Pancreatic β-cells communicate via intermittent release of ATP

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Hellman, Bo, Helène Dansk, and Eva Grapengiesser. Pancreatic β-cells communicate via intermittent release of ATP. *Am J Physiol Endocrinol Metab* 286: E759–E765, 2004. First published January 13, 2004; 10.1152/ajpendo.00452.2003.—The role of external ATP for intercellular communication was studied in glucose-stimulated pancreatic β-cells isolated from ob/ob mice. Digital image analyses with fura-2 revealed spontaneous transients of cytoplasmic Ca²⁺ appearing in synchrony in the absence of cell contacts. After removal of slow oscillations with methoxysterapamil, addition of ATP (0.1–100 μM) resulted in prompt firing of a transient, followed by suppression of the generation and synchronization of spontaneously occurring transients. It was possible to trigger transients during the suppressive phase by raising the concentration of ATP. The dual action of ATP was mimicked by ADP or 2-methylthio-ATP but not by AMP or UTP. The number of spontaneous transients and their synchronization were reduced in the presence of the dephosphorylating agent apyrase. Additional evidence that intermittent release of ATP participates in the generation of spontaneous Ca²⁺ transients was obtained from the suppression observed from use of antagonists of the purinoceptors [suramin (0.3–30 μM), pyridoxalphosphate-6-azophenyl-2,4-disulfonic acid (PPADS; 10–30 μM) and 2-deoxy-N-methyladenosine (MRS 2179; 0.3–30 μM)] or from counteracting β-cell release of ATP by inhibiting exocytosis with 100 nM epinephrine, 100 nM somatostatin, or lowering the temperature below 30°C. The data indicate that ATP has time-dependent actions (prompt stimulation followed by inhibition) on the generation of Ca²⁺ transients mediated by P2Y receptors. It is proposed that β-cells both receive a neural ATP signal with coordinating effects on their Ca²⁺ oscillations and propagate this message to adjacent cells via intermittent release of ATP combined with gap junction coupling.

**GLUCOSE STIMULATION OF INSULIN RELEASE** is mediated by increase of the cytoplasmic Ca²⁺ concentration ([Ca²⁺]i) in pancreatic β-cells. This increase is usually manifested as slow oscillations (0.1–0.5 min⁻¹) from the basal level, due to periodic entry of Ca²⁺ through voltage-dependent channels (26). Besides generating such oscillations, rise of the glucose concentration promotes spontaneous transients elicited by d-myoinositol 1,4,5-trisphosphate (IP₃)-induced mobilization of Ca²⁺ from the endoplasmic reticulum (17, 34).

The purine nucleotide ATP is known to act as an extracellular messenger, generating its effects via a family of distinct cell surface receptors (7, 45). It was early established that external ATP stimulates the release of insulin from pieces of rabbit pancreas (9) and the efflux of radioactivity from isolated mouse islets preloaded with ⁴²Ca²⁺ (20). Subsequent studies indicated that ATP mobilization of intracellular Ca²⁺ stores is coupled to a rapid breakdown of phosphatidylinositol 4,5-bisphosphate (27). It was found by measuring [Ca²⁺], that activation of purinoceptors elicits distinct transients in insulin-secreting RINm5F cells (10), as well as in mouse β-cells (4). If sufficiently pronounced, a transient increase of [Ca²⁺], is known to induce a temporary interruption of the Ca²⁺ entry into glucose-stimulated β-cells by activating a repolarizing K⁺ current (12). This effect, together with the observation that the transients often appear in synchrony in the absence of cell contacts, has resulted in the proposal that transients entrain the [Ca²⁺], oscillations and, consequently, pulses of insulin release into a rhythm common for the islets in the pancreas (16, 17, 25, 35). We have recently been able to demonstrate that an increase in the number of synchronized transients has a coordinating effect on the [Ca²⁺], oscillations (15).

It was suggested from insulin release studies with the isolated perfused rat pancreas that the islets communicate via nonadrenergic, noncholinergic (NANC) neurons (44). Evidence has been provided that both nitric oxide (16, 25) and carbon monoxide (35) elicit transients of [Ca²⁺], often occurring in synchrony in β-cells lacking physical contact. Another candidate for a neurotransmitter that generates transients with a coordinating effect on the [Ca²⁺], oscillations is ATP. This nucleotide has been reported to propagate mechanically induced [Ca²⁺], rises in confluent monolayers of rat insulinoma cells (10) and normal mouse β-cells (4). Previous studies in our laboratory have shown that the purinoceptor antagonist suramin has a suppressive action on spontaneous [Ca²⁺], transients in β-cells from ob/ob mice (17). However, there are also reports that dephosphorylation of external ATP with apyrase fails to affect the propagation of glucose-induced rises of [Ca²⁺], in rat β-cells (4).

The purpose of the present study was to examine whether external ATP acts as a diffusible messenger generating the [Ca²⁺], transients supposed to coordinate the rhythmicity of the β-cells within and among the islets in the pancreas. In support for this idea, we now demonstrate that β-cells not only are recipients of an ATP signal inducing [Ca²⁺], transients but can also propagate this message to neighboring cells via intermittent release of ATP.

**MATERIALS AND METHODS**

**Chemicals.** Reagents of analytical grade and deionized water were used. ATP (ultrgrade), ADP, UTP, apyrase, carbachol, suramin, epinephrine, somatostatin, glucagon, 8-bromoadenosine 3',5'-cyclic monophosphate (8-BrcAMP), and methoxysterapamil were obtained from Sigma Chemical (St. Louis, MO). Tocris Cookson (Bristol, UK) was the supplier of 2-methylthio-ATP (2MeSATP), 2-deoxy-N-methyladenosine-3,5-bisphosphate (MRS 2179), and pyridoxalphosphate-6-azophenyl-2,4-disulfonic acid (PPADS). Boehringer Mannheim (Mannheim, Germany) was the origin of collagenase, HEPES, and bovine serum albumin. The acetoxyethyl ester of fura-2 ([fura-2

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rapidly induced a transient, and MRS 2179 removed this effect (B).

**Table 1. Percentage of cells generating \([Ca^{2+}]_i\) transients before and after addition of various nucleotides**

<table>
<thead>
<tr>
<th>Test Substance Glucagon</th>
<th>Control period</th>
<th>Test period</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATP (10 nM)</td>
<td>–</td>
<td>5 ± 2</td>
<td>14 ± 9</td>
</tr>
<tr>
<td>ATP (100 nM)</td>
<td>–</td>
<td>2 ± 1</td>
<td>87 ± 5</td>
</tr>
<tr>
<td>ATP (1 μM)</td>
<td>–</td>
<td>2 ± 1</td>
<td>98 ± 2</td>
</tr>
<tr>
<td>ADP (10 nM)</td>
<td>–</td>
<td>3 ± 2</td>
<td>5 ± 3</td>
</tr>
<tr>
<td>ADP (100 nM)</td>
<td>–</td>
<td>3 ± 2</td>
<td>78 ± 5</td>
</tr>
<tr>
<td>UTP (1 μM)</td>
<td>–</td>
<td>2 ± 2</td>
<td>2 ± 2</td>
</tr>
<tr>
<td>UTP (10 μM)</td>
<td>–</td>
<td>2 ± 2</td>
<td>5 ± 3</td>
</tr>
<tr>
<td>ATP (10 nM)</td>
<td>+</td>
<td>24 ± 8</td>
<td>29 ± 6</td>
</tr>
<tr>
<td>ATP (100 nM)</td>
<td>+</td>
<td>26 ± 3</td>
<td>64 ± 6</td>
</tr>
<tr>
<td>AMP (1 μM)</td>
<td>+</td>
<td>38 ± 6</td>
<td>31 ± 8</td>
</tr>
<tr>
<td>UTP (1 μM)</td>
<td>+</td>
<td>28 ± 5</td>
<td>31 ± 5</td>
</tr>
<tr>
<td>UTP (10 μM)</td>
<td>+</td>
<td>27 ± 2</td>
<td>28 ± 5</td>
</tr>
<tr>
<td>2MeSATP (10 nM)</td>
<td>+</td>
<td>28 ± 9</td>
<td>42 ± 6</td>
</tr>
</tbody>
</table>

Superfusion for 10–12 min with a medium containing 20 mM glucose and 50 μM methoxyverapamil was followed by exposure to various nucleotides in the absence and presence of 20 nM glucagon. 2MeSATP, 2-methyl-thio-ATP. Data refer to observations made during 60 s before (control period) and after (test period) addition of substances. Values are means ± SE for nos. of experiments within parentheses. *P < 0.01; †P < 0.001.

Institutes of Health, Guide for the Care and Use of Laboratory Animals. Adult ob/ob mice were taken from a noninbred colony (24) and killed by decapitation. Islets were isolated with the aid of collagenase from the splenic part of the pancreas. These islets contain >90% β-cells, which have a normal secretory response to glucose (21). Single cells and small aggregates were prepared by shaking the islets in a Ca\( ^{2+} \)-deficient medium. After suspension in RPMI 1640 medium supplemented with 10% fetal calf serum, 100 IU/ml penicillin, 100 μg/ml streptomycin, and 30 μg/ml gentamicin, the cells were allowed to attach to the central part of circular coverslips during 2–5

Fig. 1. Measurements of cytoplasmic Ca\( ^{2+} \) concentration ([Ca\( ^{2+} \)]\(_i\)) in 2 β-cells (separated by a distance of 34 μm) oscillating in response to 20 mM glucose during superfusion with a glucagon-containing medium (20 nM) lacking methoxyverapamil. The [Ca\( ^{2+} \)]\(_i\) oscillations have superimposed transients, showing a high degree of synchronization (time difference between corresponding peaks <2 s). Introduction of 0.3 mM ATP into the medium induced a premature oscillation, followed by disappearance of transients with preservation of ordinary oscillatory activity. Similar effects of ATP were seen in 5 experiments.

AM) and thapsigargin were purchased from Molecular Probes (Eugene, OR).

**Preparation of β-cells.** The experiments were approved by a local animal welfare committee and conducted according to the National Institutes of Health, Guide for the Care and Use of Laboratory Animals. Adult ob/ob mice were taken from a noninbred colony (24) and killed by decapitation. Islets were isolated with the aid of collagenase from the splenic part of the pancreas. These islets contain >90% β-cells, which have a normal secretory response to glucose (21). Single cells and small aggregates were prepared by shaking the islets in a Ca\( ^{2+} \)-deficient medium. After suspension in RPMI 1640 medium supplemented with 10% fetal calf serum, 100 IU/ml penicillin, 100 μg/ml streptomycin, and 30 μg/ml gentamicin, the cells were allowed to attach to the central part of circular coverslips during 2–5

Fig. 2. Effects of nucleotides on the generation of [Ca\( ^{2+} \)]\(_i\) transients during superfusion with a medium containing 20 mM glucose and 50 μM methoxyverapamil but lacking glucagon. Addition of ATP (A and B) and ADP (C) but not UTP (D) rapidly induced a transient, and MRS 2179 removed this effect (B). Results are representative of 4–5 experiments.

Fig. 3. Effects of nucleotides on the generation of [Ca\( ^{2+} \)]\(_i\) transients during superfusion with a medium containing 20 mM glucose, 20 mM glucagon, and 50 μM methoxyverapamil. Prolonged exposure to ATP (A and B) and 2MeSATP (C) but not to UTP (D) had a suppressive action on spontaneous transients. However, it was still possible to induce a transient by raising the concentration of the nucleotide. Results are representative of 3–7 experiments.
The identification of the Fc in an atmosphere of 5% CO₂ in humidified days of culture at 37°C of a climate box maintained at 37°C. For studying the effects of were performed with an inverted microscope (Nikon Diaphot) by use media of similar composition did not affect the results. The studies ed that switching between fi turbance of slow oscillations. It was veri/

/HEPES, with pH adjusted to 7.40 with NaOH. After rinsing, the cells

C pump. The cells were superfused with a medium containing 20 mM

incubation in the presence of 3 mM glucose. The coverslips with the

cells generating transients within 60 s after addition of ATP

Superfusion for 10–12 min with a medium containing 20 mM glucose, 20 mM glucose, and 50 μM methoxyverapamil (control period) was followed by exposure to various nucleotides. A test period of 10 min starts 60 s after addition of nucleotides. Values are means ± SE for nos. of experiments within parentheses. *P < 0.005; †P < 0.001.

Table 2. Inhibitory effects of various nucleotides on generation of \( [Ca^{2+}]_i \), transients

<table>
<thead>
<tr>
<th>Test Substance</th>
<th>Glucagon</th>
<th>Control period</th>
<th>Test period</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATP (10 nM)</td>
<td>+</td>
<td>2.44 ± 0.63</td>
<td>2.09 ± 0.51</td>
<td>−0.35 ± 0.22 (9)</td>
</tr>
<tr>
<td>ATP (100 nM)</td>
<td>+</td>
<td>2.38 ± 0.34</td>
<td>0.56 ± 0.14</td>
<td>−1.81 ± 0.29 (8)†</td>
</tr>
<tr>
<td>2MeSATP (10 nM)</td>
<td>+</td>
<td>1.91 ± 0.43</td>
<td>0.27 ± 0.12</td>
<td>−1.64 ± 0.34 (7)*</td>
</tr>
<tr>
<td>UTP (1 μM)</td>
<td>+</td>
<td>2.11 ± 0.64</td>
<td>2.23 ± 0.34</td>
<td>0.12 ± 0.31 (5)</td>
</tr>
<tr>
<td>UTP (10 μM)</td>
<td>+</td>
<td>2.13 ± 0.48</td>
<td>1.75 ± 0.26</td>
<td>−0.38 ± 0.42 (7)</td>
</tr>
</tbody>
</table>

Superfusion for 10–12 min with a medium containing 20 mM glucose, 20 mM glucose, and 50 μM methoxyverapamil (control period) was followed by exposure during a similar period to various test substances. PPADS, pyridoxal-phosphate-6-azophenyl-2,4-disulfonic acid; MRS 2179, 2-deoxy-N-methyl-adenosine-3,5-bisphosphate. Values are means ± SE for nos. of experiments within parentheses. *P < 0.025; †P < 0.005; ‡P < 0.001.

Table 3. Effects of purinergic antagonists, apyrase, epinephrine, and somatostatin on generation of \( [Ca^{2+}]_i \), transients

<table>
<thead>
<tr>
<th>Test Substance</th>
<th>Glucagon</th>
<th>Control period</th>
<th>Test period</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Suramin (25 μM)</td>
<td>+</td>
<td>3.62 ± 0.68</td>
<td>2.01 ± 0.49</td>
<td>−1.60 ± 0.36 (5)*</td>
</tr>
<tr>
<td>PPADS (10 μM)</td>
<td>+</td>
<td>2.09 ± 0.58</td>
<td>0.49 ± 0.09</td>
<td>−1.61 ± 0.51 (6)*</td>
</tr>
<tr>
<td>MRS 2179 (0.3 μM)</td>
<td>+</td>
<td>2.60 ± 0.46</td>
<td>1.32 ± 0.17</td>
<td>−1.28 ± 0.34 (5)*</td>
</tr>
<tr>
<td>MRS 2179 (1 μM)</td>
<td>+</td>
<td>2.72 ± 0.59</td>
<td>0.81 ± 0.24</td>
<td>−1.91 ± 0.38 (7)†</td>
</tr>
<tr>
<td>MRS 2179 (30 μM)</td>
<td>+</td>
<td>2.52 ± 0.61</td>
<td>0.11 ± 0.05</td>
<td>−2.41 ± 0.66 (5)*</td>
</tr>
<tr>
<td>Apyrase, grade 3 (2 U/ml)</td>
<td>+</td>
<td>3.74 ± 0.28</td>
<td>2.25 ± 0.29</td>
<td>−1.49 ± 0.34 (7)†</td>
</tr>
<tr>
<td>Apyrase, grade 5 (2 U/ml)</td>
<td>+</td>
<td>3.30 ± 0.20</td>
<td>1.11 ± 0.11</td>
<td>−2.19 ± 0.22 (4)‡</td>
</tr>
<tr>
<td>Epinephrine (100 nM)</td>
<td>+</td>
<td>2.13 ± 0.42</td>
<td>0.20 ± 0.11</td>
<td>−1.93 ± 0.38 (6)*</td>
</tr>
<tr>
<td>Somatostatin (100 nM)</td>
<td>+</td>
<td>2.43 ± 0.30</td>
<td>0.31 ± 0.21</td>
<td>−2.13 ± 0.31 (6)†</td>
</tr>
</tbody>
</table>

Results

Spontaneous appearance of transients. Glucose stimulation of β-cells resulted in oscillations of \( [Ca^{2+}]_i \), sometimes superimposed by short-lived transients. These transients were more frequent in the presence of glucagon and were often well synchronized in adjacent β-cells, lacking contact (Fig. 1). When the voltage-dependent Ca²⁺ entry was blocked with 50 μM methoxyverapamil, the oscillations disappeared, and the transients were generated from the basal level at irregular intervals (see Figs. 2–7).

Prompt generation of transients after addition of ATP. When added to β-cells oscillating in response to glucose, ATP induced a premature \( [Ca^{2+}]_i \) oscillation, sometimes superimposed by transients (Fig. 1). After methoxyverapamil suppression of the Ca²⁺ entry, the early effect of ATP (100 nM or more) was restricted to \( [Ca^{2+}]_i \) transient, similar to transients occurring spontaneously (Fig. 2, A and B). The percentage of cells generating transients within 60 s after addition of ATP

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and other nucleotides to methoxyverapamil-containing media, supplemented or not with glucagon, is shown in Table 1. ADP mimicked ATP in rapidly inducing transients (Fig. 2C), whereas UTP lacked effect (Fig. 2D). In the studies of the mechanisms for the ATP induction of [Ca^{2+}]_i transients, we found that the effect disappeared in the presence of the purinoceptor antagonist MRS 2179 (Fig. 2B).

Suppression of spontaneous transients with ATP and purinoceptor antagonists. When ATP was added at a concentration of 100 nM or more, the initial generation of a transient was followed by disappearance of the spontaneously occurring transients (Fig. 3, A and B). Table 2 summarizes how various nucleotides affect the generation of [Ca^{2+}]_i transients during a 10-min period, starting 60 s after their introduction into the superfusion medium. The analog 2MeSATP was a more effective inhibitor than ATP, suppressing most of the transients at a concentration of 10 nM (Fig. 3C). There was no inhibition in the presence of 1 or 10 μM UTP (Fig. 3D). During ATP suppression of the spontaneous [Ca^{2+}]_i transients, it was possible to trigger a [Ca^{2+}]_i transient by a 100-fold increase of the nucleotide concentration (Fig. 3, A and B). Loss of transients during prolonged exposure to ATP was accompanied by a decreased synchronization. When we studied how transients appearing after 60 s were affected by 100 nM ATP in cells separated by a distance <30 μm, it was found that the synchronization index decreased from 0.53 ± 0.08 to 0.11 ± 0.07 (P < 0.001; n = 19).

The effects of different purinoceptor antagonists on the β-cell firing of spontaneous [Ca^{2+}]_i transients are presented in Table 3. Reduced firing of transients was seen after addition of suramin (not shown), MRS 2179 (Fig. 4, A and B), and PPADS (Fig. 4C). The transients were particularly sensitive to the receptor antagonist MRS 2179, with an IC_{50} of ~0.3 μM. Exposure to MRS 2179 and PPADS did not prevent generation of transients mediated by carbachol stimulation of muscarinic receptors (Fig. 4, B and C). After omission of 1 μM MRS 2179, there was a reappearance of spontaneous [Ca^{2+}]_i transients and also of those obtained in response to addition of 1 μM ATP (Fig. 4A).

Suppression of spontaneous transients with apyrase. Addition of apyrase (2 U/ml) for dephosphorylating external ATP (Fig. 4). The effects of different purinoceptor antagonists on the generation of [Ca^{2+}]_i transients during superfusion with a medium containing 20 mM glucose, 20 nM glucagon, and 50 μM methoxyverapamil. Both MRS 2179 (MRS; A and B) and PPADS (C) had a suppressive action under conditions when the response to 10 μM carbachol was unaffected. Results are representative of 3–7 experiments.

Fig. 4. Effects of purinoceptor antagonists on the generation of [Ca^{2+}]_i transients during superfusion with a medium containing 20 mM glucose, 20 nM glucagon, and 50 μM methoxyverapamil. Both MRS 2179 (MRS; A and B) and PPADS (C) had a suppressive action under conditions when the response to 10 μM carbachol was unaffected. Results are representative of 3–7 experiments.

Fig. 5. Suppressive action of 2 U/ml apyrase on the generation of [Ca^{2+}]_i transients during superfusion with a medium containing 20 mM glucose, 20 nM glucagon, and 50 μM methoxyverapamil. Arrows, transients appearing in synchrony in cells separated by a distance of 10 μm. Results are representative of 5 experiments.

Fig. 6. Suppression of spontaneously occurring [Ca^{2+}]_i transients after inhibiting exocytosis with epinephrine (A) or somatostatin (B) during superfusion with a medium containing 20 mM glucose, 20 nM glucagon, and 50 μM methoxyverapamil. The β-cells maintained ability to respond to 1 μM ATP after disappearance of transients. Results are representative of 6 experiments.
and ADP had a suppressive action on the generation of spontaneous [Ca\(^{2+}\)]\(_i\) transients (Fig. 5). With the most effective apyrase preparation, >60% of the transients disappeared (Table 3). When the synchronization of transients in adjacent cells (<30 μm apart) was studied before and during the exposure to apyrase, the synchronization index was found to decrease from 0.55 ± 0.06 to 0.15 ± 0.06 (P < 0.001; n = 21).

**Suppression of spontaneous transients after inhibition of exocytosis.** We tested how procedures known to inhibit exocytosis by cooling during superfusion with a medium containing 20 mM glucose, 20 nM glucagon, and 50 μM methoxyverapamil. β-Cells maintained ability to respond to 1 μM ATP at a temperature of 24°C. Results are representative of 6 experiments.

Measurements of the unidirectional efflux of \(^{45}\text{Ca}^{2+}\) in a Ca\(^{2+}\)-deficient medium provide a sensitive means for studying the mobilization of intracellular Ca\(^{2+}\) stores in pancreatic β-cells. With this approach, it was early observed that external ATP, like activation of muscarinic receptors, promptly induces a peak of radioactive efflux from mouse islets conditioned by previous exposure to 20 mM glucose (20). Subsequent studies with insulin-secreting RINm5F cells (1, 5, 33) and isolated rat islets (5) indicated that external ATP stimulates the generation of IP\(_3\), an effect explaining how ATP can mobilize Ca\(^{2+}\) from thapsigargin-sensitive stores with resulting increase of [Ca\(^{2+}\)]\(_i\) (37, 43). We now observe that the prompt [Ca\(^{2+}\)]\(_i\) transient triggered by addition of ATP resembles transients occurring spontaneously in glucose-stimulated β-cells. Irrespective of whether glucagon was added or not to counteract the depletion of cAMP known to occur in isolated β-cells (40), the transients were induced with a concentration of ATP as low as 100 nM. This concentration compares favorably with that (200 nM) which stimulates the efflux of \(^{45}\text{Ca}^{2+}\) from islets of ob/ob mice superfused with a Ca\(^{2+}\)-deficient medium (20).

Studies of insulin release have indicated that the pancreatic β-cells are equipped with both P1 (28) and P2 (29) purinoceptors. Whereas external ATP stimulates the secretory activity in the rat (29) and human (13), it has been reported to inhibit insulin release in mice (37). The species differences in the ATP action may at least in part reflect the types of purinoceptors involved. With immunohistochemistry, it was reported that mouse β-cells express both G protein-coupled P2Y receptors and P2X ligand-gated ion channel receptors (11). Comparing the early responses to various purinoceptor agonists, we now demonstrate that ADP is as effective as ATP in promptly triggering transients and that UTP lacks such an effect. The rank order of agonist stimulation, in combination with the observation that the effect was markedly suppressed by the purinoceptor antagonist MRS 2179 (6), indicates a role for P2Y receptors in the generation of [Ca\(^{2+}\)]\(_i\) transients.

The stimulatory action of ATP was usually restricted to prompt firing of a single transient. Subsequent exposure to extracellular ATP (0.1–100 μM) resulted in suppression of the spontaneously occurring [Ca\(^{2+}\)]\(_i\) transients. This suppression may reflect desensitization of the purinoceptors, implying that most of the spontaneous transients are mediated by ATP/ADP.

There are several arguments that the spontaneously occurring

![Fig. 7. Suppression of spontaneously occurring [Ca\(^{2+}\)]\(_i\) transients after inhibiting exocytosis by cooling during superfusion with a medium containing 20 mM glucose, 20 nM glucagon, and 50 μM methoxyverapamil. β-Cells maintained ability to respond to 1 μM ATP at a temperature of 24°C. Results are representative of 6 experiments.](http://ajpendo.physiology.org/)

**Fig. 8. Model for ATP induction of synchronized [Ca\(^{2+}\)]\(_i\) transients with coordinating effects on β-cell rhythmicity. For details see final paragraph of text.**
transients are generated by activation of purinoceptors. 1) The nucleotide specificity for the inhibitory effect is similar to that observed for triggering the initial transient. 2) Purinoceptor antagonists have a suppressive action on spontaneous transients. The most pronounced inhibition was seen with MRS 2179, an antagonist known to interact essentially with P2Y1 receptors (6). 3) A majority of the spontaneous transients disappear in the presence of the dephosphorylating agent apyrase. 4) Transients remaining after addition of apyrase or prolonged exposure to ATP are no longer synchronized.

Pancreatic β-cells communicate via both gap junctions (8) and diffusible messengers (4, 15–17, 25, 35, 42). When evaluating how external ATP affects the function of the β-cells, it should be kept in mind that the nucleotide is a component of the secretory granules (31, 32). Impaired increases of [Ca²⁺], mimicking the transients elicited by ATP, have sufficient amplitude and duration to induce exocytosis (46) and consequently release of ATP. It is plausible that a message conveyed from a specific focus, like a NANC neuron, is received by some β-cells in an islet and then propagates to adjacent cells via short-lived activation of exocytosis.

In evaluating the role of exocytosis for propagating [Ca²⁺] transients, we tested whether the inhibition of insulin release seen in the presence of epinephrine or somatostatin or lowering the temperature below 30°C had any effect. In each alternative, most of the spontaneously occurring transients disappeared with maintenance of a β-cell response to the addition of ATP. Although various mechanisms, including impairment of cAMP generation, may contribute to the inhibition of insulin release evoked by epinephrine and somatostatin (14), the predominant effect is probably activation of the protein phosphatase calcineurin (37, 38). Epinephrine and somatostatin also removed most of the [Ca²⁺] transients when the experiments were performed in the presence of a permeable analog of cAMP. Previous studies have shown that insulin release is a process with high temperature sensitivity (2, 39). When cooling was used for inhibition of exocytosis, there was a similar suppression of the [Ca²⁺] transients with maintenance of the response to ATP as seen in the presence of epinephrine and somatostatin. Taken together, three established procedures for inhibition of exocytosis promote the disappearance of spontaneous [Ca²⁺] transients, as expected if the secretory granules are a major source for ATP with coordinating effects on the β-cell activity.

The observation that sustained exposure to ATP triggers an initial transient, followed by suppression of the spontaneously occurring ones, directs the attention to the kinetics of its release. Like neural discharge, the release of ATP from the β-cells can be expected to be discontinuous. Studies of single rat β-cells have indicated brief events of secretory activity (30, 36) with fluctuations in the micromolar range of the ATP located at the cell surface (23). Intermittent release may be important both for preventing ATP suppression of [Ca²⁺] transients and for countering the inhibition of insulin release seen when primary mouse β-cells (37) and pseudosets of mouse insulinoma cells (22) are exposed to the nucleotide. We now demonstrate that transients of [Ca²⁺], can also be elicited during the suppressive phase by suddenly raising the concentration of the nucleotide. Accordingly, the suppression obtained during sustained exposure to low concentrations of ATP may facilitate the identification of an ATP message by removing the background noise. When discussing how intermittent release of ATP affects the [Ca²⁺] oscillations, attention should be paid to the autocrine feedback effects of the nucleotide.

The present observations suggest that external ATP is important for the concerted appearance of the [Ca²⁺], transients supposed to entrain the oscillatory activity of the β-cells (15). A model for how ATP coordinates the [Ca²⁺] oscillations required for pulsatile release of insulin from the pancreas is presented in Fig. 8. ATP, released alone or together with other neurotransmitters, generates synchronized [Ca²⁺] transients, which entrain the rhythmicity of the β-cells by interrupting their electrical activity. Another effect of the transients is to activate exocytosis, a process resulting in intermittent release of ATP. The β-cells can therefore both receive an ATP message with coordinating effects on the secretory activity and propagate this message to other cells within an islet.

**REFERENCES**


