Are 5-Hydroxytryptamine-preloaded β-Cells an Appropriate Physiologic Model System for Establishing That Insulin Stimulates Insulin Secretion?

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The concept that insulin exerts biochemical and physiologic effects on the cell that secretes it, the pancreatic β-cell, has been suggested based on a number of observations. Over 30 years ago (1, 2), a negative feedback effect of insulin on its own secretion was proposed. A large number of subsequent studies (3–9) in which insulin or connecting peptide levels were measured arrived at a similar conclusion. We recently reported (10), in agreement with several previous studies (29, 30), that the phosphatidylinositol 3-kinase (PI3K) inhibitor wortmannin amplified glucose-induced insulin secretion. This conclusion was supported most recently that insulin stimulates insulin secretion from the β-cell (21). Amperometrically measured spikes of 5-hydroxytryptamine (5HT) release from β-cells preincubated in 0.5 mM 5HT for 16 h provided the experimental support for this concept. This method takes advantage of the fact that amperometric measurements of 5HT release from preloaded β-cells can be used as a surrogate marker for insulin release and has been utilized by several groups (21–26). It is based upon earlier studies in which 5HT was found to be localized in islet secretory granules and thought to be cosecreted with insulin (27). Not all reports, however, agreed with this concept (28). Unfortunately in none of the electrophysiologic studies (21–26) was glucose-induced insulin secretion actually assessed to corroborate the amperometric analyses, and despite previous studies demonstrating an adverse effect of 5HT on insulin secretion (29, 30), the possibility that 5HT preloading might negatively affect secretion was not examined in any of these amperometry studies.

In an attempt to resolve the opposite concepts with regard to the inhibitory or stimulatory effects of insulin on β-cell secretion, we conducted studies with islets treated with 0.5 mM 5HT. In agreement with several previous studies (29, 30), we observed that 5HT exerts profound acute and long lasting inhibitory effects on glucose-induced insulin secretion from perfused rat islets. It also abolished tolbutamide-induced secretion as well. The concept that amperometric measurements of 5HT release from β-cells reflect the physiologic regulation of insulin secretion should be reconsidered.

**EXPERIMENTAL PROCEDURES**

**Islet Isolation**—The detailed methodologies employed to assess insulin output from collagenase-isolated rat islets have been described previously (10, 31). Male Harlan Sprague-Dawley rats (350–475 g) were purchased from Charles River Laboratories, Inc. (Wilmington, MA). All animals were treated in a manner that complied with the NIH Guidelines for the Care and Use of Laboratory Animals (41). The animals were fed ad libitum. After intraperitoneal Nembutal (pentobarbital sodium, 50 mg/kg; Abbott, North Chicago, IL)-induced anes-

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† The abbreviations used are: PI3K, phosphatidylinositol 3-kinase; 5HT, 5-hydroxytryptamine; KRB, Krebs-Ringer bicarbonate.

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improvement in P3K signaling in β-cells might be responsible for the hyperinsulinemia noted in a variety of insulin-resistant states including obesity and type 2 diabetes. Thus, whereas disruption of PI3K signaling results in insulin resistance in peripheral insulin-dependent tissues (16, 17), the same biochemical alteration in the β-cell results in compensatory hyperinsulinemia. This elegant communications network allows the β-cell to match the degree of insulin resistance and the secretion of insulin thus maintaining glucose tolerance. This concept presupposes that insulin exerts a negative feedback effect on its own secretion and utilizes the same biochemical signaling systems described in liver, muscle, and adipose tissues (18–20).

In contrast to the numerous studies demonstrating that insulin inhibits its own secretion (1–9), it has been suggested most recently that insulin stimulates insulin secretion from the β-cell (21). Amperometrically measured spikes of 5-hydroxytryptamine (5HT) release from β-cells preincubated in 0.5 mM 5HT for 16 h provided the experimental support for this concept. This method takes advantage of the fact that amperometric measurements of 5HT release from preloaded β-cells can be used as a surrogate marker for insulin release and has been utilized by several groups (21–26). It is based upon earlier studies in which 5HT was found to be localized in islet secretory granules and thought to be cosecreted with insulin (27). Not all reports, however, agreed with this concept (28). Unfortunately in none of the electrophysiologic studies (21–26) was glucose-induced insulin secretion actually assessed to corroborate the amperometric analyses, and despite previous studies demonstrating an adverse effect of 5HT on insulin secretion (29, 30), the possibility that 5HT preloading might negatively affect secretion was not examined in any of these amperometry studies.

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The release and oxidation of 5-hydroxytryptamine from 5-hydroxytryptamine-preloaded β-cells has been used as a surrogate marker for insulin secretion. Findings made using this methodology have been used to support the concept that insulin stimulates its own release. In the present studies, the effects of 5-hydroxytryptamine on stimulated insulin secretion from isolated perfused rat islets was determined. When added together with stimulatory glucose, 5-hydroxytryptamine (0.5 mM) significantly reduced both phases of 8 mM glucose-induced secretion and reduced the first phase of 15 mM glucose-induced release by 60% without any effect on sustained insulin release rates. Preloading of β-cells with 0.5 mM 5-hydroxytryptamine for 3 h resulted in a more severe impairment of 15 mM glucose-induced secretion. First and second phase release rates were reduced by 70 and 55%, respectively. In addition, this pretreatment protocol also abolished 200 μM tolbutamide-induced insulin secretion from perfused islets. These findings confirm that 5-hydroxytryptamine is a powerful inhibitor of stimulated insulin secretion. The responses of 5-hydroxytryptamine-preloaded β-cells may not accurately reflect the biochemical events occurring during the physiologic regulation of insulin secretion. The suggestion that insulin stimulates its own secretion based exclusively on amperometric measurements should be reconsidered.
themia, islets were isolated by collagenase digestion and hand-picked, using a glass loop pipette, under a stereo microscope. They were free of exocrine contamination.

Preloading Studies with 5HT—After isolation, groups of 16–18 islets were loaded onto nylon filters (Tetko, Inc., Briarcliff Manor, NY), placed in small glass vials, and incubated for 3 h in 400 μl of a Krebs-Ringer bicarbonate (KRB) solution containing 0.5 mM 5HT plus 5 mM glucose. This solution, oxygenated and warmed (37°C), was gently added to the vial with islets. The vial was capped with a rubber stopper and gassed with 95% O2, 5% CO2. The vials were again gently oxygenated vial with islets. The vial was capped with a rubber stopper and gassed with 95% O2, 5% CO2 and maintained at 37°C in the figure legends and under “Results.” Perifusion solutions were gassed with 95% O2, 5% CO2 and maintained at 37°C. Insulin released into the medium was measured by radioimmunoassay (32).

Reagents—Hanks’ solution was used for the islet isolation. The perifusion medium consisted of 115 mM NaCl, 5 mM KCl, 2.2 mM CaCl2, 1 mM MgCl2, 24 mM NaHCO3, and 0.17 g/dl bovine serum albumin. The [125I]-labeled insulin used for the insulin assay was purchased from PerkinElmer Life Sciences. Bovine serum albumin (radioimmunoassay grade), glucose, 5-HT hydrochloride, and the salts used to make the Hanks’ solution and perifusion medium were purchased from Sigma. Rat insulin standard (lot 615-ZS-157) was the generous gift of Dr. Gerald Gold, Eli Lilly Co. (Indianapolis, IN). Collagenase (Type P) was obtained from Roche Molecular Biochemicals.

Statistics—Statistical significance was determined using the Student’s t test for unpaired data or analysis of variance in conjunction with the Newman-Keuls test for unpaired data. A p value less than or equal to 0.05 was taken as significant. Values presented in the figures and under “Results” represent means ± S.E. of at least three observations.

RESULTS

Acute 5HT Exposure Studies—In the initial series of experiments islets were perifused immediately after isolation. In response to 8 mM glucose, insulin secretory rates increased most significantly during the final 20 min of the perifusion (Fig. 1, top panel). For example, 20, 30, or 40 min after the onset of stimulation with 8 mM glucose, secretory rates averaged 159 ± 14, 199 ± 25, or 214 ± 18 pg/islet/min (n = 6). The inclusion of 5HT during the stimulatory period with 8 mM glucose significantly reduced islet insulin secretory responses. For example, 20, 30, or 40 min after the onset of stimulation with 8 mM glucose, secretory rates now averaged 87 ± 20, 120 ± 15, or 112 ± 12 pg/islet/min (n = 4). Peak first phase release noted during the initial minutes of 8 mM glucose stimulation was also impaired by the inclusion of 0.5 mM 5HT. They averaged 89 ± 7 pg/islet/min from control islets and 62 ± 6 pg/islet/min from islets stimulated with the combination of 8 mM glucose plus 5HT.

When stimulated with 15 mM glucose, control islets responded with a brisk biphasic insulin secretory response (Fig. 1, bottom panel). Peak first phase secretion averaged 177 ± 21 pg/islet/min whereas release rates measured 35–40 min after the onset of stimulation increased to 834 ± 80 pg/islet/min (n = 8). The presence of 0.5 mM 5HT during stimulation with 15 mM glucose significantly reduced peak first phase secretion, which fell from 177 ± 21 pg/islet/min from control islets to 76 ± 9 pg/islet/min (n = 6) in the presence of 5HT, a reduction of ~60%. Rates of insulin secretion during the final 20 min of stimulation with 15 mM glucose (745 ± 32 pg/islet/min) were comparable with those from control islets.

Effects of Prior Exposure to 5HT on Stimulated Insulin Secretion—In the amperometric studies in which 5HT oxidation was used as the index of insulin secretion (21–26), cells were pretreated with 0.5 mM 5HT for 4–16 h and stimulated with various agonists including tolbutamide. In the next series of studies, we explored the impact of prior exposure to 5HT on insulin secretion. Two different protocols were employed. In the first series of studies, 0.5 mM 5HT was included together with 3 mM glucose only during the initial 30-min stabilization period of the perifusion with 3 mM glucose. Similar to the amperometric studies conducted with 5HT-perfused islets (21, 24, 25), there was no washout period prior to stimulation (Fig. 2). The results are given in Fig. 2 and demonstrate that a brief prior exposure to 0.5 mM 5HT exerted a significant and sustained inhibitory effect on 15 mM glucose-induced secretion. Most dramatic was the reduction in peak first phase secretion, which was decreased to 49 ± 14 pg/islet/min (n = 4). The impact of 5HT on secretion was evident during the entire 40-min stimu-
tamide, the response to 15 mM glucose was significantly re-
made when 5HT-preloaded islets were stimulated with tolbu-
tom during the final 5 min of stimulation. Similar to the findings
were then stimulated with 15 mM glucose alone for 40 min. The 15 m M
combination of 3 mM glucose plus 0.5 mM 5-HT for 30 min. Both groups
closed circles
of release. The second group (open circles) were the same as those depicted in Fig. 1. The asterisk indicates a significant difference between groups at this time point.

The results are presented in Fig. 3. In response to 200 μM tolbutamide a small increase in secretion from control islets was observed only during the initial few minutes of stimulation (Fig. 3, top panel). This agrees with previous studies that have demonstrated a marked glucose dependence for the insulin stimulatory effect of sulfonylurea (36–38). After prior exposure to 0.5 mM 5HT for 3 h, this minimal secretory response was abolished.

In response to 15 mM glucose, control islets responded with a biphasic insulin secretory response (Fig. 3, bottom panel). First phase release averaged 196 ± 21 pg/islet/min whereas sustained rates of secretion averaged 570 ± 57 pg/islet/min (n = 9) during the final 5 min of stimulation. Similar to the findings made when 5HT-preloaded islets were stimulated with tolbutamide, the response to 15 mM glucose was significantly reduced by a 3-h exposure to 5HT. Most dramatic was the reduction in the first phase response, which declined from 196 ± 21 pg/islet/min to 60 ± 16 pg/islet/min (n = 5). The adverse effect of 5HT preexposure on glucose-induced insulin release was sustained for at least 70 min. Release rates measured 35–40 min after the onset of 15 mM glucose were still significantly less than control islet responses. They averaged 240 ± 38 pg/islet/min in the 5HT-pretreated islets at this time point.

**DISCUSSION**

In an attempt to improve the time resolution between the electrical and secretory events that participate in insulin release, several groups have employed amperometry (21–24, 26). This technique has also been applied to insulin receptor substrate-1 gene-disrupted β-cells as well (25). Initially employed in studies using adrenal chromaffin cells (39), secretion is detected with this methodology by a carbon fiber electrode placed next to the cell of interest. As described by Ashcroft and co-workers (23) “the electrode is held at or above the oxidation potential of the secreted compound and secretion monitored by the current associated with the oxidation of the vesicle contents.” The method is only applicable to readily oxidized compounds, but unfortunately, insulin is not such a compound. To circumvent this shortcoming of insulin, these groups have preloaded β-cells for 4–16 h with 5HT, a highly electroactive compound (21–24, 26). Because it appears to be confined to secretory granules, it has been assumed in these amperometric studies that the secretion of 5HT accurately reflects the physiologic secretion of insulin. Unfortunately, in none of these aforementioned β-cell amperometric studies was insulin secretion actually measured. It was assumed to occur in parallel
with the amperometric changes induced by the oxidation of 5HT. In the present series of experiments, insulin secretion from perfused rat islets was measured during acute exposure to or after a 30-min to 3-h preloading period with 5HT. The level of 5HT (0.5 mM) employed was identical to that used in the amperometric studies. The exposure times to 5HT were not as prolonged. However, considering the time-dependent inhibitory actions of 5HT noted in our studies, the adverse effects of more prolonged exposure to 5HT on stimulated secretion may be even more severe than demonstrated here.

In response to 8 mM glucose, a modest insulin secretory response was evoked from control rat islets. Second phase release rates after 40 min of stimulation were increased 4–6-fold above prestimulatory rates whereas the initial response, although modest, was approximately doubled. The inclusion of 5HT together with 8 mM glucose significantly reduced both phases of 8 mM glucose-induced secretion. Using a higher glucose level (15 mM) resulted in a more pronounced first phase response and a large rising second phase response from control islets. Inclusion of 5HT during acute stimulation with 15 mM glucose reduced the peak first phase response by about 60%. Sustained second phase release rates were comparable with control values.

Preexposure to 0.5 mM 5HT for either 30 min or 3 h resulted in a profound suppression of both phases of 15 mM glucose-induced release. Thus, in agreement with previous studies using mouse islets (29, 30), 5HT is a powerful inhibitor of the secretion process. Thus, in agreement with previous studies using mouse islets (29, 30), 5HT is a powerful inhibitor of the release process and that results obtained with 5HT to be interpreted cautiously.

In conclusion, our studies and several previous reports (29, 30) demonstrate that prior exposure of β-cells to 5HT results in a profound time-dependent suppression of the insulin secretory process. The concept that the secretion of 5HT from preloaded β-cells reflects physiologic secretion is not supported by these or other studies (28–30). It is premature to conclude that insulin, under physiologic conditions, stimulates its own secretion based exclusively on studies using amperometric measurement of 5HT release without substantial corroborating data including actual measurements of insulin secretion from 5HT-preloaded β-cells.

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