Intra- and Intercellular Ca$^{2+}$ Signaling in Paraneurons and Other Secretory Cells

Tomio Kanno

Yanaihara Institute Inc., Fujinomiya, 418-0011 Japan

Summary: Paraneurons are endocrine and sensory cells which share structural, functional, and metabolic features with neurons. They produce identical with or related to neurotransmitters or neurohormones, which are synthesized and secreted by regulated secretion. They are receptoconductile-secretory in function, which is shared by specific proteins distributed at proper regions of cell membrane. A substantial advance has been made in the molecular machinery underlying protein sorting and transport within the endoplasmic reticulum and Golgi apparatus, and the mechanism of targeted membrane fusion by constitutive secretion. Various patterns of [Ca$^{2+}$]$_c$ dynamics play cardinal signaling roles in stimulus-secretion coupling in individual secretory cells. Long-lived recurrent Ca$^{2+}$ spikes or oscillation may maintain prolonged secretory responses, ATP synthesis in mitochondria, cell growth, differentiation, and division. In the neurons and the paraneurons of neuroectodermal origin, action potentials propagate along a conductile region to the secretory region of each cell and hardly be transmitted to the adjacent cells. In the paraneurons of gut endodermal origin, intracellular signaling including Ca$^{2+}$ spikes can be propagated to the adjacent cells, and in turn may maintain coordination of individual cells forming a cell society. [Japanese Journal of Physiology, 48, 219–227, 1998]

Key words: paraneuron, Ca$^{2+}$ signaling, stimulus-secretion coupling, adrenal chromaffin cells, islet B cells.

Paraneurons are endocrine and sensory cells that share structural, functional, and metabolic features with neurons. They produce substances identical with or related to neurotransmitters (including neuromodulators) or neurohormones, and they are receptosecretory in function [1–5]. In neurons, the sequence of events in stimulus-secretion coupling can be divided into three main steps: the reception of stimuli, the conduction of excitation, and the extrusion of messenger substances by exocytosis in the regulated secretion [6]. In paraneurons, the sequence is believed to show essentially identical regional differentiation in the cell membranes, provided that the conductile region of paraneurons is short enough to transmit the net depolarization [4, 7–9]. At the input regions of paraneurons, the activation of various receptors by appropriate stimuli converges into an elevation of [Ca$^{2+}$]$_c$, which functions as a common intracellular signaling to induce exocytosis at the output regions. To include all these events, the term “stimulus-secretion coupling” was proposed by Douglas and Rubin [10–17].

More than three decades ago, we analyzed the initial event in the coupling by recording intracellular potential in dissociated adrenal chromaffin cells in primary culture [15, 17–20]. The method is now standard practice and much used in patch-clamp analysis of the behavior of ion channels, also in the membrane-capacitance approach to the detection of exocytosis [21]. The [Ca$^{2+}$]$_c$ does not remain necessarily stable, but can exhibit rhythmic oscillations in periods ranging from tens of seconds to several minutes. In recent years, the oscillatory Ca$^{2+}$ signaling has been recorded in various types of cells with the development of the Ca$^{2+}$-sensing fluorescent dyes by Tsien and his colleagues [22]. Rhythmic phenomena are responsible for the creation, stabilization, and mainte-
nance of a certain kind of biological order [23–25]. Possible cellular mechanisms of receptor-controlled oscillatory Ca\(^{2+}\) signaling have been profoundly investigated [25–27].

It has become evident in the past three decades that the elevation of [Ca\(^{2+}\)]\(_c\), is the signal not only in stimulus-secretion coupling, but also in ATP synthesis in the mitochondria, cell growth, differentiation, and division. These responses in various temporal sequences in individual secretory cells can be amplified when the short-lived rise in [Ca\(^{2+}\)]\(_c\) transforms to long-lived recurrent Ca\(^{2+}\) spikes or oscillation. The Ca\(^{2+}\) signaling may also play a large role in the spatial coordination of individual cells forming a cell society.

In this review, I will concentrate on the roles of Ca\(^{2+}\) signaling in spatial and temporal coordination in the cell societies of two typical paraneurons—chromaffin cells in the adrenal medulla and endocrine cells in the pancreatic islets—since these paraneurons are capable of generating action potentials in common, mainly Na\(^+\) spikes in the former versus Ca\(^{2+}\) spikes in the latter.

**Adrenal Chromaffin Cells**

It is well established that acetylcholine (ACh) released from the terminals of splanchnic neurons plays a key role in inducing secretion of catecholamines in the adrenal chromaffin cells. In most species, adrenal chromaffin cells have nicotinic and muscarinic receptors, both of which can stimulate catecholamine secretion [28, 29]. However, variations between species are based mainly on the presence of nicotinic and/or muscarinic receptors [28, 29]. In bovine cells, the activation of nicotinic receptors is entirely responsible for ACh-induced secretion. In the cultured chromaffin cells of gerbil, more than three decades ago we showed that ACh-induced activation of nicotinic receptors is immediately followed by an opening of the ion channel-receptor complex allowing the entry of Na\(^+\) and, to a lesser extent, Ca\(^{2+}\) [18–20]. The entry of Na\(^+\) results in a mild depolarization of the chromaffin cell membrane sufficient to activate fast voltage-dependent, tetrodotoxin-sensitive channels [30]. The opening of these highly selective Na\(^+\) channels, in combination with the receptor ion channel-mediated depolarization, induces the activation of voltage-dependent, dihydropyridine-sensitive slow Ca\(^{2+}\) channels [31]. The opening of both types of channels causes the firing of action potentials and the final entry of Ca\(^{2+}\) from the extracellular fluid [32]. Na\(^+\) entry through voltage-dependent channels plays a facilitatory but not obligatory role in the stimulation of secretion by low levels of agonists [33]. The resultant rise in the free Ca\(^{2+}\) concentration in the cytosol, [Ca\(^{2+}\)]\(_c\), is the trigger for exocytosis of chromaffin granules [14–17, 29].

The activation of chromaffin cells by a wide variety of secretagogues acting on specific receptors suggests the existence of multiple pathways regulating stimulus-secretion coupling. The activation of receptors for muscarine, histamine, and angiotensin II elicits Ca\(^{2+}\)-dependent exocytosis of chromaffin granules via the rapid formation of inositol 1,4,5-trisphosphate (Ins\((1,4,5)P_3\)), which then increases the [Ca\(^{2+}\)]\(_c\) [34, 35]. The Ins\((1,4,5)P_3\)-mediated pathway is not activated by nicotine [29].

Besides the cardinal role of ACh, evidence that pitiuiary adenylate cyclase-activating polypeptide (PACAP) may also play a sizable role in regulating catecholamine secretion in the adrenal chromaffin cells has been increasing. This view is supported by the following findings [36]: (1) The adrenal gland contains the second highest concentration of PACAP among peripheral organs; (2) Endogenous PACAP levels in the adrenal medulla are 24-fold higher than in the cortex; (3) PACAP-binding sites are abundant in the cortex; (4) PACAP increases catecholamine secretion, intracellular cyclic AMP, Ins\((1,4,5)P_3\), and [Ca\(^{2+}\)]\(_c\); (5) PACAP is much more potent than vasoactive intestinal polypeptide (VIP) in stimulating catecholamine secretion and the production of both Ins\((1,4,5)P_1\) and cyclic AMP; and (6) Unlike ACh, which induces a rapid increase in catecholamine secretion with a rapid recovery, PACAP causes long-lasting secretion at concentrations as low as 10 nM. Results obtained in our recent studies indicate that PACAP causes both Ca\(^{2+}\) release, mainly from caffeine-sensitive Ca\(^{2+}\) stores, and Ca\(^{2+}\) influx via L-type Ca\(^{2+}\) channels activated by membrane depolarization that depends on protein kinase C-mediated Na\(^+\) influx [36]. They also suggest that PACAP induces release from ryanodine/caffeine stores through a novel intracellular mechanism independent of both Ins\((1,4,5)P_3\) and cyclic AMP [37].

The stimulus-secretion coupling activated by ACh, histamine, and angiotensin II can be facilitated or inhibited by other neurotransmitters such as gamma-aminobutyrate (GABA) and substance P, and by many other modulators such as somatostatin, mammalian neurokinins, neuropeptide Y, opioid peptides, and atrial natriuretic peptides (ANP). Except for substance P and ANP [29], the specific and active receptors for individual peptides are not yet convincingly confirmed. Nicotinic agonist-induced [Ca\(^{2+}\)]\(_c\) response in bovine chromaffin cell is inhibited by substance P and related peptides by direct interaction with the nico-
Ca\(^{2+}\) Signaling in Paraneurons

tinic receptor-ion channel complex (see Suzuki et al. [38] for references). The nicotinic agonist-induced catecholamine secretion in chromaffin cells is known to be inhibited by direct interaction with the complex. The muscarinic agonist-induced [Ca\(^{2+}\)]\(_i\) response in rat chromaffin cell may consist of two components: initial Ca\(^{2+}\) release from intracellular store followed by Ca\(^{2+}\) entry [39].

A large fraction of rat adrenal chromaffin cells (about 60\%) showed spontaneous recurrent Ca\(^{2+}\) spikes and spontaneous action potentials, whereas gerbil, bovine, and guinea pig adrenal chromaffin cells remained stable in a resting state. The discharge of spontaneous recurrent action potentials and spontaneous oscillations in rat chromaffin cells were inhibited by GABA in a reversible manner through the activation of GABA\(_A\) receptor and a subsequent increase in Cl\(^{-}\) conductance [40]. The spontaneous recurrent Ca\(^{2+}\) spikes may be generated through activation of an intracellular pacemaker, and the following propagation is sustained by the discharge of Ca\(^{2+}\) from the stores into the cytoplasm; the entry during action potentials may serve mainly for the refilling of intracellular stores [41, 42]. In bovine adrenal chromaffin cells, stimulation of the GABA\(_A\) receptor caused activation of the Cl\(^{-}\) channels, inward whole-cell currents, a burst of action potential discharge, elevation of [Ca\(^{2+}\)]\(_i\), and catecholamine secretion, whereas stimulation of GABA\(_B\) receptors inhibited high K\(^+\)-induced [Ca\(^{2+}\)]\(_i\), elevation and voltage-activated Ca\(^{2+}\) currents (see Buski et al. [40] for references).

Adrenal chromaffin cells consist of at least three types: adrenaline (A)-secreting cell, noradrenaline (NA)-secreting cell, and small granule chromaffin (SGC) cell (small intensely fluorescent (SIF) cell) [43]. We have recently studied the ontogenic maturation of nicotinic and muscarinic receptor-induced Ca\(^{2+}\) signaling with reference to secretion of the rat adrenal chromaffin cells during developmental stages [44]. The study was carried out by combining conventional digital Ca\(^{2+}\) imaging techniques with immuno-histochemistry and electron microscopy and provided the following results. In the adult chromaffin cells, most cells showed a single rise in [Ca\(^{2+}\)]\(_i\) only after nicotine stimulation, but not after methacholine. These chromaffin cells, although showing different response patterns of agonist-induced [Ca\(^{2+}\)]\(_i\), rises, were immunoreactive for dopamine \(\beta\)-hydroxylase (DBH) and for phenylethanolamine N-methyltransferase (PNMT), and they were identified as A cells. These results suggest that both nicotinic and muscarinic receptor-mediated signaling may function in most A cells, whereas very few A cells with only nicotinic receptors may lack muscarinic receptors or contain, if present, immature muscarinic receptors or immature [Ca\(^{2+}\)]\(_i\) transduction pathways. The changes in [Ca\(^{2+}\)]\(_i\) in the NA cells and SGC cells could not be analyzed because the number of NA cells amounted to less than 1\%, and the number of SGC cells was almost nothing in total chromaffin cells isolated with collagenase from the gland of postnatal week one onward. The [Ca\(^{2+}\)]\(_i\) response in developing adrenal chromaffin cells after stimulation with methacholine and nicotine showed three phases: (1) No effect on the chromaffin cells at embryonic day 16 after the stimulation, (2) only a nicotine effect on the chromaffin cells at embryonic day 19, and (3) both nicotine and methacholine effects or only a nicotine effect on the chromaffin cells from birth onward. These chromaffin cells, analyzed by a digital imaging technique, were immunopositive for DBH and PNMT and thus were considered mixed cells from birth to postnatal day 2 or 3, and A cells from postnatal day 2 or 3 onward.

An image analysis of spatial and temporal dynamics of [Ca\(^{2+}\)]\(_i\), during continuous stimulation with carbachol (CCh) at different concentrations gave us a clue to the mode of intra- and intercellular Ca\(^{2+}\) propagation [45]. When a cluster of chromaffin cells of guinea pig was continuously stimulated with a low concentration of CCh (1 \(\mu\)M), the [Ca\(^{2+}\)]\(_i\) in a cell (initial cell) began to increase in the cellular region beneath the plasma membrane facing the extracellular environment in the direction of intercellular margin. The [Ca\(^{2+}\)]\(_i\), in the second and third cells adjacent to the first cell in the cluster began to increase after a delay of about 2 min. A possible explanation for the distinct delay may be in the processes of independent [Ca\(^{2+}\)]\(_i\) elevation in individual cells in the cluster. The explanation can be proven if and when the heterogeneities in the sensitivity of cholinergic receptors and in the efficiency of receptor-coupled Ca\(^{2+}\) entry are certified in individual cells in the cluster. An image analysis of spatial and temporal dynamics of [Ca\(^{2+}\)]\(_i\) revealed further that the distribution of receptors for carbamyl-\(\beta\)-methylcholine or for bradykinin may be heterogeneous on individual cells in the cluster [46]. The explanation may be supported by the morphological findings, in which intercellular communications, such as gap junction, have not been found in the adrenal medulla, although short desmosomes were shown in the bovine adrenal medulla [47].

Neither the spontaneous recurrent Ca\(^{2+}\) spikes nor the agonist-induced single Ca\(^{2+}\) spike is able to propagate to the adjacent chromaffin cells. The intracellular Ca\(^{2+}\) signaling in the chromaffin cell cannot possibly interfere with that in the adjacent adrenal paraneu-
rons. The independent signal transduction in individual chromaffin cells is the property in common with neurons. It accurately corresponds to the ontogeny and the phylogeny: The chromaffin cells and the sympathetic ganglion cells derive from the neural crest. The property secures the independent signal transmission and protects against cross-talk among these cells.

**B Cells in an Islet**

The major control of insulin secretion is exerted by a feedback effect of plasma glucose directly on the B cells forming an islet in the pancreas. The mechanism of glucose-induced [Ca$^{2+}$]$\textsubscript{i}$ increase is different from ACh-induced [Ca$^{2+}$]$\textsubscript{i}$ increase in adrenal chromaffin cells, and unique among various paraneurons: (1) Glucose is transported by facilitated diffusion across the B cell membrane via a glucose transporter (GLUT 2); (2) Glucose is metabolized and ATP is generated; (3) ATP closes ATP-sensitive K$^+$ channels; (4) The resultant decrease in K$^+$ efflux depolarizes the cell membrane, then opens voltage-sensitive Ca$^{2+}$ channels through which Ca$^{2+}$ enters the cell (see Kitasato et al. [48] for references). In these processes, neither an extracellular receptor nor protein kinase C (PKC)–mediated phosphorylation events are involved [49].

Insulin secretion is also controlled by a variety of secretagogues, intracellular signaling processes that are diversified in individual secretagogues. Among these, ACh and cholecystokinin octapeptide (CCK-8) exert their insulinitropic effect via heterotrimeric G protein–coupled receptors [50, 51], G$\alpha$ [52]. The binding of ACh or CCK-8 with G$\alpha$ causes PKC translocation [52] and phospholipase C (PLC) activation. PLC hydrolyzes inositol 4,5-biphosphate to 1,2-diacylglycerol (DAG) and Ins(1,4,5)P$\textsubscript{3}$. DAG activates PKC, whereas Ins(1,4,5)P$\textsubscript{3}$ increases Ca$^{2+}$ release from the endoplasmic reticulum to elevate [Ca$^{2+}$]$\textsubscript{i}$. It is also proposed that the activation of PLC is coupled to different G proteins: the pertussis toxin (PT)-sensitive $\alpha_\text{G}$ in regard to carbachol, and the PT-insensitive $\alpha_{G11}$ in regard to CCK-8 [53]. The ACh- or CCK-8–induced signal transduction in the B cell is basically the same as the signal transduction induced by an activation of receptors for muscarine, histamine, and angiotensin II in adrenal chromaffin cells.

In contrast to the independent Ca$^{2+}$ signaling in individual adrenal chromaffin cells, the Ca$^{2+}$ signaling in a B cell can immediately propagate to adjacent B cells in the pancreatic islet. Fast (~2.5/min) and slow (~0.3/min) [Ca$^{2+}$]$\textsubscript{i}$ oscillations induced by continuous stimulation with glucose are well synchronized among B cells forming the islet [54]. We have recently recorded ratiometric images of [Ca$^{2+}$]$\textsubscript{i}$ in individual cells with a confocal UV-laser microscope in the Indo-1-loaded islets isolated from mice [55]. The individual cells were identified by recording [Ca$^{2+}$]$\textsubscript{i}$ dynamics in response to glucose, and then confirmed in the identical focal plane after fixation of the islet preparation that had previously permeabilized and stained by the indirect immunofluorescence method against insulin. Almost all cells identified as insulin-positive (B) cells by their distinct immunofluorescence responded to the increase in glucose concentration from 3 to 11 mM with an increase in [Ca$^{2+}$]$\textsubscript{i}$. The [Ca$^{2+}$]$\textsubscript{i}$ dynamics of similar patterns were recorded in most B cells in the confocal plane of the islet. The glucose-induced response of periodic [Ca$^{2+}$]$\textsubscript{i}$ increases at 34°C consisted of two main phases: In the initial phase, the [Ca$^{2+}$]$\textsubscript{i}$, climbed from a resting level (about 100 nM on average) to a peak level of about 400 nM on average, followed by a slow declivity; in the second phase, recurrent Ca$^{2+}$ spikes continued as long as glucose remained at 11 mM. The initial phase and the recurrent Ca$^{2+}$ spiking in the second phase recorded in a B cell in the presence of 11 mM glucose synchronized with the phases in other B cells forming the same islet, although small differences were noted among the amplitudes of [Ca$^{2+}$]$\textsubscript{i}$ increases in these cells. The B cell having the earliest and largest initial phase seems to be the functional pacemaker from which the initial [Ca$^{2+}$]$\textsubscript{i}$ rise may propagate to the adjacent B cells. In adjacent A cells in the same islet, however, the rise in glucose caused only slight or a decreasing [Ca$^{2+}$]$\textsubscript{i}$ response, as already reported [56].

The pattern of [Ca$^{2+}$]$\textsubscript{i}$ increase in response to glucose transformed when the ambient temperature was decreased to 26°C, from 34°C: Continuous stimulation with 11 mM glucose induced regular slow [Ca$^{2+}$]$\textsubscript{i}$ oscillations. The [Ca$^{2+}$]$\textsubscript{i}$ oscillations in individual B cells were well synchronized with one another in the same islet, although small differences existed among the amplitudes of [Ca$^{2+}$]$\textsubscript{i}$ oscillations in these cells. These [Ca$^{2+}$]$\textsubscript{i}$ dynamics may be intra- and intercellular signaling to induce pulsatile insulin secretion in normal humans and animals [54, 57, 58]. The synchronous [Ca$^{2+}$]$\textsubscript{i}$ oscillations among B cells are based on two congenital properties: electrical excitability and the gap junctions through which depolarizing current can be propagated. The asynchronous Ca$^{2+}$ signaling in the adrenal chromaffin cells may certainly be due to lack of gap junction. The diffusion of Ca$^{2+}$ and Ins(1,4,5)P$\textsubscript{3}$ through the gap junctions may result in asynchronous but coordinative signaling in electrically inexcitable secretory cells, such as pancreatic acinar cells.
Pancreatic Acinar Cells Forming an Acinus

To elucidate the mode of signal transduction through the gap junctions, we have recorded ratiometric images of [Ca^{2+}]_{i} in individual cells with a conventional and a confocal UV-laser microscope in the pancreatic acini [59–61]. Ratiometric imaging of [Ca^{2+}]_{i}, dynamics by UV-laser scanning confocal microscopy enabled us to investigate intra- and intercellular signaling in individual acinar cells forming the same acinus. Continuous stimulation of mouse acini with CCK-8 at a low physiological concentration (15 pm) induced a faint [Ca^{2+}]_{i} rise in the perimeter of an acinar cell (first cell), followed by a uniform maximum rise in [Ca^{2+}]_{i} in the entire confocal plane of the first cell and by onset of a rise in [Ca^{2+}]_{i} in the second cell adjacent to the primary cell. A time lapse (<18 s) occurred between the maximum [Ca^{2+}]_{i} rise in the first cell and that in the second cell. The fade-out of a [Ca^{2+}]_{i} rise in these two cells was followed by the fade-in of [Ca^{2+}]_{i} rise in the third and fourth cells adjacent to the first and second cells. During the continuous stimulation, this sequence of [Ca^{2+}]_{i} dynamics was repeated in individual acinar cells with different intervals. The temporal display of [Ca^{2+}]_{i} dynamics showed that the amplitude, the shape of the spike, and the interval were variants in individual cells forming the acinus, and that the recurrent spikes recorded from individual cells were not synchronous with one another. The variations in the CCK-8-induced recurrent spikes in individual cells were further emphasized when the spikes were inhibited with 100 mM 2-aminoethoxydiphenyl borate (2APB), an inhibitory modulator of Ins(1,4,5)P_{3}-mediated release from intracellular Ca^{2+} stores [62–64].

These results are compatible with our view [62, 65]. The CCK receptor and G_{q} protein may certainly be in the basallateral membrane of the acinar cell. The binding of CCK-8 with its receptor may activate phosphatidylinositol-specific phospholipase C (PI-PLC), which hydrolyzes phosphatidylinositol 4,5-diphosphate, and subsequently a raised level of Ins(1,4,5)P_{3}, sufficient to release Ca^{2+} from intracellular stores and to trigger an increase in [Ca^{2+}]_{i}. An increase in [Ca^{2+}]_{i} and a decrease in [Ca^{2+}]_{i} in the stores led to the cessation of Ca^{2+} release from the stores. The partly or completely empty stores cause Ca^{2+} entry through the store-operated Ca^{2+} channels [66] to refill the stores. When the stores refill to a set level, Ins(1,4,5)P_{3} resumes its effectiveness in releasing Ca^{2+} into the cytoplasm; therefore the cycle of [Ca^{2+}]_{i} spiking continues. The asynchronous but coordinative signaling in pancreatic acinar cells forming the acinus can be explained by the model proposed by Yule et al. [67] that Ca^{2+} acts as coagonist with Ins(1,4,5)P_{3} to potentiate the Ca^{2+}-releasing action of Ins(1,4,5)P_{3} and, by diffusion of the two molecules through gap junctions, underlies intercellular signaling for coordination of the acinar cells. The time lapse (<18 s) between the [Ca^{2+}]_{i} rise in the first cell and that in the adjacent two cells may be the time consumed by diffusion of the two molecules through gap junctions. The lack of electrical excitability and of a voltage-dependent Ca^{2+} channel may be responsible for the asynchrony of Ca^{2+} spiking among cells forming the acinus.

Signal Transmission in Paraneuron Society

Many neurons and endocrine cells were known to show a positive formaldehyde-induced fluorescence (FIF) reaction after the administration of amine precursors (such as L-dopa), which were then decarboxylated to amines (such as dopamine). Pearse [68] categorized cells having the “Amine Precursor Uptake and Decarboxylation” ability into the “APUD series.” In 1975, he included some FIF-negative cells in the APUD series [69]. In this year, Fujita and Kobayashi [1] proposed the term “paraneuron” to describe cells that produce substances identical or related to neurotransmitters or neurohormones and store these substances in granules (criterion 1); it is a receptosecretory in function (criterion 2). Criterion 1 corresponds to the characteristics preserved by regulated (nonconstitutive) secretion. The neurons and paraneurons are of neuroectodermal origin and synthesize and store not only classical neurotransmitters such as catecholamines, but also ATP; various peptides, including opioid peptides; and chromogranins [28, 29, 70].

Criterion 2 in neuron and paraneuron seems to correspond to the functional characteristics of the three different regions of neuron and paraneuron membrane. The spatial differentiation in the plasma membrane of neuron was proposed by Grundfest [6] as follows: (1) The receptive region is electrically excitable and responds to specific stimuli with a characteristic electrogensis that may be depolarizing (excitatory) or hyperpolarizing (inhibitory); (2) When the net electrogensis of the receptive region is depolarizing, it can initiate spikes in the conductile, spike-generating region; and (3) These signals, reaching the terminal secretory region of the cell, causes release of the transmitter. The signals open the voltage-dependent Ca^{2+} channels, and the resultant elevation of [Ca^{2+}]_{i} causes exocytosis at the terminal secretory region (Fig. 1, N).
Fig. 1. Schematic representation of the cellular events and Ca\textsuperscript{2+} signaling in stimulus-secretion coupling in a neuron and paraneurons. Action potentials (AP) propagate along a conductile (axonal) region to the secretory region of a neuron (N). In a small granule chromaffin (SGC) cell, action potentials as well as a Ca\textsuperscript{2+} wave can be transmitted to a secretory region. In a noradrenaline (NA)-secreting cell and an adrenaline (A)-secreting cell, the integration of action potentials and a Ca\textsuperscript{2+} wave may result in various patterns of [Ca\textsuperscript{2+}], dynamics. In a B cell forming a pancreatic islet, the glucose-induced electrical activity and [Ca\textsuperscript{2+}], increase can rapidly be propagated to the adjacent B cells and be modified by humoral factors and neurotransmitters. Action potentials and [Ca\textsuperscript{2+}], dynamics are depicted on the schemata of paraneurons, which are modified and reproduced, with permission, from Fujita et al. [4]. See text for discussion.

Although neurons and adrenal paraneurons are of neuroectodermal origin, the spatial differentiation may be less or least prominent in the adrenal paraneurons containing adrenaline (A cells) or noradrenaline (NA cells). These cells are equipped with a wide variety of receptors specific for ACh (nicotinic and muscarinic receptors), histamine, bradykinin, PACAP, VIP, substance P, angiotensin II, GABA, ATP, and others [28, 29, 70]. The binding of these secretagogues with their specific receptors induces electrogenesis, slow depolarization and/or action potential, and activation of Ca\textsuperscript{2+} channels and transporters. The integration of these events converges into various types of [Ca\textsuperscript{2+}], dynamics. In the absence of these secretagogues, spontaneous action potentials and spontaneous [Ca\textsuperscript{2+}], oscillations are recorded in a large fraction of the cells in rat [39, 40], but not in bovine [36], guinea pig [45], and gerbil [18]. Since the regional segregation of these receptors and ion channels are not discriminated in these nonpolarized cells, the recep- toconductile-secretory regions seem to be intermingled with one another to form a mosaic constituent of the plasma membrane (Fig. 1, A and NA). The SGC cell, however, has a certain degree of morphological differentiation, although its recep- toconductile-secretory function remains almost unknown. Assuming that the SGC cell may be intermediate in function between the neuron and the adrenal A and NA cells, we re-present a schema that the ACh and other secretagogues may evoke slow depolarization and action potential at the recep- toconductile region and, in turn, [Ca\textsuperscript{2+}], dynamics at the secretory region (Fig. 1, SGC). The recep- toconductile-secretory function is shared by specific proteins distributed at proper regions. A sizeable advance has been made in the molecular machinery underlying protein sorting and transport within the endoplasmic reticulum and the Golgi apparatus, and in the mechanism of targeted membrane fusion by the constitutive secretion [71–73]. The principles employed for most intracellular transport and sorting events may be common in various paraneurons.

Endocrine cells in the pancreatic islets are of gut endodermal origin, and they synthesize and store pep- tidergic hormones. The islet B cell bears the basic
characteristic in the receptor-conductile-secretory process in common with the neuroectodermal paraneurons: ACh and CCK-8 activate Gq-coupled receptors and [Ca\textsuperscript{2+}]-dependent exocytosis. However, the B cell also preserves characteristics of endodermal origin: The major secretagogue, glucose, enters B cells via the GLUT 2, generates ATP, and closes the ATP-sensitive K\textsuperscript{+} channel. The glucose-induced signal transduction is further modified by the other signaling systems in common with the other paraneurons. An additional characteristic of endodermal origin is preserved in the intercellular communication via gap junctions, which provide the ways for propagation of Ca\textsuperscript{2+} and electricity from a B cell to adjacent B cells. The propagation results in synchronization in oscillatory [Ca\textsuperscript{2+}]\textsubscript{i} dynamics and in [Ca\textsuperscript{2+}]\textsubscript{c} dependent exocytosis in the B cell society.

REFERENCES

26. Li YX, Keizer J, Stojilkovic SS, and Rinzler J: Ca\textsuperscript{2+} excitability of the ER membrane: an explanation for IP\textsubscript{3}-induced Ca\textsuperscript{2+} oscillations. Am J Physiol 269: C1079–C1092, 1995


59. Habara Y and Kanno T: Dose-dependency in spatial
Ca^{2+} Signaling in Paraneurons

68. Pearse AGE: Common cytochemical and ultrastructural characteristics of cells producing polypeptide hormones (APUD series) and their relevance to thyroid and ultimobranchial C cells and calcitonin. Proc Soc (Biol) 170: 71–80, 1968