Quantal Secretion from Adrenal Medulla: All-or-None Release of Storage Vesicle Content

Abstract. *Neurogenic secretion of catecholamines from the adrenal medulla in rabbits, induced by administration of insulin, caused decreases in both the dopamine-β-hydroxylase activity and the catecholamine content of the storage vesicle fraction. After sedimentation through a sucrose density gradient, the storage vesicles obtained from insulin-treated animals had the same density and the same ratio of dopamine-β-hydroxylase to catecholamine as did vesicles from untreated animals. These and other data indicate that neurogenic secretion from the adrenal medulla occurs by an all-or-none release from the storage vesicles.*

Evidence from several laboratories indicates that, during secretion, the catecholamine storage vesicles of the adrenal medulla release their contents directly to the exterior of the cell (*1, 2*). Whether the material that is secreted (catecholamines, adenine nucleotides, dopamine-β-hydroxylase, and other soluble proteins) is derived from a population of vesicles that release a portion of their contents or from a smaller population of the same vesicles that secrete their total contents has not previously been determined for the chromaffin cell or for any other secretory cell or nerve ending. It is generally accepted that release of neurotransmitters at chemical synapses is quantal (*3*), but this has not been demonstrated in other secretory cells.

When the storage vesicles of the rabbit adrenal medulla are lysed in distilled water, approximately 50 percent of the dopamine-β-hydroxylase (DBO) is tightly bound to the particulate fraction and the remainder of the enzyme is found in the soluble fraction of the lysates (*2*). During neurogenic stimulation, the soluble dopamine-β-hydroxylase and catecholamines (CA) are released from the gland in the same proportion as that found in the soluble fraction of lysed vesicles. Following secretion there is a much smaller decrease in particle-bound DBO of the vesicle fraction than in soluble DBO and in catecholamines, so that the ratios of total DBO to catecholamines or total DBO to soluble DBO are significantly increased (*2*). The two most obvious explanations for these results are either a partial secretion of the storage vesicle contents or a total secretion of vesicle contents with retention of empty vesicle membranes.

Isopycnic centrifugation in a sucrose density gradient can distinguish vesicles with normal catecholamine content from partially depleted vesicles obtained from animals treated with reserpine and from vesicle membranes obtained by water lysis (*4*). Comparison of the ratios of DBO to CA in the various fractions obtained after isopycnic centrifugation of vesicles from untreated animals and from animals treated with insulin provides evidence that each of the vesicles that secretes, releases its total content.

Neurogenic secretion of catecholamines from the adrenal medulla was elicited by administering insulin (40 international units per kilogram of body weight) via ear vein to adult New Zealand White rabbits. Animals that received reserpine were given .1 mg/kg. The animals were killed 4 hours after administration of insulin or 24 hours after reserpine. The adrenal glands were removed, blotted dry, weighed, and then homogenized in 20 volumes of ice-cold 0.3*M* sucrose. Cell debris was removed by centrifugation at 800*g* for 10 minutes, and the storage vesicle fraction was collected by centrifugation at 26,000*g* for 20 minutes. The pellet was resuspended in 1.0 ml of 0.3*M* sucrose and 0.5 ml of this suspension was layered over a 1.0 to 2.25*M* linear sucrose density gradient (volume 4.9 ml) and centrifuged at 100,000*g* for 3 hours in a Spinco SW-50 rotor. The centrifuge tubes were then punctured and 20 fractions containing 12 drops each were collected and assayed for DBO and CA by methods already described (*5*).

In these gradients 73 to 78 percent of the total catecholamines were found in the bottom half of the gradient forming a single peak (fractions A and B, Fig. 1). The storage vesicles from the insulin-treated animals equilibrated at the same density as did vesicles from the control group, but vesicles from the reserpine-treated animals equilibrated at a lower density (Table 1 and Fig. 1). The L values (see legend to Table 1 for definitions) for seven reserpine-treated rabbits were 0.63 ± 0.03 and 0.67 ± 0.01, respectively, for the DBO and the CA peaks. Just below the interphase between the 1.0*M* and 0.3*M* sucrose a second peak of enzyme activity with little or no catecholamines was found (fraction D). When the storage vesicles were centrifuged through a 0.3 to 1.5*M* linear sucrose density gradient, the median density of fraction D corresponded to 1.2*M* sucrose. Vesicle membranes prepared from water-lysed vesicles equilibrated in the same regions as fraction D in both types of sucrose density gradients. The DBO present in fraction C was mostly particle-bound. Fraction E contained soluble DBO and CA, presumably released during preparation of the 0.5 ml of vesicles layered over the gradient.

If a vesicle releases only a fraction of its soluble contents each time it participates in the secretory process, one would expect to find an upward displacement of the DBO and the CA peak, or the appearance of additional

Table 1. Distribution of dopamine-β-hydroxylase and catecholamines in sucrose density gradients. The dopamine-β-hydroxylase (DBO) activity is expressed as nanomoles of product formed times 100 per pair of glands per hour. Catecholamines (CA) are expressed as micrograms per pair of glands. The values are the means ± the standard error; numbers in parentheses are the numbers of animals in each group. L is the penetration of the catecholamines and dopamine-β-hydroxylase activity into the gradient and is the distance of the respective peaks from the top of the gradient divided by the total length of the gradient. The other letters refer to the fractions in Fig. 1.

Fraction	DBO (nanomoles × 100)	Particulate DBO (%)	CA (μg)	DBO/CA
	Controls (6)			
A + B	824 ± 50	61	61 ± 4	14 ± 1
C	66 ± 3	91	6 ± 1	13 ± 2
D	123 ± 9	78	4 ± 4	32 ± 5
E	90 ± 10		7 ± 0.9	13 ± 1
Total	1100 ± 100	68	77 ± 10	15 ± 1
L values	0.73 ± 0.02		0.76 ± 0.03	
	Insulin-treated animals (5)			
A + B	104 ± 10	54	8.7 ± 0.2	10 ± 2
C	45 ± 9	84	1.0 ± 0.04	49 ± 11
D	142 ± 25	84	0.81 ± 0.04	180 ± 4
E	27 ± 6		1.5 ± 0.2	18 ± 3
Total	318 ± 55	70	12 ± 0.5	26 ± 4
L values	0.69 ± 0.01		0.78 ± 0.02	

peaks with a lower density. The ratio of DBO to CA in the vesicles should increase, since all of the membrane-bound DBO remains constant while the soluble DBO and CA are released. This is, in fact, what is observed after reserpine treatment that caused a 50 percent decrease in total CA but no change in DBO (*4*).

If each vesicle releases its total soluble contents upon secretion, one would expect to find a decrease in both DBO and CA of fractions A and B, no changes in the ratio of DBO to CA, and no change in the density of the remaining vesicles. If the empty vesicle membranes are essentially similar to vesicle membranes obtained by water lysis of normal storage vesicles, we should see an increase in fraction D. If the whole vesicle, including its membrane, is extruded during secretion, or if their membranes are altered or immediately destroyed after secretion so that they do not sediment at 26,000*g*, no increase in fraction D would be expected.

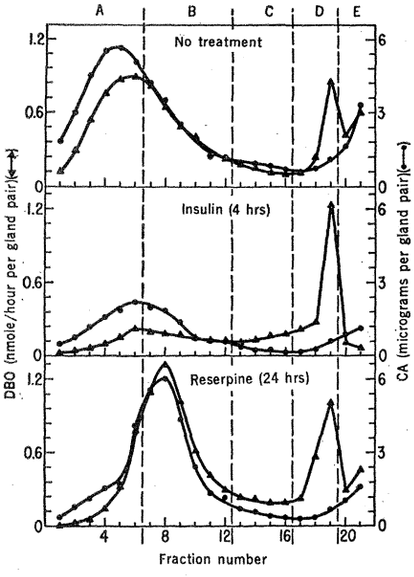

Fig. 1. Sedimentation of storage vesicle fractions of adrenal medulla through sucrose density gradients. The patterns are typical for those obtained for each pair of glands from six controls, five insulin-treated, and seven reserpine-treated animals. In this figure the catecholamine and dopamine-β-hydroxylase content of the adrenal glands from the insulin-treated animals were 16 and 43 percent, respectively, of the mean control values. The glands from the reserpine-treated animals contained 45 and 85 percent, respectively, of the normal catecholamine and dopamine-β-hydroxylase content. Controls were always run simultaneously with the reserpine-treated and insulin-treated animals.

After neurogenic stimulation, distribution of catecholamines and enzyme is what one would predict for an all-or-none release from those granules that did participate in the secretory response (Table 1). For each fraction, percentages of membrane-bound DBO in insulin-treated rabbits were similar to those of the controls. There was a decrease in the total amounts of CA and DBO but no change in density of the vesicles (fraction A plus B) or in their ratios of DBO to CA. If each of the vesicles had secreted only a portion of their contents with no loss of particulate DBO and no change in their density, then the calculated ratios of DBO to CA in this fraction are six times the observed values.

The ratio of DBO to CA of vesicles from insulin-treated animals for the total fraction placed on the gradient was approximately twice that of the controls but was much lower than the value calculated if all of the bound DBO had sedimented with the 26,000*g* fraction. The fact that DBO in fraction D from the insulin-treated rabbits was the same as that of the controls, but was lower in all other fractions, suggests that a portion of the bound DBO lost from fractions A and B was present in fraction D. The amount of enzyme lost from fractions A and B can be accounted for by release of soluble enzyme together with catecholamines during secretion and by fragmentation of the vesicle membrane so that a large fraction of it no longer sediments at 26,000*g*. When the supernatant fraction obtained after removal of the 26,000*g* vesicle fraction was centrifuged at 100,000*g*, the pellets obtained from the insulin-treated rabbits contained increased amounts of DBO, so that all of the particle-bound DBO that is lost from the 26,000*g* pellet could be found in the 100,000*g* pellet. Concurrently, the amount of soluble DBO in the 100,000*g* supernatant fraction obtained from insulin-treated rabbits is less than that found in controls (*4*). Thus, all of the particulate enzyme is retained by the gland and only soluble enzyme is lost. The data do not eliminate the possibility that the entire granule was extruded into the interstitial space and that the particle-bound DBO was trapped. However, this seems unlikely when considered in conjunction with electron microscopic studies that show an increase in electron translucent vesicles within the medullary cells after secretion and no structures corresponding to vesicle membranes in the extracellular spaces (*6*).

Our data indicate that the contents of each vesicle are released in an all-or-none fashion. The mode in which intravesicular secretory products are released from different cells and nerve terminals present remarkable similarities (*1*). The quantum hypothesis for release of neurotransmitters proposed by Del Castillo and Katz (*7*) has been confirmed in all chemical synapses that have been studied (*3*), but their proposal that the synaptic vesicles may be the quantum subcellular unit still lacks experimental evidence. Folkow *et al.* and Stjarne *et al.* (*8*) have argued against the proposal that the total noradrenergic synaptic vesicles are the subcellular unit for quantal release from the synaptic terminal plexus. They have calculated that only 3 to 10 percent of the content of a single vesicle would be released from each varicosity on maximum stimulation of the nerves to skeletal muscle blood vessels, but this assumes a uniform response from all varicosities. Similar calculations by us for the release of catecholamines from the cat's adrenal medulla for each maximum stimulus applied to the first splanchnic nerve is of the order of one vesicle content per pulse per chromaffin cell (*4*).

Although release of neurotransmitters at synapses is quantized, it does not necessarily follow that the storage vesicles are the immediate source of the released transmitter or that it is necessary to have total release of the contents of the storage vesicle. On the other hand, total secretion of the contents of storage vesicles, is, per se, quantal release. Recent evidence indicates that dopamine-β-hydroxylase in noradrenergic nerves and nerve terminals may be within the same storage vesicles as noradrenaline (*9*). Studies similar to ours, when applied to noradrenergically innervated tissues, may determine whether release from sympathetic varicosities proceeds by exocytosis and whether the synaptic vesicles are the subcellular units of quantal noradrenaline release.

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Micelle Formation between 5-Hydroxytryptamine and Adenosine Triphosphate in Platelet Storage Organelles

Abstract. *As judged by analytical ultracentrifugation, 5-hydroxytryptamine and adenosine-5′-triphosphate form micelles in artificial mixtures and also in storage organelles containing 5-hydroxytryptamine of blood platelets of rabbits. Their average apparent molecular weights depend on the concentration and on the molar ratio of the two constituents. The 5-hydroxytryptamine and adenosine triphosphate of these 5-hydroxytryptamine organelles may be stored in vivo together as micelles with apparent molecular weights of several hundred thousands or more.*

In the blood platelets, 5-hydroxytryptamine (5HT) and adenosine-5′-triphosphate (ATP) are mainly localized in special intracellular organelles, which, in rabbits, also contain histamine (*1*). In these organelles the content of 5HT and ATP relative to their volume is very high, exceeding 20 percent (weight to volume) for 5HT and 25 percent (weight to volume) for ATP (*2*). These compounds, if present in a monomolecular solution or even if dissolved as an undissociated salt containing 2 or 3 moles of 5HT and 1 mole of ATP, would give rise to an osmotic pressure exceeding that of plasma, where the isolated organelles are relatively stable.

The involvement of ATP in the storage of 5HT by platelets has been discussed (*3*, *4*), and the formation of 5HT-ATP complexes of low molecular weight in vitro was postulated based on results of electrometric titration (*5*). Our experiments give evidence for micelle formation between 5HT and ATP in artificial mixtures as well as in organelles of blood platelets containing 5HT.

Apparent molecular weights were determined at 20°C by equilibrium centrifugation in a Spinco analytical ultracentrifuge equipped with schlieren optics; a 12-mm centerpiece was used for the 1 percent solution, and a 3-mm centerpiece was used for all other experiments. The solutions were layered on a cushion of silicone oil. After equilibrium was achieved, we determined apparent molecular weights from the concentration gradient (*6*) at three different spots in the cell, each representing different concentrations. Solutions of ATP and 5HT oxalate in 0.15*M* sodium-potassium-phosphate buffer (*p*H 7.2) were prepared. The 5HT organelles of blood platelets of rabbits were isolated by density-gradient centrifugation in Urografin (*7*). The isolated organelles were thoroughly washed with Tyrode buffer to remove the Urografin; they were then disrupted by freezing in 0.6 ml of water, and the membranes were centrifuged at 159,000*g*. The supernatant solution was evaporated under normal pressure at about 10°C in a stream of nitrogen to a volume of approximately 0.015 ml; the remaining solution was immediately subjected to analytical ultracentrifugation. In the two experiments, the organelles of 36 and of 69 rabbits were pooled, with final concentrations of 5HT plus ATP of 4 and 15 percent, respectively.

The dependence of micelle weight on concentration of solutions of 5HT and ATP in molar ratios of 2 to 1 is demonstrated in Fig. 1. In mixtures of 5HT plus ATP (curve 3), the apparent molecular weights are about an order of magnitude higher than those of the single solutes (curves 1 and 2). Self-association in water occurs in one-component systems, that is, between molecules of mixed hydrophilic-hydrophobic structure such as soaps (*8*), detergents (*9*), and organic dyes (*10*), and also between many proteins (insulin) (*11*) and aromatic bases such as purines (*12*). Association in mixtures (5HT plus ATP) may be substantially higher than in the solutions of the single compounds. A separation of a second liquid phase of high viscosity is obtained on cooling to 2°C a solution (30 percent by weight) of 5HT and ATP in a molar ratio of 2 to 1. Concentrations of 5HT and ATP up to 90 percent and more have been found in the separated phase, the molar ratio between 5HT and ATP being about 2.2. Evidently, formation of micelles between 5HT and ATP has occurred.

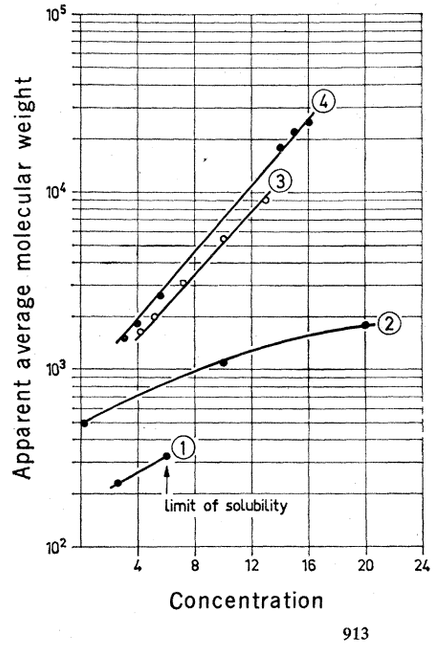

Fig. 1. Dependence of apparent molecular weight on concentration (percent by weight): Curve 1, 5HT oxalate; curve 2, ATP; curve 3, (circles), 5HT oxalate plus ATP, molar ratio 2 (*p*H about 2) and (square), 5HT chloride plus ATP, molar ratio 2 (*p*H adjusted to 6); curve 4, fluid from 5HT organelles (*p*H about 6).