg/kg sc) and then perfused through the ascending aorta with 100 ml of oxygenated phosphate-buffered saline (PBS) followed by 4% paraformaldehyde-0.5% glutaraldehyde in phosphate buffer. The pancreas was isolated and processed for both light and transmission electron microscope. Also, groups of 50 islets were fixed immediately after isolation or after an incubation period of 1 h under different conditions (see Preparation and Perfusion of Islets). These islets were fixed in a PBS solution containing 4% paraformaldehyde-2.5% glutaraldehyde and processed in the same way as the whole pancreas.

Light microscopy. After fixation, samples were washed in PBS buffer, dehydrated with graded ethanol series, and embedded in paraffin following conventional methods.

STREPTAVIDIN-PEROXIDASE METHOD. Paraffin sections (5 μm thick) were mounted in silanized slides and allowed to dry overnight before immunohistochemical staining. After xylene deparaffinization, sections were hydrated and incubated for 10 min in 3% H2O2 followed by 20 min in 0.3% H2O2 in methanol. They were then washed in Tris-buffered saline (TBS) and incubated for 10 min in a 0.1 mol/l sodium borohydride solution in TBS, pH 7.5. Sections were then washed and incubated in 3% normal goat serum (NGS) with 0.06% Triton X-100, at room temperature. Sections were sequentially incubated [anti-taurine antibody (1:200 in blocking buffer diluted 1:9, pH 7.5), blocking buffer (pH 8.2)] and washed in double-distilled, deionized water. Free aldehydes were reduced by use of 0.4% sodium borohydride in TBS, pH 7.5, for 10 min. The nonspecific protein-binding sites were blocked by incubation with TBS, pH 7.4, supplemented with 3% NGS, and 0.06% Triton X-100, at room temperature. Sections were sequentially incubated [anti-taurine antibody (1:200 in blocking buffer diluted 1:9) overnight at 4°C; blocking buffer diluted 1:9, pH 7.5, followed by the same buffer at pH 8.2 (10 min each); 15 nm gold-conjugated goat anti-rabbit IgG 1:40 in TBS (pH 8.2) supplemented with 0.1% bovine serum albumin and 0.006% Triton X-100, at room temperature for 2 h; TBS (pH 7.6) followed by PBS (pH 7.4); 1% glutaraldehyde in PBS at room temperature for 10 min] and washed in double-distilled, deionized water. Samples were stained with lead citrate and uranyl acetate and examined under a Zeiss EM10 electron microscope.

Immunostaining control. The specificity of the immunocytochemical procedures was tested by one of the following control procedures: omitting the primary antibody, using nonimmune serum instead of the primary antibody, incubating with an inappropriate secondary antibody after the incubation with the primary antibody at optimal titers, or immunolabeling on 4% paraformaldehyde-fixed samples. This last control procedure did not give taurine immunoreaction, because the anti-taurine antibody used here was prepared using taurine conjugated by glutaraldehyde to poly-l-lysine as immunogen, and thus it only recognizes haptenic taurine on glutaraldehyde-fixed samples (11). As positive controls for light microscopy, taurine immunostaining was analyzed in rat cerebellar sections, where it was localized in Purkinje cells, as previously described (36, 37). The primary antibody used in this study has previously been utilized to detect taurine in several tissues (10, 35) and extensively character-
ized to confirm its specificity by the ELISA method, showing that it virtually does not cross-react with other related amino acids (11).

**Preparation and Perfusion of Islets**

Islets were isolated by collagenase digestion of the rat pancreas (35) in Hanks’ solution, followed by hand-picking under stereomicroscope. Samples of exocrine tissue were also taken at the same time, although contamination with partially digested islets cannot be excluded. Afterward, islets were transferred to a perfusion system (70-μl chamber dead space) and perfused at 37°C with Krebs-Ringer bicarbonate [KRB composition in mmol/l: 115 NaCl, 4.7 KCl, 1.2 KH2PO4, 1.2 MgSO4, 2.56 CaCl2, 5 NaHCO3, 20 HEPES, and 0.5% (wt/vol) fatty acid-free BSA], adjusted to pH 7.4, and equilibrated with air. Islets were perfused with 3 mmol/l glucose-KRB for 45 min; afterward, the medium was switched to one containing the test solution for 15 or 30 min. Finally, the perfusion with 3 mmol/l glucose was resumed for 30 additional min. Hyposmotic solutions were obtained by lowering the NaCl content in 25 mmol/l, and their osmolarities were systematically checked with a microosmometer (model 3MO, Advanced Instruments, Norwood, MA). The effect of taurine on insulin secretion was studied in groups of 40 islets perfused at 0.5 ml/min. To study amino acid release, groups of 300–550 islets were perfused at 0.25 ml/min. The amino acid efflux was expressed as a percentage of the mean concentration value obtained during a time period of 15 min, which was considered to be 100% and which preceded the stimulation period.

Results are expressed as means ± SE. Statistical differences were assessed by one-way or two-way analysis of variance, followed, when necessary, by the Neuman–Keuls test for multiple comparisons. Differences between pairs of means were addressed by a two-tailed Student’s *t*-test.

**RESULTS**

**Amino Acid Content in the Pancreas (Endocrine vs. Exocrine)**

The free amino acid content of isolated islets and pancreatic exocrine tissue is shown in Table 1. Six of the twelve amino acids identified in our study were found to be much more concentrated in the endocrine than in the exocrine pancreas. Four of them (aspartate, taurine, glycine, and GABA) were clearly predominant in islets at concentrations several times higher than the other amino acids. The amino acid values obtained in the exocrine tissue did not appear to be contaminated with partially digested islets, inasmuch as GABA, a marker of endocrine pancreas, is found at very low concentration.

**Taurine Localization**

Immunocytochemical experiments were performed to study the localization of taurine in the pancreas and to identify the nature of the taurine-immunoreactive cells within the islets. The majority of the immunostaining reaction was located in the islets, as shown in a general view of the pancreas under light microscopy (Fig. 1A). In the endocrine pancreas, taurine was located in a ring of immunolabeled cells surrounding the weakly stained center of the islet (Fig. 1, A and B). Within these cells, taurine was found in both the nucleus and the cytoplasm (Fig. 1B). Endothelial cells from the capillaries of the endocrine pancreas were also intensely immunostained (arrows, Fig. 1B). On the other hand, within the exocrine pancreas (Fig. 1C), taurine immunolabeling was mainly localized in wall vessel cells. Smooth muscle cells and endothelial cells from muscular vessels were intensely immunostained. On the contrary, all acinar cells were unstained, and the cells from pancreatic ducts were weakly labeled (Fig. 1C).

To identify the nature of the taurine-immunoreactive cells within the islet, double immunofluorescence against taurine and insulin, glucagon, or somatostatin was performed (Fig. 2). Taurine immunoreactivity was detected using a rhodamine-conjugated secondary an-

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**Table 1. Free amino acid content in endocrine and exocrine pancreas**

<table>
<thead>
<tr>
<th>Amino Acids</th>
<th>Concentration, mmol/l</th>
<th>pmol/μg protein</th>
<th>pmol/μg protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asp</td>
<td>12.19 ± 1.08</td>
<td>39.90 ± 3.66*</td>
<td>6.54 ± 1.46</td>
</tr>
<tr>
<td>Glu</td>
<td>2.88 ± 0.18</td>
<td>8.71 ± 0.51*</td>
<td>2.79 ± 0.51</td>
</tr>
<tr>
<td>Ser</td>
<td>3.08 ± 0.37</td>
<td>9.22 ± 1.03*</td>
<td>3.22 ± 0.60</td>
</tr>
<tr>
<td>Gln</td>
<td>0.17 ± 0.03</td>
<td>0.54 ± 0.10</td>
<td>0.74 ± 0.11</td>
</tr>
<tr>
<td>His</td>
<td>0.39 ± 0.11</td>
<td>1.36 ± 0.59</td>
<td>0.47 ± 0.10</td>
</tr>
<tr>
<td>Gly</td>
<td>6.07 ± 0.53</td>
<td>18.30 ± 1.31*</td>
<td>9.35 ± 1.54</td>
</tr>
<tr>
<td>Thr</td>
<td>0.76 ± 0.10</td>
<td>2.29 ± 0.39</td>
<td>1.12 ± 0.14</td>
</tr>
<tr>
<td>Arg</td>
<td>0.48 ± 0.17</td>
<td>1.71 ± 0.90</td>
<td>1.54 ± 0.39</td>
</tr>
<tr>
<td>Tau</td>
<td>8.08 ± 0.44</td>
<td>25.92 ± 1.98*</td>
<td>6.57 ± 2.44</td>
</tr>
<tr>
<td>Ala</td>
<td>1.59 ± 0.27</td>
<td>5.26 ± 1.42</td>
<td>3.06 ± 0.56</td>
</tr>
<tr>
<td>Tyr</td>
<td>0.28 ± 0.04</td>
<td>0.91 ± 0.14</td>
<td>1.01 ± 0.32</td>
</tr>
<tr>
<td>GABA</td>
<td>4.81 ± 0.32</td>
<td>15.86 ± 1.27*</td>
<td>0.39 ± 0.12</td>
</tr>
</tbody>
</table>

Values are means ± SE. Values of islets correspond to samples of 40–100 fresh isolated islets from different rats. Mean protein content: 0.98 ± 0.05 μg protein/islet (n = 49). Little contamination of exocrine tissue with islets can be evaluated by the small amount of GABA in this fraction. *P < 0.01 (two-tailed Student’s *t*-test), endocrine vs. exocrine amino acid content.
tibody that is seen in red color (Fig. 2A), and insulin, glucagon and somatostatin were detected using FITC-conjugated secondary antibodies that are seen in green color (Fig. 2B). The overlay of the two immunoreactivities (the red and green colors) yields a yellow color, indicating colocalization of taurine with the corresponding islet hormone (Fig. 2C). While taurine immunoreactivity (red) was observed in the mantle, a positive green signal to insulin was observed in the core of the islet, showing that taurine and insulin were not colocalized in the same cells. On the other hand, both glucagon and somatostatin immunolabeling (green) had a peripheral location. Glucagon-positive cells were also immunoreactive to taurine, showing an intense yellow color (Fig. 2C). On the contrary, most of the somatostatin-positive cells remained unstained, although some of them were also partially immunoreactive to taurine (Fig. 2C).

We also investigated the immunolabeling of taurine and endocrine hormones in isolated islets. This was done before and after an incubation period of 1 h in static conditions with 3 or 20 mmol/l glucose-KRB (n = 6). Islets presented the same immunofluorescence localization as found in the whole pancreas preparations, indicating that the isolation and incubation processes preserved the integrity of external layers of the islets and their taurine and hormone content (Fig. 2D). Furthermore, high glucose levels, which released taurine, did not modify the taurine distribution pattern. Moreover, to assess whether β-cells are able to take up taurine, this amino acid was added at a concentration of 10 mmol/l in two of the groups (50 islets/group) incubated with 3 and 20 mmol/l glucose (n = 6). After 1 h of incubation, no qualitative differences in taurine immunolabeling were found between samples incubated with or without taurine, preserving the dramatic disparity between the periphery and the core of the islet, and indicating, therefore, that β-cells do not take up taurine under these conditions.

β- and α-Cells were identified, under the electron microscope, on the basis of their distribution, shape, and granular morphology (Fig. 3). The gold-immunocytochemical method confirmed the results obtained using light-microscopic procedures. β-Cells showed in some cases a weak taurine labeling, whereas α-cells were intensely immunostained. The gold particles were scattered throughout most of the cell cytoplasm and nucleus without a well-defined labeling pattern. Whereas the secretory granules of the endocrine cells remained unstained, gold particles were localized over other cell structures, such as mitochondria or endoplasmic reticulum.
Fig. 2. Double immunolabeling of taurine and insulin, glucagon, or somatostatin in rat pancreatic islets. A: taurine immunolabeling (red color). The three columns correspond to different islets in a whole pancreas preparation. Taurine localizes in the islet periphery and in the inner vessels of the islet. B: hormone labeling (green color) of the corresponding islets. Insulin has a central location, in contrast to a peripheral situation of glucagon and somatostatin. C: colocalization of taurine and the hormones is demonstrated in overlays, in which yellow color indicates coincident labeling. Whereas taurine and insulin do not show colocalization, most of glucagon-positive immunolabeling colocalizes with taurine. Only a few somatostatin-containing cells also present taurine immunoreactivity. D: colocalization of taurine and the hormones, as in C, in groups of isolated islets incubated for 1 h in 3 mmol/l glucose. Calibration bars: 50 μm (A, B, and C); 175 μm (D).
Effect of Taurine on Insulin Secretion

We first tested whether taurine (10 mmol/l) had any effect on insulin release at nonstimulating glucose levels (3 mmol/l). The basal insulin levels (0.37 ± 0.03 ng·ml⁻¹·40 islets⁻¹, n = 9) were not significantly affected by taurine application during 30 min (mean values of three consecutive 10-min periods in ng·ml⁻¹·40 islets⁻¹: 0.42 ± 0.18; 0.35 ± 0.04; 0.32 ± 0.05; these values compared with basal levels at P > 0.05, by two-tailed Student’s t-test, n = 2).

On the other hand, to test whether taurine was able to modulate the glucose-stimulated insulin release, we perifused taurine concomitantly with 20 mmol/l glucose in a group of 40 islets. Control experiments without taurine were simultaneously conducted in another group of 40 islets from the same rat (Fig. 4). For the prestimulatory period, 3 mmol/l glucose was used. When the glucose concentration in the perifusion solution was changed from 3 to 20 mmol/l, insulin release increased ~16 times above basal levels, with the characteristic biphasic pattern of secretion in both groups of islets. Taurine did not modify the first phase of secretion (minutes 9–18, two-way ANOVA, P = 0.123, n = 9), but it decreased significantly the rate of insulin release during the second phase (minutes 20–36; two-way ANOVA, P < 0.0001, n = 9). This represents an
overall reduction of 17.2 ± 5.3% during the second phase. Insulin secretion returned to basal values when the perifusion with the 3 mmol/l glucose solution was resumed.

Are Taurine and Other Amino Acids Released by Glucose Stimulation?

Another group of perifusion experiments were carried out to determine whether a glucose stimulation inducing insulin secretion affected the extracellular levels of taurine and other amino acids. For this purpose, the levels of amino acids and insulin were measured in the effluent of perifused islets (groups of 300–550 islets). Glucose stimulation (20 mmol/l) did not evoke significant changes in most of the amino acids analyzed, as shown for taurine in Fig. 5B (minutes 1–45; one-way ANOVA, \( P = 0.378, n = 7 \)). A lack of sensitivity in the amino acid analysis method appears unlikely to explain the failure to detect changes in the extracellular amino acid concentration when raising glucose, because arginine levels, which are found at a much lower concentration than taurine levels (Table 2), showed a very consistent extracellular increase when stimulated with 20 mmol/l glucose, which paralleled the biphasic pattern of insulin secretion (Fig. 5C; minutes 1–45; one-way ANOVA, \( P = 0.009, n = 7 \)), and also because the HPLC system used in this study is able to detect amino acid levels with a sensitivity of \( \sim 10 \text{ nmol/l} \).

The possibility that taurine was released from \( \alpha \)-cells by glucagon-secreting stimuli, i.e., a glucose reduction or arginine application, has been checked in some preliminary experiments. A decrease of the glucose concentration from 20 to 3 mmol/l, which yielded a conspicuous increase in glucagon release (not shown), did not change the taurine concentration in the collected effluent, as can be seen in Fig. 5B. In those experiments in which we used the stimulation with arginine (20 mmol/l), it was impossible to measure taurine and some other amino acids, because the signal of arginine saturated the fluorescence detector during the elution time of taurine, alanine, tyrosine, and GABA.

An Hyposmotic Stimulus Induces Taurine Release in Isolated Islets

Taurine behaves as an osmolyte in a great number of biological systems, so we explored whether taurine is released from the islet cells in response to a reduction in the osmolar strength of the perifusion medium. For this purpose, groups of perifused islets (300–350), equilibrated with 3 mmol/l glucose for 30 min, were challenged for 15 min with a hypotonic KRB, made by reducing the concentration of NaCl by 25 mmol/l.

As it is shown in Fig. 6A, hyposmolarity (17.2 ± 0.5% reduction of the standard medium osmolarity, \( n = 4 \)) induced a phasic stimulation (one-way ANOVA, \( P < 0.0001, n = 4 \)) of insulin release that peaked after 3 min of hypotonic application (39 ± 9%, \( n = 4 \)), decreas-
ing thereafter below basal levels. After reintroduction of a standard isotonic medium, insulin levels very slowly trended to basal values. Amino acid efflux reached steady values during the perifusion period with 3 mmol/l glucose. Under the hypotonic conditions, a significant and phasic release of both taurine (Fig. 6B) and GABA (Fig. 6C) was observed (34 and 52% overall increase, respectively; one-way ANOVA, \( P < 0.001, n = 4 \)). The release of these two amino acids peaked within the first 5 min of hypotonic medium perfusion (74±19 and 92±18% for taurine and GABA, respectively, \( n = 4 \)), followed by a decrease to basal values during the rest of the stimulatory period (Fig. 6, B and C). Figure 6D shows the evolution of glycine levels during the experiment as representative of most of the other amino acids that were not affected by the hypotonic challenge.

**DISCUSSION**

**Amino Acid Profile of Islets**

In addition to serving as metabolic fuels and/or precursors for protein synthesis, some amino acids might act as intracellular messengers or paracrine signals. Glutamate could belong to both categories (1, 38), whereas GABA and taurine could be included in the second class (13, 14, 24, 51, 62).

Different authors (10, 27, 46) have found, as in this study, a high content of taurine in the endocrine pancreas and a much lower content in the exocrine part. Similarly, an elevated concentration of GABA has also been previously reported, and it is now well established (20, 22, 24, 42).

Our study shows that aspartate, taurine, glycine, and GABA are the predominant free amino acids in freshly isolated rat islets. Aspartate and taurine were also found among the most prominent in previous reports (27, 46), but not glycine or GABA. In striking contrast with these studies, but in coincidence with more recent data (41, 42), we found relatively low values for glutamate and glutamine. Because taurine is restricted to the peripheral layer of the islet (see Cellular Localization of Taurine), its concentration might reach 40–80 mmol/l, which is within the higher levels found in other mammalian tissues (30).

**Cellular Localization of Taurine**

We have localized taurine immunoreactivity predominantly in the peripheral cell layers of rat islets.

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**Table 2. Amino acid concentration in the collected effluent**

<table>
<thead>
<tr>
<th>Amino Acids</th>
<th>Concentration in Perifusate (( n = 6 ))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asp</td>
<td>0.199 ± 0.010</td>
</tr>
<tr>
<td>Glu</td>
<td>0.168 ± 0.018</td>
</tr>
<tr>
<td>Ser</td>
<td>0.813 ± 0.143</td>
</tr>
<tr>
<td>Gln</td>
<td>0.055 ± 0.004</td>
</tr>
<tr>
<td>His</td>
<td>0.133 ± 0.022</td>
</tr>
<tr>
<td>Gly</td>
<td>2.679 ± 0.127</td>
</tr>
<tr>
<td>Thr</td>
<td>0.181 ± 0.013</td>
</tr>
<tr>
<td>Arg</td>
<td>0.106 ± 0.015</td>
</tr>
<tr>
<td>Tau</td>
<td>0.588 ± 0.057</td>
</tr>
<tr>
<td>Ala</td>
<td>1.998 ± 0.033</td>
</tr>
<tr>
<td>Tyr</td>
<td>0.087 ± 0.009</td>
</tr>
<tr>
<td>GABA</td>
<td>0.206 ± 0.030</td>
</tr>
</tbody>
</table>

Concentration values (means ± SE in \( \mu \)mol/l) were calculated for groups of 400 islets perfused at 225 \( \mu \)l/min for 30 min with a 3 mmol/l glucose-Krebs-Ringer bicarbonate solution.

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**Fig. 6.** Insulin (A), taurine (B), and GABA (C) were phasically released by perfusing solutions whose osmolarity was reduced by 50 mosmol/l (17.2 ± 0.5%, \( n = 4 \)). Effluent levels of other amino acids, such as glycine (D), were not affected by the hypotonic challenge. Experiments were conducted with groups of 300–350 islets perfused at 225 \( \mu \)l/min (\( n = 4 \); basal glucose = 3 mmol/l). Horizontal black bars, period of application of hyposmotic solution. Horizontal long-dashed lines, mean value of baseline period before hyposmotic stimulus, expressed as 100%.

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within the pancreatic tissue. This taurine distribution is preserved in the isolated islets, even after 1 h of incubation with 3 or 20 mmol/l glucose-KRB (Fig. 2C). Double immunostaining of taurine and each of the main islet hormones (insulin, glucagon, and somatostatin) has shown that taurine is contained mainly in \( \alpha \)-cells and, in minor proportion, in \( \delta \)-cells. Colocalization with other islet hormones has not been studied, but it cannot be discarded. In fact, in some samples, the localization and abundance of taurine-positive cells that are negative to glucagon and somatostatin suggest that they could be cells containing the pancreatic polypeptide (data not shown). To our knowledge, the specific localization of taurine in certain types of endocrine cells described in this work had not been reported before and suggests that this amino acid might play a physiological role in this structure. On the other hand, electron microscopy shows that taurine is not associated with secretory granules, indicating that a hypothetical release of this amino acid would take place mainly via a nonexocytotic mechanism.

The lack of taurine in \( \beta \)-cells does not appear to be due to a technical pitfall for two reasons, first because the high affinity and specificity of the antibody used have been widely confirmed in different tissues (10, 11, 35–37), and second, because other cell types in endocrine and exocrine pancreas in the same tissue slice are strongly labeled. However, we have to consider the possibility that taurine was lost during the preparation process. It has been proved that the fixation technique keeps most of the taurine in the tissue (11), but the possibility still exists that taurine shifts rapidly from one compartment to another, i.e., from \( \beta \)- to \( \alpha \)-cells. In fact, taurine in cerebellum accumulates in Purkinje cells and moves forward and backward to/from glial cells when osmotic conditions of the medium change (45). The cerebellar sections that we used as positive controls of the technique presented a pattern of taurine labeling that was always normal.

**Amino Acid Efflux**

GABA and glutamate were proposed to play a modulatory or paracrine role in the islets, because functional specific receptors for them were found in different cell types (1, 7, 8, 51, 52, 59, 63). Taurine, as a weak GABA\(_A\) receptor agonist (16, 47), and GABA could play a tonic modulatory role on glucagon and somatostatin secretion through the stimulation of the GABA\(_A\) receptors localized on their respective cell types (56). Although there is no evidence of glutamate release by glucose stimulation, GABA can be released from purified and cultured \( \beta \)-cells and from isolated islets, although it is controversial whether or not the release is regulated by glucose (21, 23, 51, 55, 56). In the present study, the stimulation of perfused islets with 20 mmol/l glucose, which produced a consistent insulin secretion, did not modify the basal efflux of any of the twelve amino acids analyzed but arginine. These data evidence the existence of different pathways or systems of secretion for GABA and insulin, because both substances are contained in \( \beta \)-cells (24, 44, 56, 60). On the other hand, preliminary experiments indicate that the glucagon secretion induced by a reduction in glucose concentration (not shown) is not accompanied by a change in the concentration of taurine in the effluent (see Fig. 5B), indicating that although glucagon and taurine are both contained in the same cells, they are not coreleased under these conditions.

To our knowledge, arginine release from glucose-stimulated islets has not been reported before, and one can ask whether it has any physiological role in vivo. Arginine is produced in the proteolytic processing of proinsulin to insulin (a maximum of three arginines per mole of proinsulin) (15). If the newly produced arginine were accumulated in the mature secretory granules, it would be released by glucose stimulation, together with insulin from granules undergoing exocytosis.

**Effect of Taurine on Insulin Secretion**

Given that taurine is mainly concentrated at high amounts in the islet \( \alpha \)-cells, we asked about the role of taurine in this structure. Taurine is not involved in metabolic routes or protein building (see Ref. 30 for review), but in excitatory tissues it has been proposed to have a modulatory role on cell excitability through activation of GABA\(_A\) receptors (16, 47) and ionic channel modulation (53).

Because functional GABA\(_A\) receptors have been localized in \( \alpha \)- and \( \delta \)-cells (7, 51, 60), and they appear to be present also in \( \beta \)-cells (7, 8, 52), it could be suggested that taurine, acting as a weak agonist of this type of receptor, could modulate hormone secretion. In fact, we found that taurine reduces the second phase of glucose-induced insulin release. A similar finding on insulin secretion has been previously reported (62) in mouse islets incubated in static conditions. In contrast with these results, it has been reported in fetal rat islets (13, 14) that taurine (0.3 and 3 mmol/l) stimulates insulin secretion and enhances the action of some secretagogues, such as leucine or arginine. However, fetal rat islets are unresponsive to glucose (14), and the mechanism of taurine action is unknown.

**Osmotic-Induced Release of Taurine and GABA**

Taurine and the other amino acids can be collected in the effluent of perfused islets, but what kind of physiological stimulus is able to evoke a prominent release of endogenous taurine in the islet? We can discard changes in glucose concentration, because these stimuli did not modify the extracellular levels of taurine in our experiments (see Fig. 5B). Another possibility is that taurine could be released from the islets as a consequence of a mechanism of regulatory volume decrease, as demonstrated in a great number of cell types (30, 45, 49, 57). Taurine fulfills some of the properties to be an osmolyte in the endocrine pancreas. This amino acid is remarkably concentrated inside some cells, and it is released by relatively mild hyposmotic conditions. Therefore, it appears reasonable to propose...
that taurine could be contributing to cell volume regulation in the islets. This possible function does not preclude other actions on insulin and/or other islet hormones, as suggested from our experiments.

Unexpectedly, GABA, but no other amino acid, was also released together with taurine from perfused islets challenged with a hyposmotic stimulus. Like taurine, GABA is highly concentrated in the islets (see Table 1) (9, 22, 42, 46). Its localization is complementary to that of taurine, being predominantly present in β-cells (20, 22, 23, 46), where it is found in the cytoplasm and associated with synaptic-like microvesicles (50, 59, 60). Besides its hypothetical role as a paracrine modulator, GABA might behave as an osmolyte to compensate volume changes occurring in β-cells. β-Cells are endowed with mechanisms of regulatory volume decrease (43). In purified β-cells and isolated islets, a 10–30% reduction of medium osmolarity led to a transient volume increase, accompanied by a concomitant transitory rise in electrical activity and insulin release (4, 6, 43).

A number of studies have demonstrated the existence of a volume-sensitive anion channel in the plasma membrane of β-cells (4, 5, 31) that could be permeable to various organic osmolytes, including taurine (3). Consistent with this suggestion, the exposure of intact islets or cultured INS-1 cells (a β-cell-derived line) to a hypertonic solution resulted in a stimulation of [3H]taurine efflux, sensitive to chloride channel antagonists (2, 26). We have shown that a hyposmotic stimulus induces a phasic release of endogenous taurine and GABA from islets perfused at 3 mmol/l glucose. In view of the fact that taurine in rat islets is mainly concentrated in non-β-cells, we cannot assume that taurine efflux plays a main role in adaptive changes of β-cell volume to variations in medium osmolality. However, GABA, which is mainly localized in β-cells (50, 51), could have such a role in this type of cell.

In conclusion, taurine and GABA are contained in different cell types of the islet, and both amino acids are released when challenged by an osmotic stress. Because cells in the islets swell as a consequence of their physiological activity (12, 43), the efflux of taurine and GABA might have an important role in the process of cell volume recovery in the endocrine pancreas.

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