Vesicular Binesis: Glucose Effect on Insulin Secretory Vesicles

Abstract. *Binesis is a process whereby the membrane of the insulin secretory vesicle in the beta cell forms a lingula that indents the vesicular membrane of an adjoining secretory vesicle or the plasma membrane. Vesicular binesis in beta cells increases when islets of Langerhans are incubated at a stimulatory glucose concentration (300 milligrams per 100 milliliters). These vesicular membrane alterations may be the morphological concomitants of activation of the insulin secretory vesicle, and indicate an active role for the vesicle and its membrane in the release mechanism.*

Emiocytosis appears to be the major mechanism for insulin secretion from the beta cell, and involves the fusion of the secretory vesicle and plasma membranes, with subsequent opening of the fused membrane to release the vesicle content. Consideration of the insulin release sequence suggests a series of processes: (i) synthesis and packaging of insulin in the secretory vesicles, (ii) activation and transport of the vesicles to the secretion site, and (iii) release of the secretory granules by emiocytosis. Superimposed upon each or all of these processes are various modulating mechanisms, which influence the total release sequence. Morphologic and functional separation of these steps could permit a detailed study of the site and mode of action of biochemical and hormonal modulators of insulin release. Little is known about the activation of the insulin secretory vesicles. In this study we report a previously unnoted morphologic characteristic of the insulin secretory vesicle and morphologic alterations at stimulatory glucose concentrations. These results suggest that the insulin secretory vesicle itself is an active component in the sequence for insulin release.

Islets of Langerhans were isolated from rats weighing 450 to 600 g by the collagenase technique (*1*). Fifty islets were incubated in Siliclad-coated centrifuge tubes in 1 ml of Krebs-Ringer bicarbonate buffer, *p*H 7.4, containing 0.5 percent bovine serum albumin and continually gassed with a mixture of 95 percent oxygen and 5 percent carbon dioxide. The islets were incubated at 37°C at 70 oscillations per minute for 60 minutes. At the end of this incubation period the media were removed for insulin assay, and the islets were fixed in formaldehyde-glutaraldehyde fixative diluted 1 : 4 with 0.1*M* cacodylate buffer, *p*H 7.4, for 1 hour at 4°C (*2*). The islets were washed in three changes of cold 0.15*M* cacodylate buffer, *p*H 7.4, for 30 minutes and post-fixed in 1.3 percent osmium tetroxide in S-collidine buffer, *p*H 7.2, for 30 minutes at 4°C. Staining en bloc was performed with 1.5 percent uranyl acetate in 0.05*M* maleate buffer, *p*H 6.2, for 30 minutes at 4°C. The islets were dehydrated in ethanol and embedded in Epon. The sections were cut on an LKB Ultratome III (LKB, Bromma, Sweden) with a diamond knife (duPont de Nemours, Wilmington, Delaware) and picked up on carbon-coated grids. Sections were stained with lead citrate (*3*) and examined in a Phillips 300 electron microscope operated at 60 kv and equipped with an anticontamination device and 30-μm objective aperture.

Since the beta cells in the islets of Langerhans display variable degrees of secretory activity, a portion of one cell was photographed at random from each islet. Micrographs from six cells or more were obtained for each experimental group. The micrographs were enlarged to a final magnification of × 32,000, and all subsequent measurements were made on the prints by a separate investigator without knowledge of their experimental origin.

Table 1. Effect of glucose on vesicular binesis in incubated islets of Langerhans. The formaldehyde-glutaraldehyde fixation used in experiments 1 to 3 is described in the text. In experiment 4, the islets were fixed with 2.5 percent glutaraldehyde in 0.08*M* cacodylate buffer, *p*H 7.4, containing 0.1*M* sucrose and $4.5 \times 10^{-3}M$ calcium chloride. Three photomicrographs in each group were examined for this experiment, and six to ten per group for experiments 1 to 3.

Glucose (mg/ml)	Vesicle count	Vesicles affected (% of total)
	Experiment 1	
0.9	1075	3.50
3.0	2420	10.50
	Experiment 2	
0.9	314	3.50
3.0	1284	11.05
	Experiment 3	
0.9	1054	2.46
3.0	863	8.11
	Experiment 4	
0.9	381	3.14
3.0	386	13.73

In initial studies we found that the membrane of the insulin secretory vesicle is frequently infolded to form a finger-like invagination or furrow that extends well into the vesicle and appears as a cleft in appropriate sections (Fig. 1, A, B, and F). The two membranes of the vesicular furrow are in close apposition. The furrows demonstrate apparently progressive stages of widening, with consequent enlargement of the furrow cavity and exposure of the cytoplasmic surface of the invaginated vesicular membrane (Fig. 1, B to E).

The secretory vesicles also undergo binesis (*4*), a process whereby the vesicular membrane forms a lingula that protrudes from one secretory vesicle and inserts into an adjoining secretory vesicle (Fig. 1, F to I). In the interaction of the lingula with the receiving vesicle, the membranes of the two secretory vesicles are separated by 80 to 100 Å, and in cross section, the lingula appears as a bimembranous concentric ring (Fig. 1, G and L). Two or more secretory vesicles demonstrating binesis in tandem (Fig. 1H), as well as binesis circumferentially into a single central vesicle (Fig. 1F), are also observed. Lingula formation and binesis of vesicles are seen in all areas of the beta cell, and are not necessarily restricted to the cell periphery. Occasionally, under conditions of marked insulin secretory activity, the closely apposed vesicular membranes fuse and open to allow the vesicles to communicate (Fig. 1M). In such a circumstance, two granule cores can be observed within a single secretory vesicle (Fig. 1, G and J).

During binesis, the membrane of the receiving vesicle does not appear to have any specialization, since many vesicles can insert into a single central vesicle. Also, the lingula does not necessarily interact with the vesicular furrow, since furrows can still be observed in some receiving vesicles during binesis. Furthermore, since some vesicles demonstrating lingula formation may also have a furrow present, the significance of the vesicular furrow in the origin of the lingula is uncertain. However, structural polarity of the vesicular lingula is indicated by the absence of vesicles demonstrating multiple lingula formation. Thus, lingula formation may represent a specific

functional alteration in the vesicle membrane necessary for binesis and subsequent vesicular membrane interaction.

Vesicular furrows and binesis were also found in beta cells of isolated islets of Langerhans fixed with glutaraldehyde alone (Table 1), as well as in perfused rat pancreas that was similarly fixed (*5*). No significant differences in morphology were observed with the use of these different fixatives.

In order to determine the effects of stimulatory glucose concentrations on the insulin secretory vesicles, photomicrographs were examined for the presence of vesicles demonstrating binesis, intravesicular concentric membrane rings, and double granules within a single vesicle. The vesicles with these characteristics were counted, and the results were expressed as a percentage of total vesicles on the photomicrographs. In four separate experiments, there was a threefold increase in the incidence of vesicles demonstrating binesis at a stimulatory glucose concentration (300 mg/100 ml) as compared to the vesicles of islets incubated at a basal glucose concentration (90 mg/100 ml). Values for insulin release (mean ± standard error of mean) by islets at basal and stimulatory glucose concentrations were 2704 ± 297 and 15,619 ± 1375 microunits per 50 islets, respectively ($P < .001$), in the four experiments.

The increase in the number of vesicles demonstrating binesis during glucose-stimulated insulin release in is-

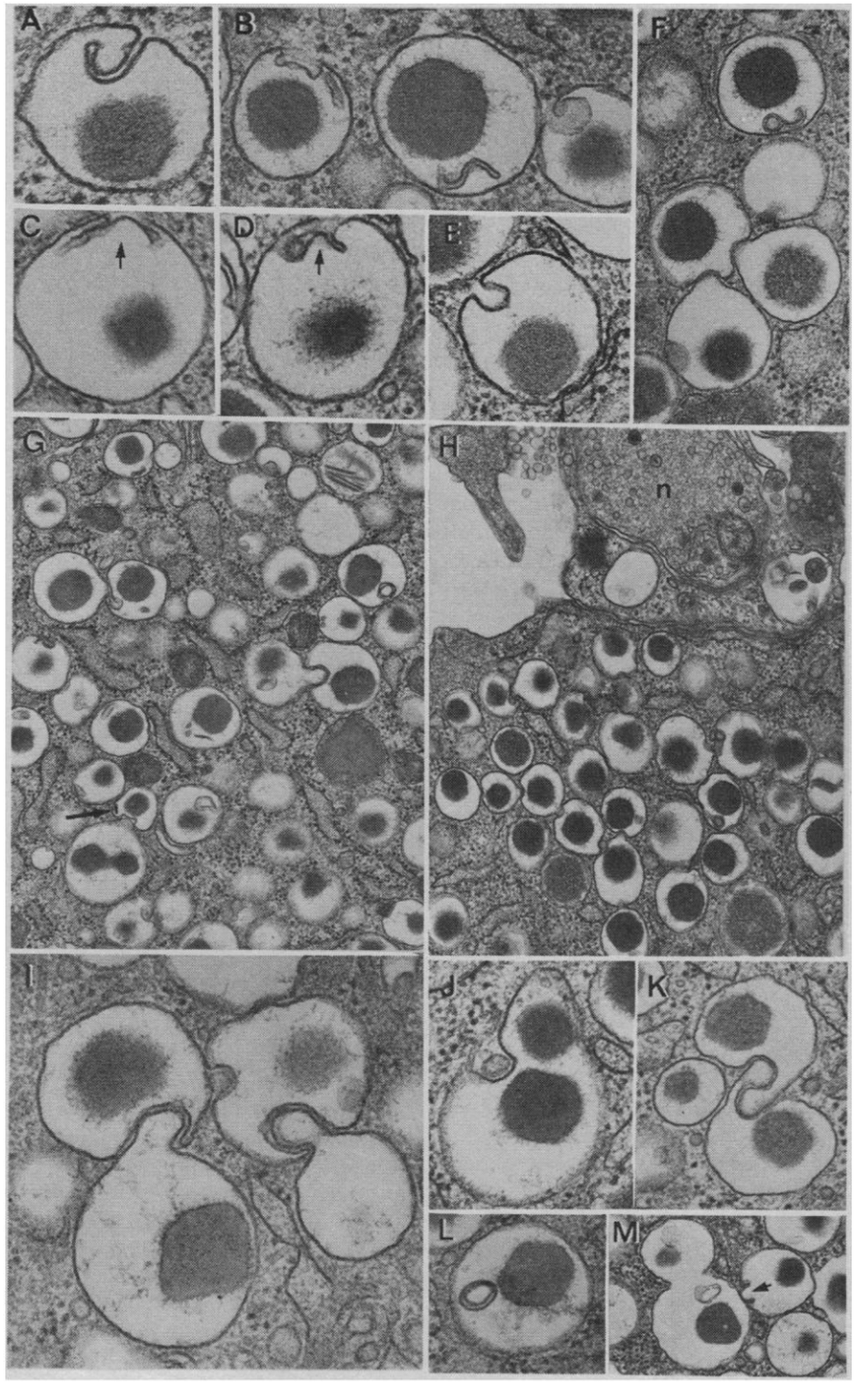

Fig. 1. Secretory vesicles in beta cells of rat islets. (A) The vesicular membrane of a secretory vesicle containing an insulin granule is invaginated to form the cleft-like vesicular furrow (× 87,500). (B) A furrow is seen in the central vesicle, and widening of the furrow cavity is seen in the two lateral vesicles. The furrow membranes in the central vesicle are in close apposition (× 52,000). (C and D) Widening of the furrow cavity and exposure of the cytoplasmic face of the furrow membrane are seen in two vesicles. Possible incipient formation of a vesicular lingula is indicated by arrows (× 62,500). (E) The lining membrane of the widening vesicular furrow cavity is continuous with the vesicular membrane (× 58,500). (F) Two secretory vesicles, one containing a pale immature secretory granule, are undergoing binesis around the circumference of another vesicle. The lingular membranes are in close apposition to the vesicle membrane (× 36,750). (G) A portion of a beta cell from an islet incubated at basal glucose concentration (0.9 mg/ml) has all of the features shown at higher magnification in other parts of the figure. Many vesicles with furrows are visible. A vesicle with a concentric bimembranous ring is seen just above the two vesicles undergoing binesis (right center). In the lower center portion, a vesicle with two granule cores is in the process of binesis; a furrow (arrow) is seen in the receiving vesicle (× 23,800). (H) A beta cell of an islet incubated at high glucose concentration (3.0 mg/ml) contains vesicles undergoing binesis in tandem. A nerve ending (*n*) with neurosecretory packets in the extracellular space is visible at top (× 22,950). (I) Two pairs of secretory vesicles are shown undergoing binesis (× 77,900). (J) A secretory vesicle contains two granule cores (×44,500). (K) Two secretory vesicles are shown undergoing "double" binesis (× 44,450). (L) The concentric bimembranous appearance of a cross section of a lingula during binesis is seen (× 43,450). (M) Two confluent vesicles are visible. The arrow points to a lingula in what may be incipient binesis (× 27,500).

lets indicates a hitherto unknown active participation of the insulin secretory vesicle and its membrane in the insulin release mechanism. While the role of binesis is still not clear, we suggest that it may represent a morphological concomitant of glucose-mediated activation of the insulin secretory vesicles occurring before emiocytosis of the vesicle contents. Similarly, the finding of binesis and vesicular fusion well within the interior of the beta cell supports the suggestion of Lacy (*6*) that insulin vesicles arrayed in tandem release their contents through a distal vesicle undergoing emiocytosis at the plasma membrane. Binesis may therefore be a useful phenomenon in the dissection of the insulin release sequence, especially since emiocytosis is a rapid process that cannot be adequately observed and quantified with ordinary transmission electron microscopy.

The observation of binesis and of its stimulation at elevated glucose concentration in islets does not appear to be influenced by the fixative employed. Rather, binesis appears to be more easily observed when the tissues are rapidly fixed by regional perfusion, or when isolated islets are used. Therefore, rapid and improved fixation may have allowed us to observe and quantitate this presumably fast and evanescent phenomenon. Indeed, inspection of published photomicrographs of rapidly fixed beta cells (fixation by regional perfusion and fixation of isolated islets) readily reveals examples of furrows and binesis by both transmission electron microscopy and by the freeze-fracture technique (*7*). Schramm *et al.* (*8*) showed that amylase granules from rat parotid gland form "pseudopods" during incubation of parotid gland slices, as well as in a cell-free preparation of secretory vesicles. The relative number of amylase granules bearing pseudopods increased during stimulus-induced secretion. Binesis may thus be of general significance in other secretory mechanisms as well.

KENNETH H. GABBAY
J. KORFF
EVELINE E. SCHNEEBERGER
Cell Biology Laboratory, Endocrine Division, Departments of Medicine and *Pathology, Children's Hospital Medical Center, Boston, Massachusetts 02115*, and *Departments of Pediatrics* and *Pathology, Harvard Medical School, Boston*

References and Notes

1. P. E. Lacy, M. M. Walker, C. J. Fink, *Diabetes* **21**, 988 (1972).
2. M. J. Karnovsky, *J. Cell Biol.* **27**, 137A (1965).
3. J. H. Venable and R. A. Coggeshall, *ibid.* **25**, 407 (1965).
4. Derived from the Greek verb βινεω, a Middle Ionian word indicating "to couple." We are grateful to A. Ramage of Cornell University for sharing his linguistic expertise and to F. Manasek for discussions.
5. K. H. Gabbay, in preparation.
6. P. E. Lacy, *Diabetes* **19**, 895 (1970).
7. W. J. Malaisse, D. L. Hager, L. Orci, *ibid.* **21**, 594 (1971), figures 1, 9, 11, and 12; A. E. Renold, *ibid.* **21**, 619 (1971), figure 1 (freeze-fracture); S. L. Howell, *Nat. New Biol.* **240**, 204 (1972); E. Van Obberghen, G. Somers, G. Devis, G. D. Vaughn, F. Malaisse-Lagae, L. Orci, W. J. Malaisse, *J. Clin. Invest.* **52**, 1041 (1973), figures 6, b and c, and 7, a to c.
8. M. Schramm, Z. Selinger, Y. Salomon, E. Eytan, S. Batzri, *Nat. New Biol.* **240**, 203 (1972).
9. Supported by USPHS grants AM-15019 and AM-16392, and by an established investigatorship of the American Heart Association to K.H.G. We thank J. Lynch and L. Fucci for technical assistance.

29 April 1974; revised 19 August 1974

Adenosine 3′,5′-Monophosphate Analogs Promote a Circular Morphology of Cultured Schwannoma Cells

Abstract. *Dibutyryl adenosine 3′,5′-monophosphate (dibutyryl cyclic AMP) and 8-bromo cyclic AMP stimulated cells cultured from a rat Schwannoma to change their morphology from irregularly shaped to flattened circular and hollow circular forms within 30 minutes. The change in shape was specifically effected by analogs of cyclic AMP and cyclic AMP elevating agents, was reversible after removal of these additives, and was prevented by vinblastine and cytochalasin b, but was not affected by actinomycin D or cycloheximide.*

Schwann cells surround peripheral nerve cell processes, acting as an electrical insulator by synthesizing a multilayered myelin sheath (*1*). Once the nervous system has formed, Schwann cells do not normally divide but during the early stages of nervous system formation, when division does occur, they sometimes undergo a neoplastic transformation, developing into a malignant Schwannoma. These tumors have been used to generate established clonal lines of Schwannoma cells which are capable of performing functions unique to the nervous system and to myelin production (*2*).

Table 1. The effect of various chemicals and culture conditions on the morphology of Schwannoma clone RN22. Cells were plated at 2×10^3 cell/cm² and incubated for 24 hours. They were then exposed to the various agents at a final concentration of 1 m*M* (except where indicated) and any effect or morphology was observed after 2 hours of incubation at 37°C. Control cells and cells not affected by the additives had a predominance of the irregular or triangular shape, whereas cells treated with cyclic AMP analogs or prostaglandin E_1 had an increase in the number of hollow and flattened circular forms. At least 500 cells were counted in each instance; where differences were suggested by the data, at least 1000 cells were counted. The cells were not fixed. Comparisons were then made between the control group and various experimental groups.

Agent or condition	Percentage of cells exhibiting these forms after 2 to 4 hours of exposure		
	Flattened circular	Hollow circular	Irregular or triangular
No additives	9	10	81
Dibutyryl cyclic AMP (DBcAMP)	15*	34*	51*
8-bromo cyclic AMP (8-Br-cAMP)	14*	44*	42*
Cyclic AMP	7	8	85
Dibutyryl cyclic guanosine monophosphate	9	9	82
8-bromo cyclic guanosine monophosphate (8-Br-cGMP)	9	12	79
Cyclic GMP	10	7	83
Butyrate	9	8	83
5′-Adenosine monophosphate	9	5	86
Adenosine	12	5	83
Serum-free medium (24 hours)	9	8	83
Papaverine (0.1 mm)	8	10	82
Prostaglandin E_1 (25 μg/ml)	12	21*	67*
Prostaglandin $F_{2\alpha}$ (25 μg/ml)	8	10	82
DBcAMP and cytochalasin b (0.1 μg/ml)	12	12	76
DBcAMP and vinblastine (10 ng/ml)	3*	12	85
8-Br-cAMP and actinomycin D (5 μg/ml)	15*	36*	49*
8-Br-cAMP and cycloheximide (5 μg/ml)	11	36*	53*
8-Br-cAMP and 8-Br-cGMP	11	42*	47*
Isoproterenol ($10^{-5}M$)	12	11	77

* Statistically significant differences at a level of $P < .05$ as measured by Student's *t*-test.