BRIEF NOTE

**A Possible Mode of Release of Secretory Granules
from Adrenomedullary Cells in Domestic Fowl**

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There have been many studies on the degranulation mechanism of adrenomedullary cells in mammals. For example, Grynszpan-Winograd [4] and Kobayashi and Serizawa [5] have demonstrated through electron microscopic observations that the secretory granules of adrenal chromaffin cells are discharged by exocytosis. On the other hand, some morphological findings have been interpreted as evidence that catecholamines are secreted into the cytoplasm and subsequently diffused to the outside of the cell [2, 8].

With regard to avian species, Fujita et al. [3] noticed no exocytotic signs in fowl even after the injection of a high dose of insulin, while Unsicker [7] reported that exocytosis was easily observed only in the adrenomedullary cells of the chaffinch. However, the degranulation mechanism of adrenomedullary cells in fowl does not seem to be absolutely resolved.

This study reinvestigates the morphological changes of adrenomedullary cells in domestic fowl after insulin injection and discusses the existence of a possible release mechanism of the secretory granules which has not previously been described.

Five Brown Leghorn cockerels (about 1.0 kg in body weight) were deprived of food for 24 hr, but had free access to water. One bird was used as control. The others were given a subcutaneous, single injection of insulin "Novo Rente" 25 I.U./kg (This insulin contains 28 units of beef insulin and 12 units of pork insulin per 1 cc.). The birds were then sacrificed after 1, 3, 9, and 12 hr. Following the insulin injection, the blood glucose level rapidly fell, reaching its lowest level at 3 hr (50 mg %). Subsequently it tended to rise, but had not reached normal level (190 mg %) at 12 hr. Adrenal glands were fixed with a modified Karnovsky's fixative (1.68% glutaraldehyde and 1.6% paraformaldehyde in phosphate buffer) containing 0.02 M *s*-collidine [1], and postfixed in 1% OsO₄. Thin sections stained with uranyl acetate and lead were observed in a JOEL 100 S electron microscope.

The secretory granules in both adrenaline- and noradrenaline-storing (A and NA) cells decreased considerably in number during and after stimulation with insulin-induced hypoglycemia, but profiles indicative of exocytosis, which were rarely found in the control (Fig. 1), did not seem to increase.

This implies that a release mechanism of the secretory granules other than exocytosis may exist in the adrenomedullary cells of the domestic fowl.

In the stimulated cells of both types, some secretory granules were found to contain a small vesicle or an electron-lucent area (Figs. 3, 4). In addition to these modified granules, many enlarged granules with a small core of decreased electron density and low density flocculent materials radiating from it, were observed (Fig. 4). The number of these profiles was greatly increased in the insulin treated fowls, as compared with the control. The enlarged granules often appeared to have fused with each other (Fig. 5) to form small vacuoles, or they united with an already formed vacuole to release their contents into it (Fig. 4). The small granule-like, moderately electron-dense mass observed in the vacuoles probably represents the released contents of a granule (Fig. 7). Unmodified secretory granules also appeared to discharge their contents into the vacuole (Figs. 2, 3).

The stimulated cells had many vacuoles of various sizes in round, oval or elongated form. The larger ones were probably formed by the fusion of smaller ones as stated by Unsicker [7] (Figs. 2, 4, 7). The vacuoles contained granule-like, moderately electron-dense mass and flocculent materials of low electron density (Figs. 2, 3, 4, 7). Study of serial thin sections revealed that vacuoles connected with the discharging secretory granules were completely separated from the cellular surface. Therefore, it is plausible to conclude that modified, enlarged or unmodified secretory granules frequently release their contents into the closed vacuoles. But large vacuoles opening into the intercellular or extracellular spaces were sometimes encountered (Fig. 6). In addition, the opening vacuoles often had

micropinocytotic pits whereas those enclosed did not.

The occurrence of a similar increase in vacuoles after insulin injection was reported in the adrenomedullary cells of fowl [3] and of rats [6], but to our knowledge, the release of secretory granules into the vacuoles has not been mentioned before. Large follicles surrounded by several chromaffin cells and filled with colloid-like substance such as Unsicker [7] reported in some untreated birds, were not found in the present study.

There is a notable difference in the electron density between the contents of the enlarged granules and vacuoles, and those of the unmodified granules. However, it is uncertain if they are otherwise identical, or whether the contents of the enlarged granules and vacuoles consist of protein only. Elfvin [2] hypothesized that the large masses of granular contents in the vacuoles were probably protein.

Based upon these observations, a possible release mechanism of secretory granules from adrenomedullary cells is proposed. Under an emergency stage such as insulin administration, either an electron-lucent area or a small vesicle occurs in the secretory granules. These modified granules become enlarged with a small core of decreased electron density and flocculent radiated materials. The enlarged granules unite with each other to produce a small vacuole. Unmodified secretory granules, as well as enlarged granules, release their contents into the small vacuole, resulting in the formation of a larger vacuole. Larger vacuoles tend to occupy the periphery of the cells and eventually open and discharge their contents into the intercellular or extracellular spaces.

The possibility that some of the vacuoles opening to the outside of the cell are products of the invaginated cellular membrane

cannot be perfectly ruled out. Further studies such as the labelling of the extracellular space are needed in order to draw a final conclusion concerning this problem.

References

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要 約

ニワトリの副腎髄質細胞の分泌顆粒の分泌過程について (短報): ナロン, チュンサマンヤート・渡辺 徹・藤岡俊健 (名古屋大学農学部家畜解剖学教室)——ニワトリにインシュリンを注射し, その 1, 3, 9, 12 時間後の副腎髄質細胞の変化を電子顕微鏡により観察した. 脱顆粒が盛んな時には, 顆粒の内容に変化が起り, 電子密度の低い内容をもつ顆粒がふえ, 相互に癒合し, 小空胞を形成する. 小空胞には顆粒が開口し, 大空胞の出現をみる. 細胞の周辺の一部の大空胞は細胞外に開いている. 少なくとも急激な分泌が起る際には, この空胞形成が, 顆粒の分泌と密に関係するものと推定された.

Explanation of Figures

- Fig. 1. Part of an A cell of the control fowl, showing exocytosis (arrow). $\times 20,000$.
- Figs. 2-7. Adrenomedullary cells of insulin-injected fowls.
- Fig. 2. Secretory granules opening into a large vacuole (arrows) in an A cell after 1 hr. $\times 20,000$.
- Fig. 3. Secretory granules opening into a small vacuole (large arrow) or containing a small vesicle (small arrows) in an NA cell after 3 hr. $\times 20,000$.
- Fig. 4. Granules with an electron-lucent area or a small vesicle (small arrows) and enlarged granules (asterisks) in an A cell after 12 hr. One enlarged granule in contact with a vacuole and one opening into a vacuole (large arrows). $\times 16,000$.
- Fig. 5. Enlarged granules united with each other in an A cell after 1 hr. $\times 20,000$.
- Fig. 6. Large vacuole opening into intercellular space in an A cell after 1 hr. $\times 20,000$.
- Fig. 7. Large vacuoles containing a small electron-dense mass (arrows) in an A cell after 9 hr.