Amperometric Detection of Quantal Secretion from Patch-clamped Rat Pancreatic β-Cells*

(Received for publication, July 24, 1995, and in revised form, October 24, 1995)

Zhuan Zhou‡ and Stanley Misler§
From the Departments of Medicine (Izwey Hospital) and Cell Biology/Physiology, Box 8217, Washington University Medical Center, St. Louis, Missouri 63110

Serotonin (5-HT) is taken up in insulin granules and co-released with insulin on stimulation of pancreatic islet β-cells. Based on these observations, we have used microcarbon fiber amperometry to examine secretogogue-induced 5-HT release from rat β-cells preloaded for 4-16 h with 5-HT and then exposed to a bath solution containing 10 μM forskolin. In response to local application of KCl (60 mM) or tolbutamide (50-200 μM), we recorded barrages of amperometric events. Each amperometric event consisted of a short pulse of current measurable at electrode voltages that catalyze 5-HT oxidation. With either secretogogue, release was calcium-dependent. On combining amperometry with perforated patch whole-cell recording, we found that barrages of such events were well coupled in time and graded in intensity with depolarization-induced Ca2+ currents and well correlated with increases in membrane capacitance. In cell-attached patch recording, amperometric events evoked by application of tolbutamide followed the closure of ATP-sensitive K+ channels and coincided with the onset of electrical activity. These experiments suggest that amperometry is a useful technique for studying, in real time, the dynamic aspects of stimulus-secretion coupling in β-cells. Their performance was facilitated by the design of a new carbon fiber electrode (ProCFE) described within.

Real time measurement of hormone release from endocrine cells, such as insulin-secreting β-cells of the pancreatic islets of Langerhans, is critical for understanding stimulus-secretion coupling normally occurring in these cells, as well as defects in secretory function, such as those occurring in non-insulin-dependent diabetes mellitus. Recent advances in amperometry, a technique for the electro-oxidization of transmitter molecules near the surface of a cell, have permitted near instantaneous measurement of exocytotic hormone release (1-3). In chromaffin cells, amperometry reveals that brief barrages of current spikes are evoked, in a calcium-dependent fashion, by several depolarizing stimuli, including nicotine agents and high K+ (1), brief voltage clamp pulses (2), and even single action potentials (4). Each spike corresponds to the near synchronous electro-oxidation of up to a million catecholamine molecules liberated from a point source on the cell surface (1). In insulin-secreting pancreatic islet β-cells, two early applications of amperometry to study secretion have been reported. In the first case, quantal release of insulin has been measured from glucose, tolbutamide-, and high K+-stimulated human islet cells based on the ability of a modified (ruthenate-coated) carbon fiber electrode to catalyze the electro-oxidation of S-S bonds between the A and B chains of insulin (5). In the second case, secretogogue-induced quantal release of the “false transmitter” serotonin (5-HT), an electro-oxidizable indolamine sequesrered and stored into insulin granules, has been measured from mouse β-cells preincubated with 5-HT and 5-HT precursors (6).

In this work, we have examined stimulus-secretion coupling in 5-HT-loaded rat β-cells by combining patch clamp electrophysiology with amperometry. We demonstrate that this technique can be used to record exocytotic secretion simultaneously with secretagogue-induced electrical activity. Using this approach, we also present evidence for (i) the direct and rapid coupling of membrane depolarization to quantal secretion and (ii) aspects of the time course of the release of a quantum. This work has been facilitated by the recent design of a polypropylene-insulated carbon fiber electrode, or ProCFE. Its geometry, high sensitivity, low noise, and mechanical stability at physiological temperatures make it particularly advantageous for combined electrophysiological and electrochemical recording from β-cells, which require minimal ambient temperatures of > 28 °C to insure secretion. Part of this data has been presented in abstract form (42).

MATERIALS AND METHODS

Cell Preparation and Treatment—Rat islets, obtained by collagenase digestion of chopped pancreases of adult male Sprague-Dawley rats, were dispersed into a collection of cells using the enzyme dispase (7). Cells were plated on glass coverslips and maintained at 37 °C in a modified CMRL medium (Life Technologies, Inc.) containing 10% heat-inactivated fetal bovine serum, 0.5% penicillin, and 0.5% streptomycin in the presence of 5% CO2, 95% air for use within 2–5 days. Four to sixteen h prior to recording, 5-OH tryptamine (5-HT) and 5-OH tryptophan were added to the culture medium to final concentrations of 0.5-1 mM each. For recording, cells were placed in a temperature-regulated chamber (30–32 °C) filled with a physiological saline solution (PSS) consisting of 138 mM NaCl, 5.5 mM KCl, 2 mM CaCl2, 1 mM MgCl2, 3.0 mM glucose, and 20 mM HEPES buffer titrated with NaOH to 7.38 pH. Forskolin (10 μM) was added to the PSS to increase intracellular cyclic AMP and enhance depolarization-secretion coupling. To assess the Ca2+ dependence of release, the PSS was modified by reducing [CaCl2]o to 0.1 mM and raising [MgCl2]o to 2.9 mM.

* This work was supported by grants from the National Institutes of Health (DK37380) and the American Heart Association (to S. M.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ Supported by a fellowship from the McDonnell Center for Cellular Neurobiology at Washington University. To whom correspondence should be addressed: Dept. of Physiology, Loyola University Medical Center, Maywood, IL 60153.

§ Established Investigator of the American Heart Association.

1 The abbreviations used are: 5-HT, 5-hydroxytryptamine; AP, action potential; AS, amperometric spike; [Ca2+]i, extracellular Ca2+ concentration; Cm, membrane capacitance; Iamp, amperometric current; Icalc, calcium current; Imembrane current; Ipulse, depolarizing current pulse; ProCFE, polypropylene-coated CFE; pCFE, polyethylene-insulated CFE; gCFE, glass/epoxy-insulated carbon fiber electrode; PSS, physiological saline solution; Q, charge transfer during an AS; Vmem, membrane potential; F, faraday(s); Ω, ohm(s).
Amperometric Measurements to Determine 5-HT Release—For amperometric measurements, two kinds of carbon fiber electrodes (CFEs) were used. A polyethylene-insulated CFE (peCFE) (4) modified from Chow et al. (2) was used for early amperometric recording (see Figs. 2 and 3). A newly developed, low noise, polypropylene-insulated CFE (ProCFE) was used for all combined patch-clamp and amperometric experiments. Use of the ProCFE permitted mechanically stable amperometry at temperatures >30 °C required to optimize the chances for secretion. The CFE serves as the input to the head stage of the amperometry monitor, a patch-clamp amplifier, which in turn holds the CFE at a designated voltage. Test solutions containing secretagogues were applied by a puffette consisting of a low resistance, glass capillary at a designated voltage. Test solutions containing secretagogues were initiated once pipette access resistance fell to ~35 MΩ, which is usually within 1–5 min after formation of the seal (9). Electrical activity and membrane currents, I

m

v

, were evoked and recorded with an EPC-9 patch clamp amplifier (Heka Electronic, Lambrecht, Germany) controlled by an Atari computer. In the voltage-clamp mode, membrane currents were evoked by stepping the membrane potential (V

m

) from a holding potential of −70 mV to a given test potential. To evoke electrical activity in the current clamp mode, cells maintained at a Vm of −70 mV through the application of a holding current up to −10 pA, were stimulated by depolarizing currents (10 to 20 pA) applied for a variable duration. Data were acquired at sample rates up to 3 kHz using a Macintosh Quadra 600 computer running Pulse Control software (10), which was connected to the patch clamp amplifiers via an ITC-16 A/D converter interface (Instrutech, Syosset, NY). Amperometric spikes (ASs) were analyzed, and histograms of their features were compiled using macros written by R. Chow and Z. Zhou using Igor software (Wavemetrix, Lake Oswego, OR). All values from multiple experiments were given as mean ± S.D.

RESULTS

Secretagogue-induced Quantal Secretion of 5-HT from Single β-Cells—Fig. 2, taken from an experiment typical of a series of 10, presents the salient features of amperometrically detected, stimulus-induced quantal release from intact β-cells loaded with 5-HT. With the ProCFE positioned at the surface of a single islet cell bathed in a modified PSS containing 0.1 mM Ca

2+

, puffer application of PSS containing 50 μM tolbutamide, and 6 mM Ca

2+

, after variable delays of 5–30 s, repeatedly evoked a vigorous discharge of brief duration ASs. Each AS represents the total oxidation current of many 5-HT molecules released as a packet from an individual vesicle (see below). In contrast, intermittent puffs of PSS containing <20 μM free Ca

2+

 and 50 μM tolbutamide were ineffective in evoking a response, suggesting that tolbutamide-induced quantal secretion is dependent on external Ca

2+

. Note that with repeated puffs of the secretogogue, the average amplitude of the individual amperometric spikes declines.

Fig. 3A depicts a sample experiment from an extensive set (n = 20) showing that a PSS enriched in K + also evokes quantal secretion from a 5-HT-loaded β-cell. High K + solutions are well known to rapidly depolarize β-cells, initiate electrical activity and intracellular Ca

2+

 transients, and provoke Ca

2+

-dependent insulin secretion. As in the case of tolbutamide, the occurrence of high K +-evoked ASs was dependent on [Ca

2+

]; no events were seen in a PSS containing 100 μM [Ca

2+

], (data not shown). Note that the delay time between the start of the high K + puff and the initiation of the AS event was markedly shorter than seen with application of tolbutamide in Fig. 2. These experiments suggest that amperometric response to application of high K + PSS provides a quick and simple way to

2 Tolbutamide is an oral hypoglycemic sulfonylurea. In β-cells, it specifically closes ATP-sensitive K + channels and promotes cell depolarization and Ca

2+

-dependent insulin secretion (16–18).
check the integrity of depolarization-secretion coupling in single β-cells. Fig. 3B provides evidence consistent with the substance underlying stimulus-induced ASs being 5-HT: high K⁺-induced amperometric spikes are seen at a DC electrode potential (Vₑ = +780 mV) sufficient to oxidize 5-HT but not at one (Vₑ = +100 mV) far below the threshold oxidation potential for 5-HT.⁴ In the best batches, about 50% of cells displayed exocytosis in response to either tolbutamide or high K⁺ puffer solutions.

Quantitation of Quantal Release Events in Rat β-Cells—Fig. 4 demonstrates detailed features of individual amperometric spikes. Panels A–C show examples of these “quantal” release signals at expanded time scales. As seen in panels B and C, in many (>20%) low frequency quantal events, widely separated from others, the major spike is preceded by a small, brief (<50-ms duration) “foot.” These feet may be analogous to “pre-fusion” events recorded in chromaffin cells and mast cells (2, 3, 28) prior to the final expansion of the fusion pore connecting the granule and plasma membranes. Panels D–E tabulate peak amplitude (6.4 ± 4.5 pA), half-height duration (6.6 ± 6.1 ms), and total charge (Q) (56 ± 38 femtocoulombs) of amperometric spike events collected from 10 cells, where Q was calculated by integrating single amperometric currents over their time courses. Assuming (i) release is occurring from a point source under the large surface area electrode tip, hence permitting complete oxidation of all the transmitter molecules, and (ii) two or four electro-oxidations per molecule,⁴ Q can be used to estimate the number of 5-HT molecules oxidized in a near synchronous manner and hence provide an estimate of the number of molecules released in a quantal event; that is: 5-HT molecules/quantum = Q(femtocoulombs/quantum) × (5-HT molecule/2 or 4 e⁻) × (e⁻/1.6 × 10⁻¹⁹ coulomb). From this, we estimate that, on average, as many as 0.88–1.76 × 10⁵ molecules of 5-HT are released as a packet. While this quantity is substantially smaller than the 2.5 × 10⁶ catecholamine molecules calculated to be released from a secretory granule of a chromaffin cell (1), it is substantially larger than the 3.5 × 10⁴ catecholamine molecules recently calculated to be released from a synaptic vesicle at an axon varicosity (11).

Depolarization-secretion Coupling in Patch-clamped Cells—In the presence of 10 μM forskolin, we combined patch-clamp electrophysiology with amperometry to examine, in single β-cells, the relationship of membrane depolarization to quantal release. It is assumed that these final steps of insulin secretion in β-cells share many common features with depolarization-secretion coupling in neurons and other excitable endocrine cells, such as adrenal chromaffin cells.

Fig. 5 demonstrates amperometric detection of quantal release evoked by prolonged membrane depolarization from a

---

⁴ While it has generally been assumed that there are two electro-oxidations/5-HT molecule (38), a recent study suggests, at least in some cases, that four electro-oxidations/5-HT molecule may be occurring (39).
Voltage-clamped cell. Rat β-cells display high voltage-activated Ca\(^{2+}\) currents detectable on depolarization to \(V_m = -30\) mV but reaching a peak value on depolarization of \(V_m = +15\) mV (see current trace in response to voltage ramp in right inset to panel A). Panel A demonstrates that repeated (3-s) pulses from \(-70\) mV to +10 mV evoke short bursts or intermittent individual amperometric events during the course of depolarization, although the quantal output declined with stimulus repetition (\(n = 11\)). From these experiments, it was clear that the quantal release was coupled with membrane depolarization. The first release events occurred with latencies as short as 30 ms after onset of the depolarization (left inset). In cells with higher and more stable rates of quantal release, it was possible to alternate several test potentials, evoking either a large or a small Ca\(^{2+}\) current, and to combine amperometry with membrane capacitance (\(C_m\)) tracking to assess exocytosis (see panel B). In β-cells, Ca\(^{2+}\) influx at \(-30\) mV is nearly 10-fold smaller than at 5 mV (see Fig. 5A, inset). Note the absence of recognizable AS events or \(\Delta C_m\) in response to a 5-s membrane depolarization to \(-30\) mV that evoked barely measurable \(I_{Ca}\). In contrast a barrage of amperometric spikes and a \(\Delta C_m\) of 290 fF were generated in response to a 5-s membrane depolarization to +5 mV, a voltage that evokes a 6–7-fold greater \(I_{Ca}\). This data, typical of those obtained from a set of three similar experiments, is consistent with the hypothesis that membrane fusion, as resolved by \(C_m\), and quantal discharge of transmitter, as resolved by amperometry, reflect the same underlying fusion process determined by Ca\(^{2+}\) entry into intact rat β-cells.\(^5\)

Combination of amperometry with current clamp recording from patch-clamped cells permits examination of quantal secretion induced by electrical activity of β-cells. In Fig. 6A, note that injection of a 20-pA depolarizing current from \(-10\) pA holding current evoked cell activity consisting of a short barrage of action potentials followed by a plateau depolarization and resulted in a barrage of amperometric events. This type of cell activity is typical for single rat β-cells. In these experiments with single cells (\(n = 3\)), we were not successful in obtaining either (i) consistent single APs in response to very brief current injections, probably due to the paucity of expression of sodium channels in the rat β-cell (12, 13) or (ii) consistent release in response to plateau depolarizations, probably \(^5\)In our cells, there were no exogenous Ca\(^{2+}\) buffers, such as Fura-2, to disturb mobility and alter cytosolic Ca\(^{2+}\) levels.
due to exhaustion of the previously loaded 5-HT. However, the feasibility for eventual success with such experiments is demonstrated by the voltage clamp experiment in Fig. 6. A single cell, within a small cluster of islet cells bathed in a PSS containing 3 mM glucose, was patched in the cell-attached mode with a pipette containing K⁺-IS and held at 0 mV. A CFE touched another region of the cell surface. Note, in the trace marked I_{on-cell}, that application by puffer pipette of a PSS containing 200 μM tolbutamide resulted in rapid closure of ATP-sensitive K⁺ channels, identified by their typical gating (burst of short openings) and characteristic amplitudes (~5 pA amplitude at pipette holding potential 0 mV) (see left bottom inset), followed by the appearance of biphasic action currents (see right bottom inset). The onset of amperometric spikes (see I_{amp} trace) coincided with the train of electrical activity. The slow time courses of individual AS events seen here, in contrast with the more rapid events in previous figures, was typical of recordings from cells within clusters. This phenomenon reflected slower diffusion of 5-HT to the sensor electrode, perhaps because significant granule release occurs at regions of cell-cell contact. As was the case with more rapid AS events, slower events were no longer evident when the holding potential of the CFE was decreased to +100 mV (data not shown), suggesting that they are produced by oxidation of a substance with a threshold of detection between +100 and +680 mV. Since rodent β-cells in clusters are often electrically coupled (41), some of the action potentials in the train may have been initiated in cells other than the one actually patched.

Amperometric Detection of Stimulus-Secretion Coupling in β-Cells—In several small diameter secretory cells, it has been demonstrated that current wave forms ("action currents"), associated with action potentials in the rest of the cell membrane, which is not voltage-damped, may be recorded extracellularly (i.e., by capacitative coupling) from cell-attached membrane patches (14, 15). In β-cells subjected to cell-attached patch recording, exposure to concentrations of glucose or tolbutamide sufficient to close most of the K⁺ (ATP) channels, often results in the onset of vigorous action current activity (7, 15–17). The amperometric current trace (I_{amp}) shows the onset of a barrage of broad ASs coincident with the train of electrical activity. The slow time courses of individual AS events seen here, in contrast with the more rapid events in previous figures, was typical of recordings from cells within clusters. This phenomenon reflected slower diffusion of 5-HT to the sensor electrode, perhaps because significant granule release occurs at regions of cell-cell contact. As was the case with more rapid AS events, slower events were no longer evident when the holding potential of the CFE was decreased to +100 mV (data not shown), suggesting that they are produced by oxidation of a substance with a threshold of detection between +100 and +680 mV. Since rodent β-cells in clusters are often electrically coupled (41), some of the action potentials in the train may have been initiated in cells other than the one actually patched.

**Fig. 6.** Quantal secretion induced by prolonged electrical activity and brief depolarizations nearly simulating single action potentials. Panel A shows that prolonged (10-s) injection of depolarizing current (~10 pA) results in (i) an initial barrage of APs followed by plateau depolarization (see V_m trace) and (ii) a concurrent barrage of amperometric spike events (see I_{amp} trace). The inset shows initial electrical activity at an expanded time scale. Panel B shows that brief (50-ms duration) square pulses of depolarization to +30 mV, repeated at 1 Hz, result in individual ASs. The inset shows that ASs occur during, as well as some ms after, the depolarization. As these recordings were made with a K⁺-IS pipette, membrane currents consist of a small initial inward current, in this case probably a combination of I_{Na}⁺ and I_{Ca}²⁺, followed by a larger outward I_{K}⁺.

**Fig. 7.** Cascade of events in stimulus-secretion coupling monitored by combined cell-attached patch recording and amperometry. A single cell, within a small cluster of islet cells bathed in a PSS containing 3 mM glucose, was patched in the cell-attached mode with a pipette containing K⁺-IS and held at 0 mV. A CFE touched another region of the cell surface. Note, in the trace marked I_{on-cell}, that application by puffer pipette of a PSS containing 200 μM tolbutamide resulted in rapid closure of ATP-sensitive K⁺ channels, identified by their typical gating (burst of short openings) and characteristic amplitudes (~5 pA amplitude at pipette holding potential 0 mV) (see left bottom inset), followed by the appearance of biphasic action currents (see right bottom inset). The onset of amperometric spikes (see I_{amp} trace) coincided with the train of electrical activity. The slow time courses of individual AS events seen here, in contrast with the more rapid events in previous figures, was typical of recordings from cells within clusters. This phenomenon reflected slower diffusion of 5-HT to the sensor electrode, perhaps because significant granule release occurs at regions of cell-cell contact. As was the case with more rapid AS events, slower events were no longer evident when the holding potential of the CFE was decreased to +100 mV (data not shown), suggesting that they are produced by oxidation of a substance with a threshold of detection between +100 and +680 mV. Since rodent β-cells in clusters are often electrically coupled (41), some of the action potentials in the train may have been initiated in cells other than the one actually patched.

*6* S. Misler, L. Falke, and K. D. Gillis, unpublished data.
DISCUSSION

We have combined electrochemical amperometry with patch clamp electrophysiology to examine, in real time, quantal secretion of 5-HT from rat β-cells during secretogogue-induced electrical activity, as well as during imposed cell depolarization. The justification for this approach is that pancreatic β-cells from a variety of species, selectively take up 5-HT out of proportion to other islet cells, sequester 5-HT into granules, and then secrete 5-HT along with insulin when exposed to insulin secretogogues (19–22). Single cell secretion of 5-HT by β-cells has been reported, in tandem, with rises in cytosolic Ca^{2+} under conditions where stimulus-secretion coupling is maximized (6). Our application of 5-HT amperometry has allowed us to examine aspects of the time course of exocytosis of a single quantum as well as the rapid (millisecond range) temporal coupling of depolarization to secretion. The advantages of this very rapid and sensitive single cell approach to the study of depolarization-secretion coupling over prior attempts, including combined perfusion and electrophysiology and membrane capacitance tracking, as well as some intrinsic limitations of this approach are discussed.

5-HT Amperometry as an Approach to Investigating Quantal Secretion in Rat Pancreatic β-Cells—In our initial experiments with amperometry of 5-HT preloaded islet cells (depicted in Figs. 1 and 2), we found that either of two insulin secretogogues known to depolarize β-cells results, after a short latency, in the appearance of a barrage of pulse-like electrochemical signals. The average charge transferred represented by the signal signifies that up to hundreds of thousands of molecules are oxidized in each unit of release (see below). The threshold oxidation potential of the release substance (>100 mV) is consistent with 5-HT being a principal species released. Furthermore, on lysis by saponin, similarly preloaded islet cells released material whose electrochemical (voltametric) profile was very similar to 5-HT (6). Our choice of amperometry to detect 5-HT, a co-secretion or false transmitter, rather than insulin, the chief secretory substance in insulin granules, was based on several factors. The first is the relative ease of fabrication of uncoated CFEs used to detect 5-HT as compared with the ruthenium dimer/oxide-precoated CFEs needed to detect insulin. The second is our experience with the higher time resolution of quantal signals and with the stability and sensitivity of the uncoated CFEs compared with the apparent lability of the precoated CFEs, perhaps due to their ability to catalyze electro-oxidation of nontransmitter substances, such as buffers, abundant in the media.

There are several reasons why the signals we recorded are likely to originate, in large part, from exocytosis of insulin granules from β-cells. First, our choice of the largest single cells visible for study (>10 μm in diameter) preselects with >80–90% probability for β-cells as previously demonstrated by cell sorting (23). This is now independently confirmed here; when so tested, most of our pre-selected cells responded to tolbutamide, a specific β-cell secretogogue. Second, as noted above, β-cells, and insulin granules in particular, selectively take up 5-HT (19–21). Third, although smaller, γ-aminobutyric acid-containing granules, roughly the size of synaptic vesicles, are present in β-cells, there is no evidence that these vesicles undergo regulated exocytosis (24). In addition, the molecular content of the quantal event we have measured here is several times larger than that of a catecholamine-containing synaptic vesicle (11), as might be expected for a granule with >125-fold greater volume than the synaptic vesicle. Fourth, the time courses of amperometrically measured quantized release of insulin from single β-cells stimulated by tolbutamide and K^{+} is similar to those observed here for release of 5-HT when differences in agonist concentration are taken into account (5). Fifth, in later experiments, we were able to identify β-cells electrophysiologically by their display, at high density, of distinctive ATP-sensitive K^{+} channels. Sixth, all amperometric signals were evoked under conditions under which insulin granule release was expected.

The use of our newly designed polypropylene insulated carbon fiber electrode (ProCFE) facilitated combination of high resolution electrophysiological and electrochemical recordings. In comparison with previously described glass/epoxy-insulated carbon fiber electrodes (geCFEs) (25–26) and peCFEs (2, 4), the newly designed ProCFE combines several critical features. These are (i) the mechanical stability, particularly at physiological temperatures, as compared with the widely used geCFE; (ii) the relatively lower noise and smaller tip dimension that previously promoted the use of a peCFE in combined electrophysiological/electrochemical recording; and (iii) the simple structure and manufacturing process. While the ProCFE consists of only a carbon fiber and polypropylene insulation, both the peCFEs and geCFEs require at least three kinds of material.

The Unitary Quantal Signals—Features of the individual 5-HT signals may offer some insight into the nature of exocytotic release of 5-HT from β-cells. The spike-like nature of individual amperometric signals, namely rapid time to peak and brief half-height duration, is similar to that of amperometric events recorded by similar techniques from chromaffin cells and widely accepted as exocytotic in origin (1, 2). This is so despite of the widely different molecular contents of the amperometric events; the average charge of individual ASs from rat β-cells, 56 femtocoulombs, corresponding to 1.75 × 10^{5} 5-HT molecules, is 3.5 times larger than that from the developing nerve terminal (11) but 20 times smaller than that from chromaffin cells. Furthermore, with our improved sensitivity recording, small, brief, but discrete signals can be seen at the rising edge of even widely isolated, low frequency events; these are reminiscent of “foot” or prefusion signals seen with chromaffin cell events (2, 27). These comparisons suggest that the kinetics of exocytosis of the false transmitter 5-HT from β-cells may be similar to the release of the physiological transmitter epinephrine from similarly sized chromaffin granules. Closer correlation of the time courses of exocytotic release of insulin and 5-HT from β-cells will be needed, especially since evidence from electron microscopy suggests that insulin may be released as a crystalline granule that subsequently must decondense.

Combined Electrophysiological/Electrochemical Studies of Depolarization-secretion and Agonist-secretion Coupling in Rat β-Cells—These experiments revealed that quantal release of 5-HT was rapidly activated by cell depolarization to voltages that evoke measurable Ca^{2+} currents in a given single cell. Release was graded with Ca^{2+} entry; depolarizations to voltages that evoke larger Ca^{2+} currents produce more frequent AS events during the course of the depolarization. Brief depolarizations evoked single amperometric events with variable latency, ranging from 5 ms after the start of a 50-ms depolarization to hundreds of ms after its cessation (see Fig. 6B). Prolonged depolarizations, either in the voltage or current clamp mode, often resulted in discharge throughout, although release frequency appeared to decline with time. Furthermore, the exocytotic nature of release was supported by correlation of the number of release events, recorded during cell depolarization.

\footnote{Based on a measured inner diameter of 322 nm (40), we estimate the 5-HT concentration of an insulin granule to be 8.5–17 mM, depending on the choice of estimate of the oxidation number for 5-HT, as compared with a catecholamine content of several hundred mM in a similarly sized chromaffin granule.}
tion, with the increase in membrane capacitance measured shortly (usually within 1–2 s) thereafter. These features were previously seen with combined amperometric and electrophysiological studies of adrenal chromaffin cells (1, 2), another excitable endocrine cell. Taken together, these results provide further evidence that these endocrine cells display voltage-activated, Ca\(^{2+}\) entry-dependent secretion resembling that seen in neurons. However, the longer latencies of release after Ca\(^{2+}\) entry suggest that, in these endocrine cells, part of the action of Ca\(^{2+}\) may be more distant from its entry site or more delayed than in neurons (2, 4, 28).

Using single rat \(\beta\)-cells recorded from in the perforated patch mode, we encountered some difficulty in (i) triggering single APs with brief depolarizing current pulses and (ii) evoking sustained electrical activity with a secretagogue such as tolbutamide. Many cells developed wobbly, plateau-like depolarizations (see Fig. 6). Other cells with more discrete APs were less than optimal for study because they failed to secrete reliably. However, better results were obtained recording from cells in clusters, particularly when cell-attached patch recording was used. In these experiments, it was possible to view aspects of an entire cascade of events involved in the induction of secretion by tolbutamide. These include rapid closure of ATP-sensitive K\(^+\) channels critical to the maintenance of the resting membrane potential, subsequent onset of electrical activity (biphasic action currents), and, coincident with the onset of electrical activity, quantized release of 5-HT. This approach should be very useful in determining the relative contribution of different steps in the stimulus-secretion cascade to the heterogeneity of agonist responsiveness seen in single cells.

Comparison with Other Approaches to Assay Stimulus-secretion Coupling: Limitations and Perspective—To be sure, the combination of electrophysiology and amperometry stands in a long tradition of approaches to relate electrical activity and secretion in \(\beta\)-cells either by simultaneous or sequential monitoring of events. Earlier approaches have included (i) single islet perfusion (followed by radioimmunoassay of the perfusate for insulin) combined with simultaneous electrical recording from a single cell within the islet (29) and (ii) single cell immunochemical assay of insulin release (by reverse hemolytic plaque assay) followed by patch clamp recording from the identified cell (12). At best, these assays have permitted resolution of insulin secretion over many seconds to minutes. More recently, membrane capacitance tracking has offered a more real time single cell assay of exocytosis. Using this approach, it has been possible in single cells (i) to quantify the dependence of release on voltage-activated entry of Ca\(^{2+}\) and other divalents cations (30–33), as well as bulk cytosolic concentrations of these ions (32–34) and (ii) to examine the roles of protein kinase and phosphatase activation in modulating Ca\(^{2+}\)-dependent release (35–37). However, major drawbacks of this approach include (i) poor resolution at the level of release of one to several granules, often requiring extended depolarization to reveal release; (ii) the requirement that the cell be voltage-clamped during the capacitance measurement, a condition that precludes real time monitoring of electrical activity induced release under physiological conditions; and (iii) uncertainty that chemical release is actually occurring. Our evidence suggests that the combination of amperometry and electrophysiology eliminates all three of the aforementioned drawbacks of capacitance tracking. However, the need for close apposition of the electrode and the release site imposes the drawback that amperometry only captures a fraction of release events. Where possible, combination of amperometry and capacitance tracking might provide an ideal approach.

A disappointing feature of the application of the highly sensitive 5-HT amperometric assay to rat \(\beta\)-cells is its lack of robustness in measuring secretion. This often precluded extended or detailed quantitative studies. To be sure, as with single cell assays, only a fraction (<50%) of \(\beta\)-cells secrete in response to secretagogues or direct stimulation. Of more concern is the rapidity with which secretion in most cells declines to barely detectable levels during even short, repetitive bouts of depolarization in the presence of cAMP-enhancing agents thought to increase the secretion-ready granule pool. While the loading of granules appears to be dependent on the bath concentration of 5-HT, it is not clear what factors contribute to 5-HT retention or redistribution after loading. At this time, it is uncertain what percentage of granules actually load with 5-HT and whether these represent a specific fraction. Electron microscopic autoradiography of random sections of 3\(^{3}\)H-5-HT-loaded \(\beta\)-cells show radioactive vesicles dispersed within much of the vesicle pool (13), but evidence from serial reconstruction is lacking. This feature of limited loading might be overcome with improved loading techniques and/or extension of current approaches to islets of other species. For example, in early experiments, canine islet cells display quantal events with 5 times the molecular content of those from rat. Although their amplitude slowly declines with time, these events remain detectable over many minutes, especially when short periods of rest are interspersed between bouts of electrical activity (42).

Acknowledgments—We thank J. Fink for cell preparation, Yan-Fang Hu for assistance in preparing the amperometric electrodes, and Dr. D. Barnett for comments on the manuscript. We also thank Dr. K. Kawagoe of Axon Instruments for noise measurements of ProCFEs.

REFERENCES

1. Wightman, R. M., Jankowski, J. A., Kennedy, R. T., Kawagoe, K. T., Schroeder, T. A., Lezczynsky, D. J., Near, J. A., Diliberto, E. J., Jr., and Viveros, O. H. (1991) Proc. Natl. Acad. Sci. U.S.A. 88, 10754–10758
2. Chow, R. H., Rüden, L. V., and Neher, E. (1992) Nature 356, 60–63
3. Toledo, G. A., Fernandez-Chacon, Z. L., and Fernandez, J. M. (1993) Nature 363, 554–558
4. Zhou, Z., and Misler, S. (1995) J. Biol. Chem. 270, 3498–3505
5. Kennedy, R. T., Huang, L., Atkinson, M. A., and Dush, P. (1993) Anal. Chem. 65, 1862–1867
6. Smith, P., Duchen, M., and Ashcroft, F. M. (1995) Pflugers Arch. Eur. J. Physiol. 430, 808–818
7. Misler, S., Falke, L. C., Gillis, K., and McDaniel, M. L. (1986) Proc. Natl. Acad. Sci. U.S.A. 83, 7119–7123
8. Hamill, O. P., Marty, A., Neher, E., Sakmann, B., and Sigworth, F. J. (1981) Pflugers Arzneim. Forsch. Eur. J. Physiol. 315, 85–100
9. Zhou, Z., and Neher, E. (1993) J. Physiol. (London) 469, 245–273
10. Herrington, J. and Bookman, R. (1993) Pulse Control Manual, University of Miami Press, Miami
11. Zhou, Z., and Misler, S. (1995) Proc. Natl. Acad. Sci. U.S.A. 92, 6933–6942
12. Hirai, M., and Matteson, D. R. (1988) J. Gen. Physiol. 91, 617–639
13. Pressel, D. M., and Misler, S. (1991) J. Membr. Biol. 124, 239–253
14. Fricker, E. M., Marty, A., and Neher, E. (1981) J. Physiol. 311, 577–597
15. Ashcroft, D. F., Harrison, E. D., and Ashcroft, S. J. H. (1984) Nature 312, 446–448
16. Rorsman, P., and Trube, G. (1985) Pflugers Arch. Eur. J. Physiol. 405, 395–399
17. Trube, G., Rorsman, P., and Ohno-Shosaku, T. (1986) Pflugers Arch. Eur. J. Physiol. 407, 493–499
18. Gillis, K. D., Gao, W. M., Hammoud, A., McDaniel, M. L., Falke, L. C., and Misler, S. (1989) Am. J. Physiol. 257, C1119-C1127
19. Ekholm, R., Ericson, L. E., and Lundquist, I. (1971) Diabetologia 7, 339–348
20. Heiman, B., Lernmark, Å., Seblin, J., and Talljedal, I.-B. (1972) Biochem. Pharmacol. 21, 695–706
21. Gyfle, E. (1978) J. Endocrinol. 78, 239–248
22. Gyfle, E. (1980) Acta Physiol. Scand. 109, 155–161
23. Pipeleers, D. G., in't Veld, P. A., Van de Venkel, M., Maes, E., Schuit, F. C., and Gepts, W. (1985) Endocrinology 117, 806–816
24. Reetz, A., Solimena, M., Matteoli, M., Fuli, F., Takéi, K., and De Camilli, P. (1991) EMBO J. 10, 1273–1280
25. Armstrong, M., and Millar, J. (1979) J. Neurosci. Meth. 1, 279–288
26. Kawagoe, K. T., Zimmerman, J. B., and Wightman, R. M. (1993) J. Neurosci. Meth. 48, 225–240
27. Zhou, Z., Misler, S., and Chow, R. H. (1995) Biophys. J. 68, A11
28. Chow, R. H., Klingsauf, J. G., and Neher, E. (1994) Proc. Natl. Acad. Sci. U.S.A. 91, 12765–12769
29. Scott, A. M., Attwater, I., and Rojas, E. (1981) Diabetologia 21, 470–475
30. Gillis, K. D., and Misler, S. (1992) Pflugers Arch. Eur. J. Physiol. 405, 121–123
31. Åmmelö, C., Eliasson, B., Bokvist, K., Larsson, O., Ashcroft, F. M., and Rorsman, P. (1993) J. Physiol. (London) 472, 665–668

Downloaded from http://www.jbc.org/ by guest on July 24, 2018
32. Barnett, D. W., and Misler, S. (1995) Pflugers Arch. Eur. J. Physiol. 430, 593–595
33. Proks, P., and Ashcroft, F. M. (1995) J. Physiol. (London), in press
34. Bokvist, K., Eliasson, L., Åmmålä, C., Renström, E., and Rorsman, P. (1995) EMBO J. 14, 50–57
35. Åmmålä, C., Ashcroft, F. M., and Rorsman, P. (1993) Nature 363, 356–358
36. Gillis, K., and Misler, S. (1993) Pflugers Arch. Eur. J. Physiol. 424, 195–197
37. Åmmålä, C., Eliasson, L., Berggren, P.-O., Honkanen, R. E., Sjöholm, A., and Rorsman, P. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 4343–4347
38. Stamford, J. A., Crespi, F., and Marsden, C. A. (1992) in Monitoring Neuronal Activity (Stamford, J. A., ed) pp. 113–145, ILP, Oxford
39. Bruns, D., and Jahn, R. (1995) Nature 377, 62–65
40. Dean, P. M. (1973) Diabetologia 9, 115–119
41. Perez-Arriandiz, M., Roy, C., Spray, D. C., and Bennett, M. V. (1991) Biophys. J. 59, 76–92
42. Zhou, Z., and Misler, S. (1995) Soc. Neurosci. Abstr. 21, 334

Quantal Release of 5-HT from β-Cells

277
Amperometric Detection of Quantal Secretion from Patch-clamped Rat Pancreatic β-Cells
Zhuan Zhou and Stanley Misler

J. Biol. Chem. 1996, 271:270-277.
doi: 10.1074/jbc.271.1.270

Access the most updated version of this article at http://www.jbc.org/content/271/1/270

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 39 references, 8 of which can be accessed free at http://www.jbc.org/content/271/1/270.full.html#ref-list-1