

A qualitative and quantitative electron microscopic study of differences in adrenomedullary adrenaline cells between golden hamsters and rats, with special reference to the Golgi apparatus

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Summary. Ultrastructural features of adrenomedullary adrenaline (A) cells in golden hamsters and rats were investigated qualitatively and quantitatively with special reference to the Golgi apparatus. The A cells displayed a characteristic follicular arrangement, with each cell showing structural polarity in hamsters, but not apparently in rats. In hamsters, the Golgi apparatus of A cells was larger (t-test: $P < 0.001$) and more frequently showed large and complexly organized structures (χ^2 -test: $P < 0.005$) compared with that of rats. Quantitative analysis of the Golgi apparatus revealed differences in the size and numerical density of Golgi vesicles in relation to the animal species and region. Two-way analysis of variance (ANOVA) confirmed species difference in the size of coated vesicles ($P < 0.005$) and interaction between species and region concerning the size of smooth-clear vesicles ($P < 0.01$) and numerical density of granular vesicles ($P < 0.05$). One-way ANOVA revealed regional differences

in the size and numerical density of smooth-clear vesicles in rats and hamsters ($P < 0.01 \sim 0.001$), and in the numerical density of coated vesicles in hamsters ($P < 0.05$). Data were further analyzed by Tukey-Kramer's method. These and other reported results suggest that, in hamster A cells, the Golgi apparatus has different structural, molecular, and functional mechanisms which are at least partly related to the distinct cellular polarity and higher concentration of peptide hormones in secretory vesicles, and that in rat A cells, in contrast, loading secretory vesicles with A during the post-Golgi stage is predominant. In conclusion, the Golgi apparatus in hamster A cells shows markedly different ultrastructural features compared with that in rat A cells.

Introduction

It has been well established that the adrenal medulla is an endocrine gland of neural origin, and that adrenomedullary chromaffin cells secrete two types of hormones—catecholamines and peptides (Carmichael and Winkler, 1985; Winkler, 1993). In addition, the hormone-storing secretory granules in these chromaffin cells have been known to contain water-soluble proteins called granins (Somogyi *et al.*, 1984; Winkler *et al.*, 1986). According to recent studies on adrenomedullary chromaffin cells, prohormones of peptides are synthesized in the rough-surfaced endoplasmic reticulum (RER) and transferred to the Golgi apparatus, and as they are transported to secretory vesicles, the processing of prohormones and maturation occur. On the other hand,

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in regard to catecholamine synthesis, only dopamine- β -hydroxylase (DBH) is contained in the vesicle through a route similar to peptides, and other reactions occur probably in the cytosol; the end product, adrenaline (A), is stored in the secretory vesicle (Carmichael and Winkler, 1985; Jamieson, 1998). However, many details remain to be clarified for the intracellular structure-function relationships and regulatory mechanisms concerning synthesis and secretion of these amines, peptides and proteins, among others (Winkler *et al.*, 1986; Sietzen *et al.*, 1987; Jamieson, 1998; Kachi *et al.*, 1998; Tooze, 1998; Bornstein *et al.*, 2000; Gillingham and Munro, 2003; Kim *et al.*, 2005).

The ultrastructure of the Golgi apparatus has been intensively investigated using different types of cells. In addition, the Golgi apparatus has been known to have two pathways, i.e. a constitutive pathway and a regulatory pathway (Gumbiner and Kelly, 1982), in addition to multiple functions: i.e. after receiving various peptides, proteins and lipids, the Golgi chemically modifies, concentrates, and redistributes those substances to the plasma membrane, secretory vesicles or cell organelles such as lysosomes (Arvan and Castle, 1998; Jamieson, 1998; Tooze, 1998; Rodriguez-Boulanger and Musch, 2005). Still, relationships among the fine structures, molecular events, and functions of the Golgi apparatus, particularly those in differentiated cells such as adrenomedullary chromaffin cells, remain to be studied.

We have investigated various structures of the adrenal medulla and their changes under normal and experimental conditions, such as the effects of time of day, pinealectomy, and sham-pinealectomy, by both light and electron microscopy (EM) using quantitative morphological methods in the rat (*Rattus norvegicus albus*), which is neither a seasonal breeder nor hibernator, and the golden hamster (*Mesocricetus auratus*), which is a seasonal breeder and a hibernator (for review see: Kachi *et al.*, 1996, 1998). The present authors have previously reported the results of such an experimental EM study on the Golgi apparatus and its constituents in the adrenomedullary A cells of rats (Kajihara *et al.*, 1997).

Since the adrenal medulla of golden hamsters shows fascinating histo-cytological and biochemical natures such as a follicular arrangement of chromaffin cells (Al-Lami, 1970; Grynszpan-Winograd, 1974), typical profiles of exocytosis in chromaffin cells (Diner, 1967; Benedeczy and Smith, 1972; Grynszpan-Winograd, 1974; Kachi *et al.*, 1985), high concentrations of enkephalins (Bohn *et al.*, 1983; Franklin, 1997) and the exclusive distribution of noradrenaline cells in the juxtacortical region (Kachi *et al.*, 1998), the ultrastructure of the hamster adrenal medulla has been studied from various approaches (see:

Grynszpan-Winograd, 1974; Al-Lami and Farman, 1975). These studies, however, were mainly qualitative. To date no quantitative EM studies in hamster A cells have been carried out, except for ours on the exocytosis (Kachi *et al.*, 1985) and the nucleus (Taji and Kachi, 1999), nor has there been a detailed analysis of the Golgi apparatus.

We therefore performed a qualitative and quantitative ultrastructural study of adrenomedullary A cells of golden hamsters in comparison with those of rats. The main focuses were on the Golgi apparatus, its regional differences and species differences. In this study, we aimed to gain insight into possible structural / functional relationships of adrenomedullary A cells and their Golgi apparatus and the significance of their species differences, and also to obtain basic EM data for future studies on these subjects including seasonal changes. This work was presented at a meeting of Japanese anatomists (Kajihara *et al.*, 1996).

Materials and Methods

Six male golden hamsters and 3 male Wistar rats were used. The golden hamsters and rats were purchased after weaning and maintained in a windowless animal room under 24 h light-dark cycle (LD 12:12) at $22 \pm 2^\circ\text{C}$. Food (MF, Oriental Company, Tokyo) with water given *ad libitum*. After 5 weeks of acclimation, both sides of the adrenal glands were extirpated at 6 AM (the late dark phase) in young adult golden hamsters and rats. After cutting the glands in half, tissues were prefixed in 2.5% GA solution in a 0.1M cacodylate buffer. After several washings with the same buffer solution, tissues were postfixed with 1% OsO_4 for 2 h, dehydrated with a series of ethanol, and then embedded in epon 812. Semithin sections of 1 μm in thickness selected from several blocks at random, were stained with toluidine blue and observed with a light microscope. Ultrathin sections were then made. After mounting silver-colored sections on grid meshes, sections were double-stained with uranyl acetate and lead citrate, and then observed under an electron microscope (JEM-100CX, JEOL, Tokyo).

For quantitative analysis of a whole A cell and the Golgi apparatus, electron micrographs were taken at a magnification of 4,800 times in almost the same number of A cells randomly selected in three animals from each group of rats and golden hamsters, and printed on paper at a final magnification of 13,000 times. For detailed observations, electron micrographs of cross sections of the Golgi apparatus were taken at a magnification of 10,000 times and an accelerating voltage of 80 kV, and printed on paper at a final magnification of 28,000 times.

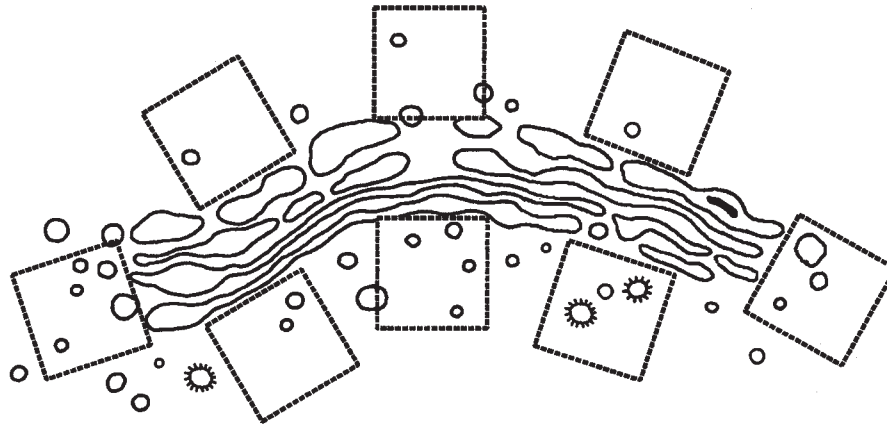


Fig. 1. Schema of the Golgi apparatus in an adrenomedullary adrenaline cell. Measured areas are shown by squares. Upper: convex (=cis) side; lower: concave (=trans) side.

On each of the electron micrographs at a magnification of 13,000 times, cross sectional areas of a whole cell, the nucleus, and the Golgi apparatus, particularly its lamellae, were measured, and from these the volume density of Golgi apparatus per each cell body was calculated. For measurement of these areas, NIH Image of public domain soft (developed at the U.S. National Institutes of Health and available on the Internet at <http://rsb.info.nih.gov/nih-image/>) was used.

On electron micrographs at a magnification of 28,000 times, detailed qualitative observations of the Golgi apparatus, the classification of its form and structure, and the measurement of density and diameter of smooth-clear vesicles, coated vesicles, and granular vesicles around the Golgi lamellae were performed. The measurement of vesicles was done separately on the convex (=cis) side, concave (=trans) side and both lateral sides in vertical sections of the Golgi lamellae in which the convex side and concave side were readily visible, these being selected from ca.15 electron micrographs of the Golgi apparatus in each animal. Vesicles contained in a 14.0 mm square (ca. 500 nm square in original length) were measured. At most, 5 places at equal distances were set as one side of each square adjacent to the outermost surface of the convex or concave side of the Golgi lamellae, and each place was additionally set on both lateral sides (Fig. 1). The areas of nuclear and mitochondrial section-profiles in a square were measured by a hitting-point counting method using a transparent plastic sheet with a regular lattice of points, each 2.0 mm distant from its nearest neighbors, and the

area which was calculated by deleting the nuclear and mitochondrial areas from the total area of square was used as the total area of the measured object.

The numerical density of vesicles per unit area was first determined, and then the numerical density of vesicles per unit volume was determined by the following formula:

$$N_v = N_a \frac{10^3}{T} \frac{T}{T+2R}$$

N_v : the numerical density of vesicles per unit volume ($N/\mu m^3$); N_a : the numerical density of vesicles per unit area ($N/\mu m^2$); T : the section thickness (nm); $2R$: the mean diameter of vesicles (nm). In the present study, T was set as 60 nm, and for $2R$, the mean diameter of vesicles in each portion of the Golgi apparatus was used in each animal species.

The measurement of vesicle diameter was also done in similar areas. As for smooth-clear vesicles and granular vesicles, diameters of all vesicles in selected areas were measured, but as for coated vesicles, all vesicles in the whole Golgi area were measured since these coated vesicles were extremely small in number.

For statistical analysis, analysis of variance (ANOVA), Tukey-Kramer's multi-comparison method (TK), Student's t-test, and the chi-square test were used.

All animal experiments in this paper followed the Guidelines for Animal Experimentation, Hirotsaki University.

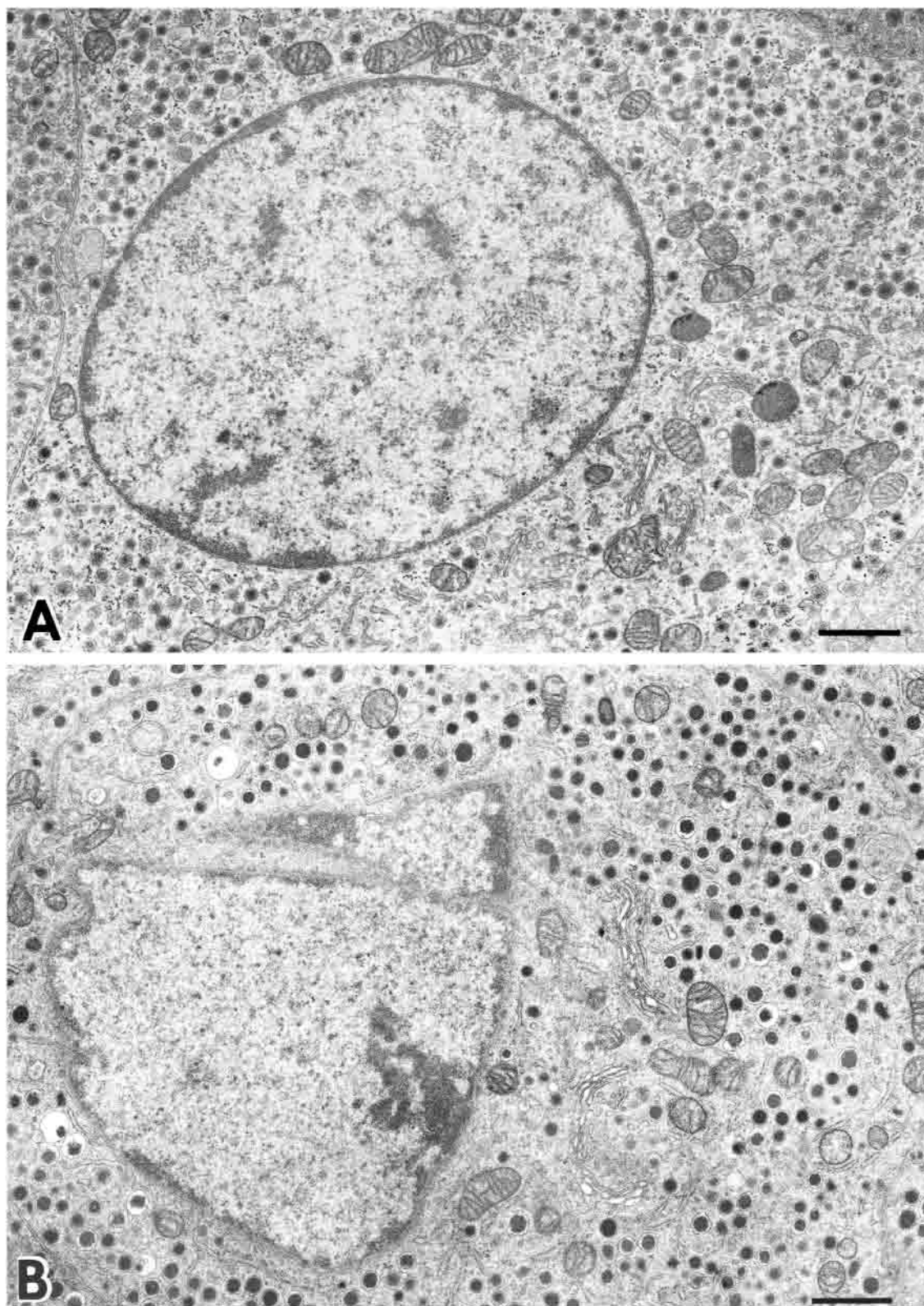


Fig. 2. Legend on the opposite page.

Results

Qualitative observations

Adrenaline cells in rats (Fig. 2A).

General observations. Adrenomedullary A cells in rats were irregularly arranged in rounded clusters or short cords and did not exhibit a follicular arrangement. Almost all nuclear profiles were circular in shape. Various sizes and forms of profiles of the RER were distributed in the cytoplasm. Large-sized RER showing lamellar accumulation were not numerous, but it was not uncommon for accumulation(s) of small-sized RER to exist in the cytoplasm on the basal-lamina side of the nucleus and the Golgi apparatus. The Golgi apparatus was medium in size, and various forms of the Golgi apparatus existed near the nucleus in many cases. Inside, and in the vicinity of the Golgi apparatus, centrosome and filamentous structures such as microtubules were occasionally seen. Mitochondria were encountered frequently and distributed inhomogeneously, more numerous near the Golgi apparatus but relatively few in the area in which granular vesicles were abundant. Granular vesicles were generally distributed almost homogeneously and densely over a broad area. The electron density of the content of vesicles varied from low to high, and granular content with low or medium density was more general. Sometimes lysosome-like corpuscles were seen, but their numbers were scanty compared with that of secretory granules. In general, no apparent polarity was seen in these structures of A cells in rats.

Golgi apparatus. The size and distribution of the Golgi apparatus varied, from small-sized ones which faced only a part of the nucleus to aggregated large-sized ones which were composed of several portions. In many cases, several profiles of a small amount of Golgi lamellae which were separated from each other largely surrounded a nucleus as a whole. Each Golgi apparatus consisted of the Golgi saccules, arranged in stacks, the trans-Golgi network, and small vesicles and vacuoles around the stacks and network. Granules were seen in the lumen of cisternae in rare cases. Small granular vesicles were frequently seen

in the vicinity of the Golgi apparatus. Various patterns of the Golgi apparatus were seen, i.e. a large Golgi apparatus consisting of many profiles of aggregated lamellae, a small Golgi apparatus consisting of only 1 to 2 profiles and others. The sizes of lamellar profiles also varied from a small type (length of the profile: ca. 250 nm) to a large type (length: more than 3 μm). Each lamella usually showed a curved profile, having a convex surface and a concave surface, but sometimes small profiles did not show such profiles. The direction of the convex or concave side also assumed various patterns. In some cases, the convex side faced toward the plasma membrane or toward the nucleus, and in other cases, several profiles of lamellae showed a ring-like arrangement as a whole. A mixed pattern was also seen. In general, the convex side faced toward the RER, and at a fairly high frequency, the concave side faced toward the nucleus.

Adrenaline cells in golden hamsters (Fig. 2B).

General observations. Adrenomedullary A cells of golden hamsters showed a follicular arrangement. In electron micrographs in which both the basal lamina facing the perivascular space and the follicular lumen were visible, it was common that the large-sized RER existed on the follicular-lumen side, and a well-developed Golgi apparatus was often seen on the basal-lamina side of the nucleus, though the RER and the Golgi apparatus were partially close to each other. Mitochondria were numerous scattered throughout the cytoplasm, being relatively more numerous near the Golgi apparatus. Inside, and in the vicinity of the Golgi area, centrosome and filamentous structures such as microtubules were seen at times. Their nucleus was ellipsoidal in outline but their nuclear profiles often showed irregular shapes, having shallow or deep invaginations of the nuclear membrane—which more frequently localized on the Golgi-apparatus side (Fig. 3B). Granular vesicles were distributed throughout the cytoplasm, but were relatively more dense on the basal-lamina side than on the follicular-lumen side. The distribution density of granular vesicles was lower in golden hamster A cells than in rat A cells. The electron density of the granular content was similarly high

Fig. 2. Adrenomedullary adrenaline cells of the rat and the golden hamster. **A:** Adrenaline cell of the rat. Several small profiles of the Golgi apparatus are seen near a circular nucleus. Scale bar=1 μm . **B:** Adrenaline cell of the golden hamster. A slightly large profile of the Golgi apparatus representing the arch-type arrangement is seen near an irregularly shaped nucleus. Scale bar=1 μm

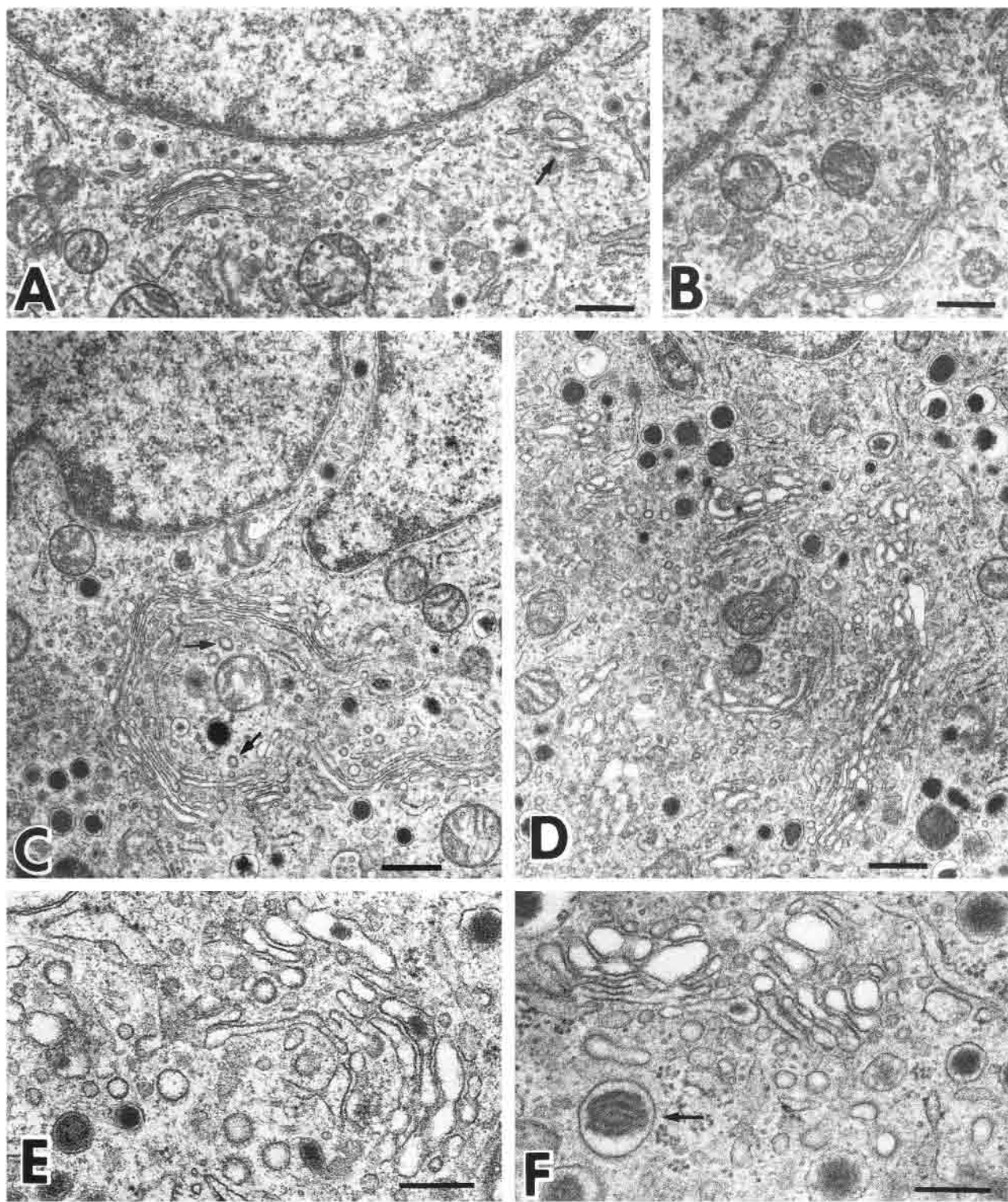


Fig. 3. Legend on the opposite page.

among most vesicles, but sometimes a small number of vesicles had contents with either a low electron density or somewhat different structures (Fig. 3F). Lysosomal bodies were occasionally seen, though very small in number. Thus, in A cells of golden hamsters, apparent polarity was seen in various intracellular structures.

Golgi apparatus. The Golgi apparatus existed mainly near the nucleus. In contrast to the rats, it was much more common for many lamellae of the Golgi apparatus to be localized on one side of the nucleus. The Golgi apparatus in golden hamsters was similar to that in rats in composition, consisting of lamellae, vesicles, etc (Fig. 3E, F). The size and form of lamellae varied, from small to large, with the curvature of lamellae being apparent in large profiles. Granules with high electron density were

often seen in a part of the cisternae (Fig. 3E, F). These granule-containing cisternae were divided into two types: 1) round granules in enlarged cisternae; 2) elongated elliptic granules in nonenlarged cisternae. The former was seen mainly on the concave side and the latter on the convex side. Small granular vesicles were often seen near the Golgi apparatus. The location of the Golgi apparatus in the cell showed apparent polarity, but the direction of the convex or concave surface of Golgi lamellae in the cell varied, and a circular arrangement of lamellar profiles or aggregated arrangement of those which took various directions were frequently encountered. However, the side of the Golgi lamellae which faced toward the RER was generally the convex or the lateral one.

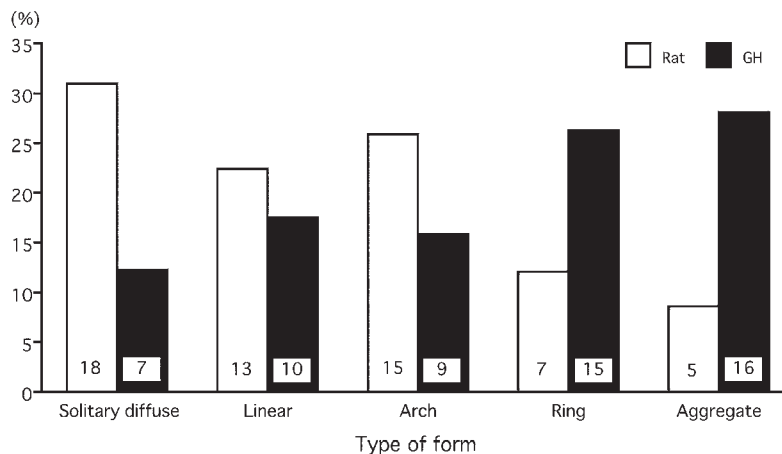


Fig. 4. Ratio of each type of Golgi apparatus form. Each number in bars represents the number of cells counted. GH: Golden hamster.

Fig. 3. Various types of the Golgi apparatus in adrenomedullary adrenaline cells of rats and golden hamsters. **A:** Solitary diffuse type of the Golgi apparatus in the rat. Two profiles of the Golgi stack which are separated from each other are present near a nucleus, and one represents a very small profile (arrow). **B:** Arch type of the Golgi apparatus in the rat. The concave side of the Golgi apparatus faces toward a nucleus and forms an arch-like shape. **C:** Ring type of the Golgi apparatus in the golden hamster. Large-sized coated vesicles (arrows) are seen inside the lamellae showing a ring-type arrangement. Invaginations of the nuclear membrane are seen. **D:** Aggregate type of the Golgi apparatus of the golden hamster. An assembly of several cross sections of large-sized Golgi lamellae which are arranged in different directions. **E:** The Golgi apparatus of the golden hamster and vesicles around the lamellae. Many large-sized coated vesicles are seen. A part of the cisternae are swollen, and granules with high electron density are seen in the cisternae. **F:** The Golgi lamellae and vesicles surrounding them in the golden hamster. Various sizes of granular vesicles are seen around the Golgi lamellae in addition to many smooth-clear vesicles. Granular contents assuming a spiral-like shape (arrow) are also seen in a vesicle. Scale bars = 0.5 μm (A, B, C, D); 0.3 μm (E, F).

Quantitative observations

Volume density of the Golgi apparatus

The volume density of the Golgi apparatus was higher in golden hamsters (Mean \pm SE, number of animals: 1.51 ± 0.11 , 21) than in rats (0.93 ± 0.09 , 22) (t-test: $P < 0.001$).

Type of the form of the Golgi apparatus

The forms of the profiles of the Golgi apparatus were classified into 5 types and the frequency of each type was examined.

- a. Solitary diffuse type (Fig. 3A): Relatively small-sized lamellae existed solitarily or distributed diffusely and sparsely in a broad area.
- b. Linear type: Several lamellae showed an almost linear arrangement.
- c. Arch type (Fig. 3B): Large lamellae showed an arch-like arrangement.
- d. Ring type (Fig. 3C): The lamellar structure was larger than that in the arch type and showed an almost ring-like arrangement.
- e. Aggregate type (Fig. 3D): Several large profiles of lamellae which were arranged in different directions aggregated and formed a large Golgi area.

As shown in Figure 4, the large types (the ring type and the aggregate type) were seen more frequently in golden hamsters (chi-square test: $P < 0.005$).

Diameter and numerical density of Golgi vesicles

Diameters and numerical densities of smooth-clear vesicles, coated vesicles and granular vesicles in the whole Golgi apparatus and in each region are shown in Figures 5 and 6, respectively. The results of statistical analyses by ANOVA are shown in Table 1.

Diameter. As shown in Table 1, species differences between rats and golden hamsters in the mean diameters of Golgi vesicles in the whole Golgi area were seen only in that of coated vesicles (two-way ANOVA: $P < 0.005$) although the mean diameter of granular vesicles tended to be slightly larger in hamsters than in rats. The mean diameter of coated vesicles was larger in hamsters than in rats, and the differences were evident on the convex (TK: $P < 0.01$) and concave sides ($P < 0.01$) (Fig. 5). Regional differences in the mean diameter of smooth-clear vesicles were seen in both species (one-way ANOVA, rat: $P < 0.001$; hamster: $P < 0.01$). The mean diameter was larger on the concave than the lateral (TK: $P < 0.01$) or the convex side ($P < 0.01$) in rats, and was larger on the concave than the convex side ($P < 0.01$) in hamsters, in which—on the lateral side—it showed a higher value than in rats ($P < 0.05$). Patterns of regional differences in the smooth vesicle diameter differed between two species (interaction by two-way ANOVA: $P < 0.01$). Neither species nor regional differences were seen in the mean diameter of granular vesicles.

Numerical density. As shown in Table 1, numerical densities of smooth-clear vesicles, coated vesicles,

Table 1. Statistical evaluation of the significance of differences in the diameter and numerical density of Golgi vesicles by means of analysis of variance (ANOVA)

		Diameter			Numerical density		
	Vesicle type:	Smooth-clear	Coated	Granular	Smooth-clear	Coated	Granular
Two-way							
Species(S)		NS	$P < 0.005$	NS	NS	NS	NS
Region (R)		$P < 0.001$	$P < 0.05$	$P < 0.05$	$P < 0.001$	$P < 0.01$	NS
S x R		$P < 0.01$	NS	NS	NS	NS	$P < 0.05$
One-way							
Region (R)	Rat	$P < 0.001$	NS	NS	$P < 0.001$	NS	NS
	GH	$P < 0.01$	NS	NS	$P < 0.005$	$P < 0.05$	NS

GH: golden hamster; NS: not significant

and granular vesicles did not show species differences throughout the Golgi apparatus, but showed regional differences in smooth-clear vesicles in both species (one-way ANOVA, rat: $P<0.001$; hamster: $P<0.005$) and in coated vesicles in golden hamsters ($P<0.05$). Mean numerical densities of smooth-clear vesicles showed gradual increases from the convex to the lateral side (TK: $P<0.01$) and from the lateral to the concave side ($P<0.05$) in rats, and a lower value on the convex than on the lateral ($P<0.05$) or the concave side ($P<0.01$) in hamsters (Fig. 6). The mean density of coated vesicles was higher on the

concave than on the convex ($P<0.05$) or the lateral side ($P<0.05$) in hamsters. The mean density of smooth-clear vesicles on the convex side was higher in hamsters than in rats ($P<0.05$), and the mean densities of coated vesicles on the lateral side and granular vesicles on the convex side were higher in rats than in hamsters ($P<0.05$ and $P<0.01$, respectively). Regional patterns of the numerical densities of granular vesicles differed between the two species (interaction by two-way ANOVA: $P<0.05$).

A summary of results is given in Table 2.

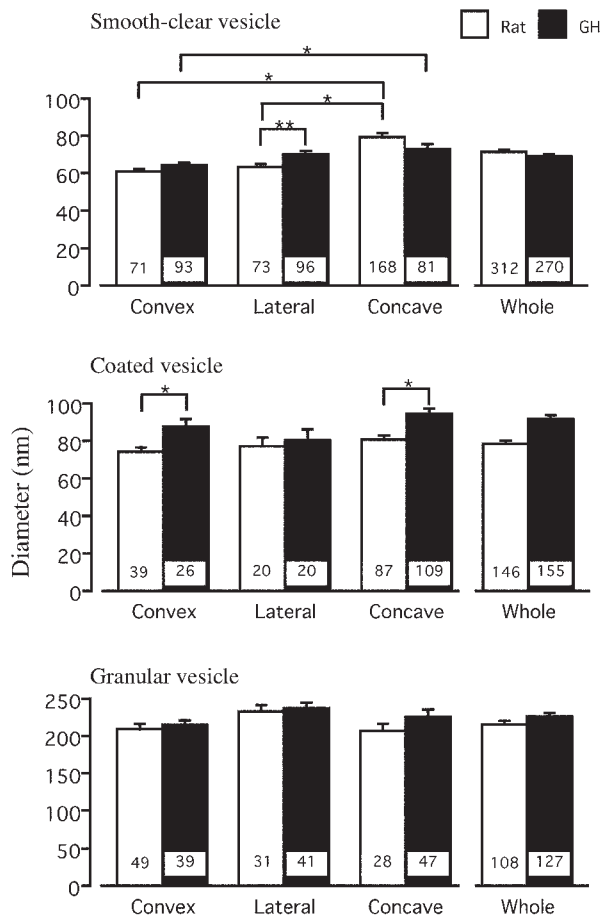


Fig. 5. Diameter of Golgi vesicles. Each bar indicates the mean \pm standard error of diameter. Each number in bars represents the number of vesicles. GH: Golden hamster. *: $P<0.01$, **: $P<0.05$ (TK)

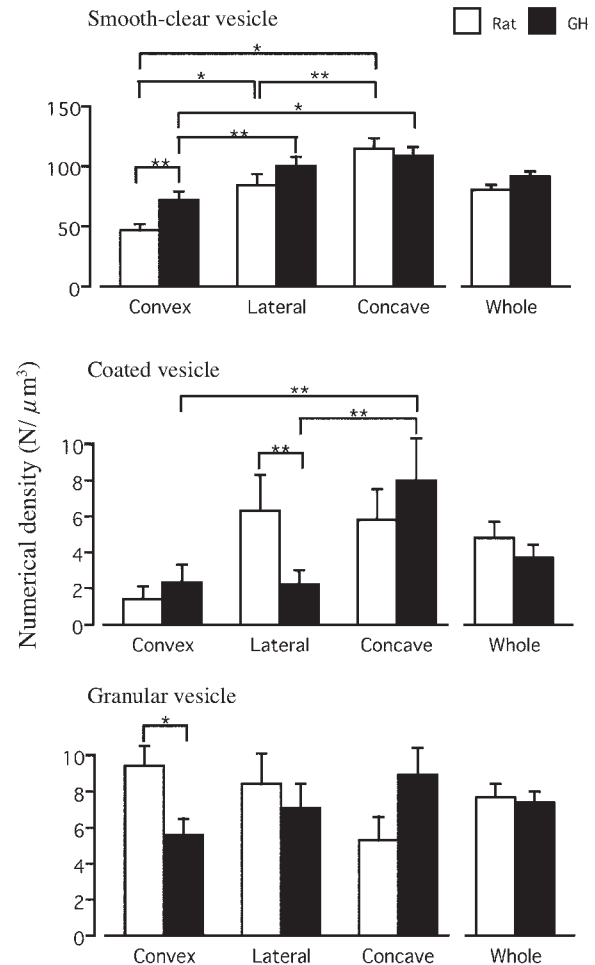


Fig. 6. Numerical density of Golgi vesicles. Each bar indicates the mean \pm standard error. Numbers of cells counted were 29 in rats and 41 in GHs, respectively. GH: Golden hamster. *: $P<0.01$, **: $P<0.05$ (TK)

Discussion

As shown in the present results, remarkable differences in various ultrastructures including the Golgi apparatus were seen in adrenomedullary A cells of golden hamsters compared with those of rats. As noted above, golden

hamsters show characteristic differences compared with rats at the histo-cytological and molecular levels in adrenal medullae. From many reported results, it seems that these various differences in adrenal medullae converge into at least two, possibly three, lines, as discussed below.

Table 2. Summary of results on the morphological features of adrenomedullary adrenaline cells.

1. Species difference			Rat	vs	GH
A. Adrenaline cell					
1) Cellular arrangement			Rounded clusters or short cords		Follicular
2) Structural polarity			– or not apparent		+
B. Golgi apparatus as a whole					
1) Size				<	
2) Frequency of large-scaled and complexly organized profiles				<	
3) Frequency of granules in Golgi cisternae			– or almost none		+
4) Diameter of coated vesicle				<	
5) Interaction (Species vs Region)					
	Diameter of smooth-clear vesicle			+	
	Numerical density of granular vesicle			+	
C. Golgi vesicles in relation to the region					
Smooth-clear	Diameter	Lateral		<	
	Numerical density	Convex		<	
Coated	Diameter	Convex or Concave		<	
	Numerical density	Lateral		>	
Granular	Numerical density	Convex		>	
2. Regional difference in Golgi vesicle					
		Vesicle type			
		Smooth-clear	Coated	Granular	
Rat	Diameter	Convex or Lateral < Concave	–	–	
	Numerical density	Convex < Lateral < Concave	–	–	
GH	Diameter	Convex < Concave	–	–	
	Numerical density	Convex < Lateral or Concave	Convex or Lateral < Concave	–	

GH: Golden hamster

Quantitative differences in cellular structures and chemical activities

It has been reported that the adrenal medullae of adult hamsters contain many cells with met- and leu-enkephalin immunoreactivity, but those of normal adult rats generally have at best only a very small amount of enkephalin immunoreactivity (Hexum *et al.*, 1980; Bohn *et al.*, 1983; Somogyi *et al.*, 1984). Franklin *et al.* (1991) reported that in the hamster adrenal, enkephalin-containing peptides levels are 400 times and preproenkephalin mRNA levels are 90 times greater than those found in the rat adrenal. Moreover, the intensity of met-enkephalin immunoreactivity observed following intracranial surgery is higher in hamster A cells than in rat A cells (Kachi *et al.*, 1993; Kimura and Kachi, 1996). Related to these, galanin immunoreactivity has been found in adrenomedullary chromaffin cells of Djungarian hamsters and guinea pigs, but not in those of rats (Zentel *et al.*, 1990). Somogyi *et al.* (1984) reported strong chromogranin-immunoreactivity in adrenal chromaffin cells of the sheep and ox, and only very weak immunoreactivity in the rat. In contrast, it is likely that the adrenaline level in the adrenal medulla is higher in rats than in hamsters (Scheving *et al.*, 1968; Lew and Quay, 1973) although the aromatic L-amino acid decarboxylase activity is higher in hamsters than in rats (Baker *et al.*, 1991). As shown in the present and previous results (Benedeczky and Smith, 1972; Grynszpan-Winograd, 1974), the Golgi apparatus and the RER in adrenomedullary A cells of golden hamsters are well developed compared with those of rats. These structural features seem to harmonize with those of chemical activities except A. This is interesting, because opioids have been reported to inhibit the secretion of catecholamines from adrenomedullary chromaffin cells (Kumakura *et al.*, 1980; Jarry *et al.*, 1989; Kitamura *et al.*, 2002). Therefore, the inverse relationship observed between the levels of peptide-related activities and A in A cells of each animal species seems to make sense.

In relation to this, it should be noted that the numerical density of coated vesicles was higher in rats than in hamsters in the lateral region of the Golgi apparatus in the present study. This may sound contradictory, because close relationships between peptide hormones and coated vesicles have been reported, as shall be discussed later. However, since a high activity level of the acid phosphatase has been demonstrated in rat A cells (Vincent *et al.*, 1982), it is very likely that at least part of these coated vesicles become primary lysosomes (Benedeczky and Smith, 1972; Grynszpan-Winograd, 1974), which may contribute to reduce the amount of structures

particularly related to the syntheses and processing of peptide hormones. In this context, it seems to be important to point out that the amount of structures and chemical activities in rat A cells are very changeable: for example, the size of the RER and enkephalin-immunoreactivity markedly increase following experimental treatments such as intracranial surgery (Kachi *et al.*, 1992, 1993, 1998), and chemical activities concerning the enkephalin synthesis also markedly increase following denervation (Schultzberg *et al.*, 1978; Lewis *et al.*, 1981; Bohn *et al.*, 1983). Therefore, it is conceivable that the activities of syntheses and processing of peptide hormones and their related structures and functions are in a strongly suppressed state under normal, physiological conditions compared with some experimentally induced conditions in rat A cells (for review see: Winkler *et al.*, 1986; Kachi *et al.*, 1998).

A more detailed discussion about the structure and function of the Golgi apparatus notes the following: It is now well established that the Golgi cisternae are heterogeneous in their composition and that secretory proteins or precursor peptides are transported from the cis (convex) to trans (concave) side of the Golgi. The generally accepted hypothesis is that the transport of substance from the RER to the Golgi and subsequent communication between its cisternae utilize discrete vesicular transport steps (Jamieson, 1998). As clearly shown in the present study, the size and the numerical density of smooth-clear vesicles in both animal species and the numerical density of coated vesicles in golden hamsters attained higher values in the concave compared with the convex and/or lateral side in the Golgi apparatus of A cells. These results indicate the existence of polarity in the transport of peptides/proteins using vesicles within the Golgi apparatus and suggest a gradual increase in vesicular contents due to the processing of materials during transport through the Golgi.

Our results also showed species differences in the quantitative nature of Golgi vesicles depending on the intra-Golgi region. Smooth vesicles on the convex and lateral sides of Golgi cisternae are considered to be transfer vesicles from the RER to the Golgi, transporting vesicles between Golgi cisternae and/or recycling vesicles released from its cistern to the RER (Gillingham and Munro, 2003) although at least part of the profiles may be those of tubules (Jamieson, 1998). Since smooth vesicles were larger in size on the lateral side and higher in numerical density on the convex side in hamster A cells than in rat A cells, these differences in structural indices seem to reflect those in the extent of traffic of peptides/proteins or the vesicle membrane itself between the RER and Golgi, at least partly.

Relationships between coated vesicles and peptide hormones have long been investigated (Tooze and Tooze, 1986). We reported that both the numerical density of Golgi coated vesicles (Kajihara *et al.*, 1997) and the intensity of met-enkephaline immunoreactivity (Kachi *et al.*, 1993, 1998) changed in parallel following experimental treatments, i.e. being higher in the sham-pinealectomized group than in normal and pinealectomized groups of rats. More recent studies have confirmed the transient appearance of lysosomal enzymes in the regulated pathway, suggesting that lysosomal enzymes are being removed from the maturing immature secretory granules (Arvan and Castle, 1998; Tooze, 1998). In relation to these findings, it has been shown that, in adrenomedullary A cells, opioids such as enkephalins are synthesized as inactive protein/peptide precursors from which the active molecules have to be liberated by proteolytic processing (Carmichael and Winkler, 1985; Winkler *et al.*, 1986; Winkler, 1993). These cleavages are likely to begin in the trans Golgi network and continue in the secretory vesicles. In addition, it is known that membrane recycling is important for returning Golgi components to the Golgi apparatus, etc., and that the vesicles mediating this retrieval originate as clathrin-coated buds on the surface of immature secretory vesicles (Carmichael and Winkler, 1985; Winkler, 1993; Arvan and Castle, 1998). Therefore, it seems to be reasonable that the Golgi coated vesicles are larger in size in hamster A cells than in rat A cells and exist more numerous on the concave side compared with the convex and lateral sides in hamster A cells.

As shown in previous and present studies, profiles of exocytosis in adrenomedullary chromaffin cells can be seen easily in hamsters, but not in rats (Diner, 1967; Benedeczký and Smith, 1972; Grynszpan-Winograd, 1974; Kachi *et al.*, 1985), and granules with high electron density in the Golgi cisternae are seen more frequently in hamster A cells than in rat A cells (Benedeczký and Smith, 1972; Grynszpan-Winograd, 1974). In addition, the granular content of secretory vesicles has been shown to be at least partly due to granins (Natori *et al.*, 1998; Tooze, 1998; Beuret *et al.*, 2004; Kim *et al.*, 2005).

In any case, considering that the whole size of the Golgi apparatus is much larger in hamster A cells than in rat A cells, it is suggested that the total numbers of smooth-clear, coated, and granular vesicles also reflect the amount of substances contained, e.g. opioids, proopioid peptides, hydrolytic enzymes, and granins, and that the synthesis, processing, and transport of peptides/proteins from RER via Golgi to secretory vesicles are more active in hamster A cells. More studies at the molecular level are obviously needed to explain more clearly such species and regional

differences in the quantitative nature of the three types of Golgi vesicles. In addition, large profiles of the Golgi apparatus with ring-type and aggregate-type arrangements were more frequently seen in hamster A cells compared with rat A cells in our results, but the chemical nature and functional significance of such unique structures are currently unknown.

Cellular polarity and regulatory mechanisms of cell activities

One of the most conspicuous characteristics in the adrenomedullary A cells of golden hamsters is a follicular arrangement of cells (Al-Lami, 1970; Grynszpan-Winograd, 1974) which is not apparent in rat A cells. Therefore, hamster A cells have distinct parts of the plasma membrane, i.e. the basal (which is covered with the basal lamina and faces the perivascular space), lateral, and apical (which has no covering with the basal lamina and faces the follicular lumen) membranes, whereas rat A cells do not show such apparent regional differentiation of the plasma membrane. Related to this, the incidence of large loop-like gap junctions between chromaffin cells has been reported to be higher in hamsters compared with rats (Grynszpan-Winograd *et al.*, 1980). Recent studies have shown that the plasma membrane of epithelial cells is segregated into chemically and functionally distinct domains, i.e. the apical domain and basolateral domain, and that these regional differences in membrane composition are maintained by newly synthesized membrane molecules sorted in the trans-Golgi network and endosomes (Drubin and Nelson, 1996; Rodríguez-Boulán and Müsch, 2005). However, the relationship between molecular mechanisms and the characteristic ultrastructure of the Golgi apparatus in hamster A cells is yet undetermined.

Due to this follicular arrangement of chromaffin cells, distinct polarity can be seen in the structures of hamster A cells. Namely, the RER exists on the follicular lumen-side, and the Golgi on the basal-lamina side of the nucleus (Grynszpan-Winograd, 1974; present study). The invagination of the nuclear membrane is present on the basal-lamina side or faces the Golgi (Taji and Kachi, 1999; present study), and large nerve endings are seen on the follicular-lumen side (Diner, 1967; Benedeczký and Smith, 1972; Grynszpan-Winograd, 1974). Granular vesicles distribute more densely in the cytoplasm near the basal lamina than in other cytoplasmic regions. In addition, it has been shown that the frequency of exocytoses is higher in the plasma membrane which faces the perivascular space than in other parts of the plasma membrane, and that the increased frequency of

exocytoses following pinealectomy occurs only in the plasma membrane which faces the perivascular space (Kachi *et al.*, 1985). These results suggest that observed phenomena are at least partly attributable to the regional differentiation of molecular mechanisms in the plasma membrane and/or the cytoplasm, such as Ca^{++} -related activities (Carmichael and Winkler, 1985; Arvan and Castle, 1998).

In any case, such conspicuous ultrastructural polarity observed in both the whole cell body and the Golgi apparatus in hamster A cells seems to harmonize with a hypothetical view that the flow of secretory substances from synthesis to storage and release has a relatively straightforward direction from the follicular-lumen side to the basal-lamina side, and the flow of the major regulatory influence at least is initiated by neural input from the follicular-lumen side.

In contrast, in rat A cells, small aggregations of RER were frequently seen on the basal-lamina side, and the concave surface of the Golgi apparatus faced toward a round-shaped nucleus in many cell profiles. Secretory vesicles, whose content had a relatively low electron density were distributed densely and almost homogeneously throughout the cytoplasm in many cell profiles. Interestingly, the numerical density of granular vesicles on the convex side of the Golgi apparatus was found to be higher in rat A cells than in hamster A cells. A possible explanation for this unexpected result could be, that in rat A cells, while the peptide hormone-related biochemical activities in the Golgi apparatus are relatively low, the A synthesis—of which the last step takes place in the cytosol, i.e. during the post-Golgi stage, (Carmichael and Winkler, 1985)—is relatively high. Therefore, it is suggested that granular vesicles observed in the vicinity of the convex surface of the Golgi apparatus are not those appearing immediately after detaching from the Golgi, but those drifting around for a while after detaching and gradually loaded with a relatively high concentration of A during the post-Golgi stage.

Concerning the main regulatory mechanisms of catecholamine biosynthesis, Axelrod and Reisine (1984) have stated that the stress-induced increase in tyrosine hydroxylase activity, the rate limiting step in dopamine synthesis, is due mainly to neuronal activity, whereas DBH activity is affected by nerve activity and the pituitary-adrenal axis, and the A-synthesizing enzyme (phenylethanolamine N-methyltransferase) activity is controlled mainly by glucocorticoids and ACTH. In addition, it has long been known that A cells in rats distribute in the juxtacortical region of the adrenal medulla, where noradrenaline cells exclusively exist in hamsters. This long-standing observation (for review see:

Kachi *et al.*, 1998) suggests that rat A cells exist in a more suitable place for regulation by glucocorticoids, compared with hamster A cells. According to Franklin (1997), proenkephalin gene expression is high in the hamster adrenal medulla in which tonic splanchnic nerve activity is required to maintain this high level of gene expression, whereas the gene expression is low in the rat adrenal medulla. Thus, all these situations of the adrenomedullary A cells in rats seem to reflect the relatively high importance of the regulation/modulation of A synthesis by hormonal mechanisms such as glucocorticoids during the post-Golgi stage in their endocrine mechanisms. In hamster A cells, on the other hand, opioids such as enkephalins, their precursors, and the regulation/modulation of their syntheses and processing by neural mechanisms seem to have a relatively high importance in their endocrine mechanisms.

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References

- Al-Lami F: Follicular arrangements in hamster adrenomedullary cells: Light and electron microscopic study. *Anat Rec* 168: 161-178 (1970).
- Al-Lami F, Farman N: Ultrastructural and histochemical study of the adrenal medulla in normal and cold-stressed Syrian hamsters. *Anat Rec* 181:113-130 (1975).
- Arvan P, Castle D: Sorting and storage during secretory granule biogenesis: looking backward and looking forward. *Biochem J* 332: 593-610 (1998).
- Axelrod J, Reisine TD: Stress hormones: Their interaction and regulation. *Science* 224: 452-459 (1984).
- Baker H, Abate C, Szabo A, Joh TH: Species-specific distribution of aromatic L-amino acid decarboxylase in the rodent adrenal gland, cerebellum, and olfactory bulb. *J Comp Neurol* 305:119-129 (1991).
- Benedeczky I, Smith AD: Ultrastructural studies on the adrenal medulla of golden hamster: origin and fate of secretory granules. *Z Zellforsch Mikrosk Anat* 124: 367-386 (1972).
- Beuret N, Stettler H, Renold A, Rutishauser J, Spiess M. Expression of regulated secretory proteins is sufficient to generate granule-like structures in constitutively secreting cells. *J Biol Chem* 279: 20242-9 (2004).
- Bohn MC, Kessler JA, Golightly L, Black IB: Appearance of enkephalin-immunoreactivity in rat adrenal medulla following treatment with nicotinic antagonists or reserpine. *Cell Tissue Res* 231: 469-479 (1983).

- Bornstein SR, Tian H, Haidan A, Bötner A, Hiroi N, Eisenhofer G, McCann SM, Chrousos GP: Deletion of tyrosine hydroxylase gene reveals functional interdependence of adrenocortical and chromaffin cell system in vivo. *Proc Nat Acad Sci* 97: 14742-14747 (2000).
- Carmichael SW, Winkler H: The adrenal chromaffin cell. *Sci Am* 253: 30-39 (1985).
- Diner O: L'expulsion des granules de la médullo-surrénale chez le Hamster. *C R Acad Sc Paris D* 265: 616-619 (1967).
- Drubin DG, Nelson WJ: Origins of cell polarity. *Cell* 84: 335-344 (1996).
- Franklin SO: Changes in proenkephalin gene expression in the developing hamster. *Dev Brain Res* 101: 239-248 (1997).
- Franklin SO, Yoburn BC, Zhu Y-S, Branch AD, Robertson HD, Inturrisi CE: Preproenkephalin mRNA and enkephalin in normal and denervated adrenals in the Syrian hamster: comparison with central nervous system tissues. *Brain Res Mol Brain Res* 10: 241-250 (1991).
- Gillingham AK, Munro S: Long coiled-coil proteins and membrane traffic. *Biochim Biophys Acta* 1641: 71-85 (2003).
- Grynspan-Winograd O: Ultrastructure of the chromaffin cell. In: *Handbook of physiology. Endocrinology section: Adrenal medulla* (Blaschko H, Sayers G, Smith AD, ed), Williams and Wilkins, Baltimore, 1974 (p. 295-308).
- Grynspan-Winograd O, Nicolas G: Intercellular junctions in the adrenal medulla: A comparative freeze-fracture study. *Tissue Cell* 12: 661-672 (1980).
- Gumbiner B, Kelly RB: Two distinct intracellular pathways transport secretory and membrane glycoproteins to the surface of pituitary tumor cells. *Cell* 28: 51-59 (1982).
- Hexum TD, Yang H-YT, Costa E: Biochemical characterization of enkephalin-like immunoreactive peptides of adrenal glands. *Life Sci* 27: 1211-1216 (1980).
- Jamieson JD: The Golgi complex: perspectives and prospectives. *Biochim Biophys Acta* 1404: 3-8 (1998).
- Jarry H, Dietrich M, Barthel A, Giesler A, Wuttke W: In vivo demonstration of a paracrine, inhibitory action of met-enkephalin on adrenomedullary catecholamine release in the rat. *Endocrinology* 125: 624-629 (1989).
- Kachi T, Banerji TK, Quay WB: Quantitative ultrastructural analysis of differences in exocytosis number in adrenomedullary adrenaline cells of golden hamsters related to time of day, pinealectomy, and intracellular region. *J Pineal Res* 2: 253-269 (1985).
- Kachi T, Takahashi G, Banerji TK, Quay WB: Rough endoplasmic reticulum in the adrenaline and noradrenaline cells of the adrenal medulla: effects of intracranial surgery and pinealectomy. *J Pineal Res* 12: 89-95 (1992).
- Kachi T, Takahashi G, Suzuki T, Kimura N, Banerji TK, Quay WB: Effects of pineal and intracranial surgery on the adrenal medulla: Quantitative morphological and immunohistochemical studies. In: *Melatonin and the pineal gland: From basic science to clinical application* (Touitou Y, Arendt J, Pévet P, ed), Elsevier, Amsterdam, 1993 (p. 277-280).
- Kachi T, Takahashi G, Suzuki T, Kimura N, Yanagisawa M, Kajihara S, Kudo H: Relationship between pineal gland and adrenal medulla. In: *A universal photoperiodic signal with diverse actions. Front Horm Res, vol. 21* (Tang PL, Pang SF, Reiter RJ, ed), Karger, Basel, 1996 (p. 51-59).
- Kachi T, Takahashi G, Suzuki T, Kimura N, Kajihara S, Kurushima M, Yamauchi T, Irie T: Dynamic and versatile structures of adrenal medulla, related to pineal and surgery. In: *Dynamic cells: Cell biology of the 21st century* (Yagihashi S, Kachi T, Wakui M, ed), Elsevier Science B. V., Amsterdam, 1998 (p. 47-58).
- Kajihara S, Sakamoto T, Kachi T: Electron microscopic observations on the Golgi apparatus in adrenomedullary adrenaline cells: comparison between rats and golden hamsters. *Acta Anat Nippon* 71: 699 (Abstract) (1996).
- Kajihara S, Takahashi G, Kachi T: Effects of pinealectomy and sham surgery on Golgi apparatus in adrenomedullary adrenaline cells. A quantitative electron microscopic study. *Hiroaki Med J* 48: 197-207 (1997).
- Kim T, Zhang C-f, Sun Z, Wu H, Loh YP: Chromogranin A deficiency in transgenic mice leads to aberrant chromaffin granule biogenesis. *J Neurosci* 25: 6958-6961 (2005).
- Kimura N, Kachi T: Effects of time of day, intracranial surgery and pineal hormone on methionine-enkephalin-like immunoreactivity in adrenal medullae of golden hamsters. *Hiroaki Med J* 48: 139-147 (1996).
- Kitamura G, Ohta T, Kai T, Kon Y, Ito S: Inhibitory effects of opioids on voltage-dependent Ca^{2+} channels and catecholamine secretion in cultured porcine adrenal chromaffin cells. *Brain Res* 942: 11-22 (2002).
- Kumakura K, Karoum F, Guidotti A, Costa E: Modulation of nicotinic receptors by opiate receptor agonists in cultured adrenal chromaffin cells. *Nature* 283: 489-492 (1980).
- Lew GM, Quay WB: Circadian rhythms in catecholamines in organs of the golden hamster. *Am J Physiol* 224: 503-508 (1973).

- Lewis RV, Stern AS, Kilpatrick DL, Gerber LD, Rossier J, Stein S, Udenfriend S: Marked increases in large enkephalin-containing polypeptides in the rat adrenal gland following denervation. *J Neurosci* 1: 80-82 (1981).
- Natori S, King A, Hellwig A, Wei β U, Iguchi H, Tsuchiya B, Kameya T, Takayanagi R, Nawata H, Huttner WB: Chromogranin B (secretogranin I), a neuroendocrine-regulated secretory protein, is sorted to exocrine secretory granules in transgenic mice. *EMBO J* 17: 3277-3289 (1998).
- Rodriguez-Boulant E, M \ddot{u} sch A: Protein sorting in the Golgi complex: Shifting paradigms. *Biochim Biophys Acta* 1744: 455-464 (2005).
- Scheving LE, Harrison WH, Pauly JE: Daily fluctuation (circadian) in levels of epinephrine in the rat suprarenal gland. *Am J Physiol* 215: 799-802 (1968).
- Schultzberg M, Lundberg JM, H \ddot{o} kfelt T, Terenius L, Elde RP, Goldstein M: Enkephalin-like immunoreactivity in gland cells and nerve terminals of the adrenal medulla. *Neuroscience* 3: 1169-1186 (1978).
- Sietzen M, Schober M, Fischer-Colbrie R, Scherman D, Sperk G, Winkler H: Rat adrenal medulla: levels of chromogranins, enkephalins, dopamine β -hydroxylase and of the amine transporter are changes by nervous activity and hypophysectomy. *Neuroscience* 22: 131-139 (1987).
- Somogyi P, Hodgson AJ, DeP Potter RW, Fischer-Colbrie R, Schober M, Winkler H, Chubb IW: Chromogranin immunoreactivity in the central nervous system. Immunochemical characterization, distribution and relationship to catecholamine and enkephalin pathways. *Brain Res Rev* 8: 193-230 (1984).
- Taji A, Kachi T: Effects of time of day and pinealectomy on adrenomedullary adrenaline cells in golden hamsters: an electron microscopic study. *Acta Anat Nippon* 75: 308 (Abstract) (1999).
- Tooze SA: Biogenesis of secretory granules in the trans-Golgi network of neuroendocrine and endocrine cells. *Biochim Biophys Acta* 1404: 231-244 (1998).
- Tooze J, Tooze SA: Clathrin-coated vesicular transport of secretory proteins during the formation of ACTH-containing secretory granules in AtT20 cells. *Cell* 49: 865-868 (1986).
- Vincent SR, Schultzberg M, Dalsgaard C-J: Fluoride-resistant acid phosphatase in the rat adrenal gland. *Brain Res* 253: 325-329 (1982).
- Winkler H: The adrenal chromaffin granule: a model for large dense core vesicles of endocrine and nervous tissue. *J Anat* 183: 237-252 (1993).
- Winkler H, Apps DK, Fischer-Colbrie R: The molecular function of adrenal chromaffin granules: Established facts and unsolved topics. *Neuroscience* 18: 261-290 (1986).
- Zentel HJ, Nohr D, M \ddot{u} ller S, Yanaihara N, Weihe E: Differential occurrence and distribution of galanin in adrenal nerve fibers and medullary cells in rodent and avian species. *Neurosci Lett* 120:167-170 (1990).