Proc. Natl. Acad. Sci. USA
Vol. 96, pp. 329–331, January 1999

Commentary

Protein kinase A takes center stage in ATP-dependent insulin secretion

*Thomas A. Blanpied and George J. Augustine**

Department of Neurobiology, Duke Medical Center, Box 3209, Durham, NC 27710

Beta cells of the pancreas are critical players in regulating the body's energy supply. Rising blood glucose levels cause these cells to secrete insulin, a hormone that directs the metabolic consumption of glucose by many different tissues. It is clear that glucose triggers insulin secretion by being metabolized to ATP within the beta cell, with the resultant increase in intracellular ATP causing insulin release. Precisely how ATP controls insulin secretion is an important question that remains unresolved. Insulin is stored in secretory granules and is released into the bloodstream when these granules fuse with the beta cell plasma membrane. As is the case for most types of regulated exocytosis, the primary trigger for insulin granule fusion is a rise in intracellular Ca^{2+} concentration, $[Ca^{2+}]_i$. This calcium enters the cell through voltage-dependent Ca^{2+} channels when glucose depolarizes the beta cell membrane potential, because beta cells possess a K^+ channel that is blocked by ATP (1, 2), and so it has long been thought that ATP promotes insulin secretion by regulating Ca^{2+} entry. Thus, when glucose is metabolized to create ATP, the ATP-sensitive K^+ channels are blocked, the membrane potential depolarizes, voltage-dependent Ca^{2+} channels open, and Ca^{2+} flows into the beta cell to trigger granule fusion. Although this explanation of ATP-dependent insulin secretion has been widely accepted (3), there may be more to this story because a number of experiments provide hints that ATP may cause insulin release independent of its ability to alter $[Ca^{2+}]_i$. For example, glucose can augment Ca^{2+}-induced insulin secretion even when drugs are used to hold ATP-sensitive K^+ channels open (4) or closed (5). Thus, the specific mechanism(s) underlying the actions of glucose and ATP in triggering insulin release from beta cells are not yet clear.

In this issue of the *Proceedings*, Takahashi *et al.* (6) provide clear evidence that ATP acts independently of its effects on $[Ca^{2+}]_i$, and they provide a provocative new suggestion for how it does so. They accomplished this by using a combination of powerful techniques to study secretion from single beta cells. Perhaps most importantly, they used amperometry to measure secretion resulting from the fusion of individual secretory granules rather than relying on more conventional patch-clamp measurements of plasma membrane capacitance. Although the capacitance technique offers outstanding time resolution and sensitivity, it has two fundamental problems. First, because cell capacitance is proportional to the total area of the plasma membrane, such measurements cannot easily distinguish exocytosis and endocytosis that overlap in time. This is an important concern, because beta cells evince a robust endocytosis that is nearly as fast as their exocytosis (7, 8). Second, although beta cells have both small vesicles and large granules that undergo Ca^{2+}-stimulated exocytosis (9, 8), capacitance measurements generally cannot distinguish between the fusion of these different types of vesicles. In fact, secretion of insulin granules may contribute proportionally less to the prominent early phases of secretion that are detected with capacitance measurements (8). Another technical development was to use a new caged calcium compound, dimethoxynitrophenyl-EGTA-4 (DMNPE-4), to trigger Ca^{2+}-dependent secretion from the beta cells. DMNPE-4 has Ca^{2+}-releasing properties that are optimal for use in single-cell studies of secretion (10). Photolysis of DMNPE-4 with UV light produces a uniform and nearly instantaneous increase in $[Ca^{2+}]_i$ throughout the cell, which avoids the more complex spatial and temporal pattern of $[Ca^{2+}]_i$ elevation associated with Ca^{2+} entry through channels.

Rapid elevation of $[Ca^{2+}]_i$ via photolysis of DMNPE4 evoked the fusion of insulin granules in two kinetic phases. These phases lasted about 3 and 30 seconds and were termed "mode 1" and "mode 2" exocytosis. By using diffusion from the recording pipette to manipulate the intracellular environment of the beta cell, Takahashi *et al.* (6) then could examine the influence of ATP on these two components of exocytosis. Their key observation is that raising the intracellular ATP concentration from 0.1 mM to 3 mM greatly potentiated the rapid, mode 1 exocytosis. Because the $[Ca^{2+}]_i$ was controlled by DMNPE-4, we know that this augmentation by ATP is independent of its action on K^+ channels or on Ca^{2+} entry. The results of this new approach therefore confirm notions of a role for ATP that is independent of its effects on $[Ca^{2+}]_i$. Interestingly, the second kinetic phase, mode 2, was not influenced by intracellular ATP.

Takahashi *et al.* then explored this fundamental observation to determine how ATP acts. The major surprise is that ATP increases secretion through phosphorylation rather than through other reactions that require ATP hydrolysis. This point was addressed by comparing the actions of two analogues of ATP, 5′-adenylyl-β,γ-imidodiphosphate (AMP-PNP) and adenosine 5′-*O*-(3-thiotriphosphate) (ATP[γS]). Although neither analogue can be hydrolyzed by ATPases, AMP-PNP cannot support phosphorylation whereas ATP[γS] can. Inside beta cells, these two analogs differed dramatically in their ability to support secretion. AMP-PNP produced none of the potentiating effect of ATP, whereas ATP[γS] not only substituted for ATP but potentiated release even more strongly than ATP. That ATP acts via phosphorylation was confirmed by showing that the effects of ATP and ATP[γS] depended on intracellular Mg^{2+} but were not inhibited by ADP, typical of phosphorylation reactions.

These results are surprising because of recent suggestions that ATP may act in beta cells by "priming" granules for release (7). Priming refers to the ATP-dependent reactions that granules apparently undergo to become competent for Ca^{2+}-stimulated fusion; the thought is that vesicle fusion requires an early ATP-dependent priming step that precedes the final, Ca^{2+}-regulated triggering step (11–13). In adrenal

Abbreviations: DMNPE-4, dimethoxynitrophenyl-EGTA-4; AMP-PNP, 5′-adenylyl-β,γ-imidodiphosphate; ATP[γS], 5′-*O*-(3-thiotriphosphate); NSF, *N*-methylmaleimide-sensitive fusion protein; PKA, protein kinase A.

The companion to this Commentary begins on page 760.

*To whom reprint requests should be addressed. e-mail: georgea@neuro.duke.edu.

PNAS is available online at www.pnas.org.

chromaffin cells, where priming was discovered (14), priming is partially caused by the *N*-methylmaleimide-sensitive fusion protein, NSF (15), an ATPase that is involved in a wide spectrum of membrane fusion reactions (16). However, the ATPase activity of NSF is blocked by ATP[γS] (17), whereas beta cell secretion is stimulated by this nucleotide. Thus, reactions very close to the fusion reaction involve ATP but do not require its hydrolysis, meaning that NSF and other ATPases do not mediate these effects of ATP in beta cells. Given the ubiquity of NSF, this protein still may have other actions in regulating Ca^{2+}-dependent secretion by ATP. However, such reactions must take place on a time scale slower than that observed by Takahashi *et al.* (6), about 2 min. This is consistent with previous observations that the slower phases of release from chromaffin cells require hydrolysis of ATP (12). It is worth noting that ATP[γS] would be expected to promote lipid phosphorylation, which also appears to be important for priming (18).

These initial ATP-dependent priming and later ATP-dependent phosphorylation reactions are likely to show quite different dynamic characteristics. The phosphorylation described by Takahashi *et al.* (6) is probably rapid and easily reversible by endogenous phosphatases, as indicated by the stimulatory effect of ATP[γS] and because removing ATP or substituting with AMP-PNP reduced release. From their rough determinations of the dependence of phosphorylation on ATP concentration, Takahashi *et al.* (6) estimate that physiological fluctuations in ATP levels may serve to regulate the extent of phosphorylation. On the other hand, granule priming by ATP hydrolysis is expected to be slower than phosphorylation and more slowly reversible and therefore should be less sensitive to rapid changes in ATP levels in the cell.

A second important point made by Takahashi *et al.* (6) is that the cAMP-dependent protein kinase A (PKA) mediates the phosphorylation reaction required for secretion in beta cells. An extensive set of experiments provides unambiguous evidence that PKA is important for the action of ATP. First, three antagonists of PKA blocked the effect of ATP. Furthermore, blocking adenylate cyclase—the enzyme that produces cAMP—blocked the action of ATP, and this was overcome by addition of exogenous cAMP. Finally, increasing cAMP levels by supplying exogenous cAMP or by stimulating adenylate cyclase with forskolin enhanced release at low but not high ATP concentrations. Thus, it appears that ATP acts in at least two steps in the PKA pathway; it serves as a source of cAMP, which is produced from ATP by the action of adenylate cyclase, and it must also serve as the phosphate donor for phosphorylation of the substrates of PKA. Together, these results show that PKA has a powerful effect on exocytosis but that its influence is restricted by the production of cAMP, by the amount of ATP available for substrate phosphorylation, and by ongoing phosphatase activity.

These conclusions about PKA are significant for a number of reasons. First, they unify thinking about the actions of ATP and PKA in beta cells. Previously, ATP and cAMP were thought to act in separate pathways, with ATP playing the central role in glucose-regulated insulin secretion and cAMP being involved in regulating secretion during the action of hormones such as glucagon (19). However, the results of Takahashi *et al.* (6) make clear that these two chemical signals are central players in the same pathway and that this pathway mediates insulin secretion. Second, these findings are important because they help to clarify the molecular nature of the direct regulation of secretion by ATP. What remains to be determined are the downstream substrates of PKA. The protein rabphilin, which is associated with synaptic vesicles (20) and appears to be involved in both the fusion and endocytosis of synaptic vesicles (21), may be an important target of PKA in neurons (22). However, rabphilin has been reported to be absent from beta cells (23). SNAP-25, a SNARE (soluble *N*-ethylmaleimide-sensitive factor-attachment protein receptor) protein important for the fusion of synaptic vesicles (24) and perhaps many other types of membranes (16), may also be a downstream component of the PKA signaling pathway (25). Third, because Takahashi *et al.* (6) found that ATP enhanced secretion produced by $[Ca^{2+}]_i$ near saturation for Ca^{2+} activation of exocytosis (7, 8, 26), PKA must do more than simply change the Ca^{2+} affinity of the fusion machinery (26). The most straightforward explanation of the results is that phosphorylation by PKA increases the number of granules available for Ca^{2+}-regulated fusion, although a final answer on this issue awaits further analysis.

The results of Takahashi *et al.* (6) may have very general significance because exocytosis in many other cell types is regulated by PKA. Synaptic transmission between neurons is known to be regulated by the activity of many protein kinases, including PKA, and PKA may be an important effector for changing the strength of synapses during various types of synaptic plasticity. For example, in *Aplysia* sensory neurons, cAMP increases the strength of synaptic transmission by enhancing neurotransmitter release (27). Interestingly PKA increases neurotransmitter release at this synapse, both by regulating Ca^{2+} entry through effects on K^+ and Ca^{2+} channels (28, 29) and by increasing the ability of entering Ca^{2+} to trigger synaptic vesicle fusion (30). These multiple effects of PKA on neurotransmitter release offer an intriguing parallel with the dual actions of ATP in beta cells and suggest that common molecular themes are shared by many secretory systems. In the hippocampus, where PKA also can modulate release independent of Ca^{2+} entry (25, 31, 32), PKA has been implicated in long-term potentiation, a form of synaptic plasticity that can be expressed as an enduring increase in neurotransmitter release (31, 33) and may serve as a cellular substrate for learning and memory. Therefore, it is possible that PKA modulation of vesicle availability is involved in both rapid and persistent alterations of neuronal communication.

In summary, the paper of Takahashi *et al.* (6) illustrates that ATP has several important roles in insulin secretion. Aside from enhancing Ca^{2+} entry by effects on K^+ and Ca^{2+} channels, ATP serves as a source of cAMP and donates a phosphate for PKA-mediated phosphorylation of protein(s) important for exocytosis. These results provide us with a clear and unified scheme to understand the ATP dependence of insulin secretion and establishes that actions of ATP independent of modulating $[Ca^{2+}]_i$ are a key component of this secretory event. This work also identifies components of the PKA signal-transduction cascade, and any of their diverse modulators, as potential sites of regulating insulin secretion, greatly expanding the list of tangible hypotheses for the etiology and treatment of non-insulin-dependent diabetes mellitus.

1. Cook, N. & Hales, C. N. (1984) *Nature (London)* **311,** 271–273.
2. Ashcroft, F. M., Harrison, D. E. & Ashcroft, S. J. H. (1984) *Nature (London)* **312,** 446–448.
3. Ashcroft, F. M. & Rorsman, P. (1989) *Prog. Biophys. Mol. Biol.* **54,** 87–143.
4. Gembal, M., Gilon, P. & Henquin, J. C. (1992) *J. Clin. Invest.* **89,** 1288–1295.
5. Aizawa, T., Sato, Y., Komatsu, M. & Hashizume, K. (1992) *Endoc. Regul.* **26,** 159–162.
6. Takahashi, N., Kadowaki, T., Yazaki, Y., Ellis-Davies, G. C. R., Miyashita, Y. & Kasai, H. (1999) *Proc. Natl. Acad. Sci. USA* **96**, 760–765.
7. Eliasson, L., Renström, E., Ding, W., Proks P. & Rorsman, P. (1997) *J. Physiol.* **503,** 399–412.
8. Takahashi, N., Kadowaki, T., Yazaki, Y., Miyashita, Y. & Kasai, H. (1997) *J. Cell Biol.* **138,** 55–64.
9. Thomas-Reetz, A. C & DeCamilli, P. (1994) *FASEB J.* **8,** 209–216.
10. Ellis-Davies, G. C. R. (1998) *Tetrahedron Lett.* **39,** 953–956.

11. Hay, J. C. & Martin, T. F. J. (1992) *J. Cell Biol.* **119,** 139–151.
12. Parsons, T. D., Coorsen, J. R., Horstmann, H. & Almers, W. (1995) *Neuron* **15,** 1085–1096.
13. Xu, T., Binz, T., Niemann, H. & Neher, E. (1998) *Nat. Neurosci.* **1,** 192–200.
14. Holz, R. W., Bittner, M. A., Peppers, S. C., Senter, R. A. & Eberhard, D. A. (1989) *J. Biol. Chem.* **264,** 5412–5419.
15. Banerjee, A., Barry, V. A., DasGupta, B. R. & Martin T. F J. (1996) *J. Biol. Chem.* **271,** 20223–20226.
16. Rothman, J. E. (1994) *Nature (London)* **372,** 55–63.
17. Sollner, T. S., Whiteheart, S. W., Brunner, M., Erdjument-Bromage, H., Geromanos, S., Tempst, P. & Rothman, J. E. (1993) *Nature (London)* **362,** 318–324.
18. Hay, J. C., Fisette, P. L., Jenkins, G. H., Fukami, K., Takenawa, T., Anderson, R. A. & Martin, T. F. J. (1995) *Nature (London)* **374,** 173–177.
19. Holz, G. G. & Habener, J. F. (1992) *Trends Biochem. Sci.* **17,** 388–393.
20. Takai, Y., Sasaki, T., Shirataki, H. & Nakanishi, H. (1996) *Genes Cells* **1,** 615–632.
21. Burns, M. E., Sasaki, T., Takai, Y. & Augustine, G. J. (1998) *J. Gen. Physiol.* **111,** 243–255.
22. Lonart, G. & Südhof, T. (1998) *J. Neurosci.* **18,** 634–640.
23. Regazzi, R., Ravazzola, M., Iezzi, M., Lang, J., Zahraoui, A., Andereggen, E., Morel, P., Takai, Y. & Wollheim, C. B. (1996) *J. Cell Sci.* **109,** 2265–2273.
24. Montecucco, C. & Schiavo, G. (1994) *Mol. Microbiol.* **13,** 1–8.
25. Trudeau, L.-E., Fang, Y. & Haydon, P. G. (1998) *Proc. Natl. Acad. Sci. USA* **95,** 7163–7168.
26. Ämmälä, C., Ashcroft, F. M. & Rorsman, P. (1993) *Nature (London)* **363,** 356–358.
27. Byrne, J. H. & Kandel, E. R. (1996) *J. Neurosci.* **16,** 425–435.
28. Brunelli, M., Edmonds, B. & Kandel, E. R. (1976) *Science* **194,** 1178–1181.
29. Siegelbaum, S. A., Camardo, J. S. & Kandel, E. R. (1982) *Nature (London)* **299,** 413–417.
30. Hochner, B., Klein, M., Schacher, S. & Kandel, E. R. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 8794–8798.
31. Weisskopf, M. G., Castillo, P. E., Zalutsky, R. A. & Nicoll, R. A. (1994) *Science* **265,** 1878–1882.
32. Capogna, M., Gähwiler, B. H. & Thompson, S. M. (1995) *J. Neurosci.* **15,** 1249–1260.
33. Lonart, G., Janz, R., Johnson, K. M. & Südhof, T. C. (1998) *Neuron* **21,** 1141–1150.