Electron Microscopic Study on the Newt Parathyroid Gland after Administration of Agents Affecting Autonomic Nerves

By

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The activity of the parathyroid gland has been investigated in different experimental models and it has been electronmicroscopically proved that the calcium level of serum is important factor in the regulation of the parathyroid gland. Furthermore, electron microscopic studies have demonstrated nerve fibers and/or nerve endings in perivascular spaces of the parathyroid gland (Roth and Munger, 1962; Munger and Roth, 1963; Capen et al., 1965; Rogers, 1965; Elliott and Arhelger, 1966; Mazzocchi et al., 1967; Nakagami, 1967; Ochi et al., 1970; Altenähr 1971; Altenähr and Seifert, 1971; Yeghiayan et al., 1972; Isono and Miyake, 1973; Shoumura, 1974; Sakuma, 1974). Recently, Chin (1974) has reported that unmyelinated nerve ending was present between chief cells as well as in perivascular spaces of the turtle parathyroid gland. A nervous influence on function of the parathyroid gland cells seems possible. It has, however, not been ultrastructurally confirmed as yet.

The present study concerns the fine structure of the newt parathyroid gland under the influence of administration of atropine sulfate or pilocarpine hydrochloride.

Materials and Methods

Eighty-eight adult newts, *Triturus pyrrhogaster*, of both sexes (7±1 g body weight) kept in a water tank at 15-17°C in November were used in this study. The newts were collected in the suburbs of Gifu City. Eight newts served as the controls. The newts were intraperitoneally given 0.2 mg/kg body weight of atropine sulfate (atropine) or 4 mg/kg body weight of pilocarpine hydrochloride (pilocarpine). The parathyroid glands of eight newts were removed without anesthesia at 1, 3, 6, 12 and 24 hr after injection of atropine or pilocarpine, res-
pectively. The tissues were fixed in 1% osmium tetroxide in 0.2 M Millonig's buffer, pH 7.4, for one hr, dehydrated in a graded acetone series, and embedded in Epon 812. Thin sections cut on a Porter-Blum MT-1 ultramicrotome were stained with uranyl acetate and lead citrate, and examined with a Hitachi HS-8 electron microscope.

The dimensions of the cell minus the nucleus were measured using a planimeter in fifty photographs (final magnification 12,000) of the parathyroid gland of each experimental newt. The number of secretory granules, heterogeneously dense bodies, vacuolar bodies and lipofuscin-like bodies was counted in each photograph, and the average values per 100 μ² of cytoplasm were calculated.

The serum calcium levels of all the animals were measured by the Yanagisawa's method (1955).

**Results**

As shown in Table 1, changes of the serum calcium concentration between the control animals and the agent treated animals were not recognized.

The newt parathyroid gland was capsulated with thin connective tissue, and was made up of packed groups of the parenchymal cells. Each group of the parenchymal cells was surrounded by connective tissue containing rich blood vessels, unmyelinated nerve fibers, connective tissue cells and collagen fibers (Fig. 1, Inset).

The parenchymal cells were divided into the basal cells containing

| Table 1. Serum calcium level (mg per 100 ml) of the control and experimental newts |
|---|---|
| **Group** | **No. of newts** | **Mean values ± S. E. serum calcium** |
| Controls | 8 | 10.3 ± 0.47 |
| Time after atropine injection |  |  |
| 1 hr | 8 | 11.2 ± 0.94 |
| 3 hr | 8 | 10.9 ± 1.03 |
| 6 hr | 8 | 11.0 ± 0.75 |
| 12 hr | 8 | 10.5 ± 0.74 |
| 24 hr | 8 | 10.6 ± 1.23 |
| Time after pilocarpine injection |  |  |
| 1 hr | 8 | 10.1 ± 1.03 |
| 3 hr | 8 | 10.3 ± 0.57 |
| 6 hr | 8 | 9.8 ± 0.98 |
| 12 hr | 8 | 10.5 ± 1.39 |
| 24 hr | 8 | 10.1 ± 0.54 |
many filaments, a few cell organelles and inclusion bodies, and the suprabasal cells having a few filaments, numerous cell organelles, secretory granules and inclusion bodies (Fig. 1), as described by the present authors (Setoguti et al., 1970a, b; Isono et al., 1971; Isono and Shoumura, 1973a, b). No remarkable changes in the fine structure of the basal cell were observed after administration of atropine or pilocarpine.

In the control newts, the suprabasal cells contained a few filaments, numerous cell organelles, secretory granules, heterogeneously dense bodies, vacuolar bodies and lipofuscin-like bodies (Fig. 1). Both inside and outside a moderately well-developed Golgi area in most cells, there were found small or large groups of secretory granules, heterogeneously dense bodies and vacuolar bodies. There were transitional forms between each of secretory granules and various bodies. Heterogeneously dense bodies were subdivided into a vesicular type filled mainly with numerous vesicles and a lysosomal type having tubular structure, dense material and lipid-like substance. The lysosomal type was more dominant than the vesicular type. Vacuolar bodies contained various number of uncoated vesicles and/or coated ones and floccular or dense material.

At 3, 6 and 12 hr after administration of atropine, enlarged intercellular spaces had a large amount of floccular substance. The elements of granular endoplasmic reticulum were so well developed after administration of atropine as compared with those of the control newts, and were frequently situated close to mitochondria at 3-24 hr after

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of newts</th>
<th>Secretory granules</th>
<th>Heterogeneously dense bodies</th>
<th>Vacuolar bodies</th>
<th>Lipofuscin-like bodies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>8</td>
<td>1.22 ± 0.15</td>
<td>2.54 ± 0.23</td>
<td>2.44 ± 0.45</td>
<td>0.72 ± 0.11</td>
</tr>
<tr>
<td>Time after atropine injection</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 hr</td>
<td>8</td>
<td>0.97 ± 0.34*</td>
<td>2.40 ± 0.65</td>
<td>2.28 ± 0.32</td>
<td>0.36 ± 0.23**</td>
</tr>
<tr>
<td>3 hr</td>
<td>8</td>
<td>0.71 ± 0.20*</td>
<td>1.39 ± 0.54*</td>
<td>2.58 ± 0.38</td>
<td>0.38 ± 0.12**</td>
</tr>
<tr>
<td>6 hr</td>
<td>8</td>
<td>0.10 ± 0.00*</td>
<td>0.33 ± 0.00*</td>
<td>5.37 ± 0.78*</td>
<td>0.46 ± 0.23</td>
</tr>
<tr>
<td>12 hr</td>
<td>8</td>
<td>0.78 ± 0.18*</td>
<td>0.69 ± 0.12*</td>
<td>3.04 ± 0.58*</td>
<td>0.65 ± 0.33</td>
</tr>
<tr>
<td>24 hr</td>
<td>8</td>
<td>1.41 ± 0.62</td>
<td>2.53 ± 0.63</td>
<td>2.22 ± 0.54</td>
<td>0.67 ± 0.37</td>
</tr>
</tbody>
</table>

| Time after pilocarpine injection |                      |                    |                              |                 |                      |
| 1 hr                            | 8            | 1.56 ± 0.53        | 2.55 ± 0.37                 | 2.40 ± 0.22     | 0.78 ± 0.45         |
| 3 hr                            | 8            | 3.89 ± 0.75*       | 3.01 ± 0.52*                | 1.18 ± 0.42**   | 1.09 ± 0.42**       |
| 6 hr                            | 8            | 2.11 ± 0.45*       | 5.03 ± 0.78*                | 1.24 ± 0.27*    | 1.24 ± 0.38**       |
| 12 hr                           | 8            | 2.02 ± 0.48*       | 2.93 ± 0.42*                | 1.16 ± 0.36*    | 0.98 ± 0.39         |
| 24 hr                           | 8            | 1.34 ± 0.29        | 2.55 ± 0.28                 | 0.92 ± 0.22*    | 0.95 ± 0.22         |

* <0.01, ** <0.05; Significantly different from control values

Table 2. Mean values ± S.E. of secretory granules and inclusion bodies per 100 μ² of the cytoplasm of the suprabasal cells in the control and experimental newts
administration of atropine. The number of mitochondria was almost unchanged in the atropine treated newts. A well-developed Golgi apparatus was distributed widely throughout the cytoplasm at 3, 6 and 12 hr after administration of atropine (Fig. 2). Secretory granule levels were decreased significantly \( (p<0.01) \) from a mean control value of 1.22 per 100 \( \mu^2 \) to 0.97, 0.71, 0.10 and 0.78 per 100 \( \mu^2 \) at 1, 3, 6 and 12 hr after administration of atropine, respectively (Fig. 2, Table 2). Heterogeneously dense body levels were decreased significantly \( (p<0.01) \) from a mean control value of 2.54 per 100 \( \mu^2 \) to 1.39, 0.33 and 0.69 per 100 \( \mu^2 \) at 3, 6 and 12 hr after administration, respectively (Fig. 2, Table 2). The vesicular type of heterogeneously dense body was more dominant than the lysosomal type, and most of the heterogeneously dense bodies consisted of the vesicular type at 3, 6 and 12 hr (Fig. 3). Vacuolar body levels were increased significantly \( (p<0.01) \) from a mean control value of 2.44 per 100 \( \mu^2 \) to 5.37 and 3.04 per 100 \( \mu^2 \) at 6 and 12 hr after administration, respectively (Fig. 4, Table 2). Lipofuscin-like body levels were decreased significantly \( (p<0.05) \) from a mean control value of 0.72 per 100 \( \mu^2 \) to 0.36 and 0.38 per 100 \( \mu^2 \) at 1 and 3 hr after administration, respectively (Table 2).

In the suprabasal cells after administration of pilocarpine, the elements of granular endoplasmic reticulum were moderately well developed, and in some cells they were not so well developed as compared with those of the control newts. The number of mitochondria was almost unchanged in the pilocarpine treated animals. The Golgi apparatus was not so well developed in most of the cells at 6, 12 and 24 hr after administration of pilocarpine (Fig. 5). Secretory granule levels were increased significantly \( (p<0.01) \) from a mean control value of 1.22 per 100 \( \mu^2 \) to 3.89, 2.11 and 2.02 per 100 \( \mu^2 \) at 3, 6 and 12 hr after administration of pilocarpine, respectively (Fig. 5, Table 2). Heterogeneously dense body levels were also increased significantly \( (p<0.01) \) from a mean control value of 2.54 per 100 \( \mu^2 \) to 3.01, 5.03 and 2.93 per 100 \( \mu^2 \) at 3, 6 and 12 hr after administration, respectively (Fig. 6, Table 2). The lysosomal type of heterogeneously dense body was more dominant than the vesicular type in the pilocarpine treated newts (Fig. 6). Vacuolar body levels were decreased from a mean control value of 2.44 per 100 \( \mu^2 \) to 1.18, 1.24, 1.16 and 0.92 per 100 \( \mu^2 \) at 3, 6, 12 and 24 hr after administration, respectively (Table 2). Lipofuscin-like body levels were increased significantly \( (p<0.05) \) from a mean control value of 0.72 per 100 \( \mu^2 \) to 1.09 and 1.24 per 100 \( \mu^2 \) at 3 and 6 hr after administration, respectively (Table 2).

In most of the suprabasal cells of the parathyroid gland under administration of agents, autophagic vacuoles and lipid droplets were not so markedly changed in number.
Discussion

It has been widely accepted that the secretory activity of the parathyroid gland is regulated by serum calcium concentration. Furthermore, many workers have described that nerve fibers and/or nerve endings were present in perivascular spaces of the parathyroid gland (Roth and Munger, 1962; Munger and Roth, 1963; Capen et al., 1965; Rogers, 1965; Elliott and Arhelger, 1966; Mazzocchi et al., 1967; Nakagami, 1967; Ochi et al., 1970; Altenåhr, 1971; Altenåhr and Seifert, 1971; Yaghiayan et al., 1972; Shoumura, 1974; Sakuma, 1974). In this study, nerve fibers were observed in perivascular spaces of the newt parathyroid gland (Fig. 1, Inset). Recently, Chin (1974) has showed nerve endings between the chief cells of the turtle parathyroid gland. Morii et al., (1963, 1965) and Fujita et al., (1975) have physiologically demonstrated the possibility concerning the nervous regulation of the parathyroid function. It has, however, not been electronmicroscopically confirmed as yet.

In the present work, remarkable changes in the fine structure of the suprabasal cells were observed after injection of atropine or pilocarpine (Fig. 2, 3, 4, 5, 6, Table 2). However, as described previously by the present authors (for reference see Isono and Shoumura, 1973b) and also in this experiment, ultrastructural alterations of the basal cells were not recognized.

In most suprabasal cells after administration of atropine, the elements of granular endoplasmic reticulum and the Golgi apparatus were so well developed as compared with the control newts (Fig. 2). These observations suggest an increase in protein synthesis by the cell of the experimental animals as compared with the control newts. Similar results have been observed in the chief cells which were in the hyperfunctional condition (Capen et al., 1965; Melson, 1966; Roth and Raisz, 1966; Capen and Young, 1967; Capen and Rowland, 1968; Isono et al., 1969; Setoguti et al., 1970b; Youshak and Capen, 1970; Furuta, 1971; Isono et al., 1971; Iwatsutsumi, 1971; Nunez et al., 1972; Isono and Shoumura, 1973b; Chin, 1974; Krstić et al., 1974; Sakuma, 1974; Fujii, 1975; Sannes and Hayes, 1975; Isono et al., 1976). In the present research, in most suprabasal cells at 1-12 hr after administration of atropine, secretory granules were decreased in number, reaching a minimum at 6 hr (Fig. 2, Table 2). This finding has been observed in the active chief cells of other animal species (Capen and Young, 1967; Capen and Rowland, 1968; Youshak and Capen, 1970; Sannes and Hayes, 1975). The decrease of secretory granules in most suprabasal cells of the experimental animals means that their extrusion was more accelerated than synthesis by injection of atropine. The present authors (Isono and Shoumura, 1973b) have published in the parathyroid gland
of the EDTA treated newts that heterogeneously dense bodies and lipofuscin-like bodies were increased while vacuolar bodies were decreased in number. In this work, however, the number of heterogeneously dense bodies at 3, 6 and 12 hr, and lipofuscin-like bodies at 6 and 12 hr was decreased while the number of vacuolar bodies at 6 and 12 hr after administration of atropine was increased (Fig. 3, 4, Table 2). Most of the heterogeneously dense bodies consisted of the vesicular type at 3, 6 and 12 hr (Fig. 3). It is thought in the atropine treated newts that most secretory granules were changed into many vacuolar bodies through heterogeneously dense bodies of the vesicular type, since there were transitional forms between each of secretory granules, heterogeneously dense bodies of the vesicular type and vacuolar bodies. Such transformation indicating a parathormone releasing-process (Isono and Shoumura, 1973b) might be accelerated by injection of atropine.

In most suprabasal cells after administration of pilocarpine, cell organelles such as granular endoplasmic reticulum and the Golgi apparatus became smaller in size than those of the control animals (Fig. 5). These findings might show the hypofunctional condition of the suprabasal cells, as reported by many authors (Montskò et al., 1963; Stoeckel and Porte, 1966; Nakagami, 1967; Hara and Nagatsu, 1968; Tanaka, 1969; Murakami, 1970; Iwatsutsumi, 1971; Isono and Shoumura, 1973a; Chin, 1974; Sakuma, 1974). In the present work, in most suprabasal cells at 3, 6 and 12 hr after administration of pilocarpine, secretory granules were increased in number (Fig. 5, Table 2). This result is consistent with the observation of Roth and Raisz (1966) and Murakami (1970). The increase of secretory granules in most suprabasal cells means that their extrusion was more suppressed than synthesis by injection of pilocarpine. In this work, heterogeneously dense bodies of the lysosomal type were increased in number at 3, 6 and 12 hr after administration of pilocarpine (Fig. 6, Table 2). Similar finding has been recognized in the parathyroid gland under the hypofunctional condition of the parathormone treated newts (Isono and Shoumura, 1973a). Furthermore, it has been reported that vacuolar bodies were increased while lipofuscin-like bodies were decreased in number (Isono and Shoumura, 1973a). In the present study, however, the number of vacuolar bodies was decreased at 3–24 hr while the number of lipofuscin-like bodies was increased at 3 and 6 hr after administration of pilocarpine (Table 2). The present authors (Setoguti et al., 1970c) have indicated in the newt parathyroid gland under artificial hibernation that reaction product of acid phosphatase was present on heterogeneously dense bodies of the lysosomal type and some of vacuolar bodies. It is thought in the pilocarpine treated newts that numerous secretory granules were changed into vacuolar bodies through a large number of heterogeneously
dense bodies of the lysosomal type, since there were transitional forms between each of secretory granules, heterogeneously dense bodies of the lysosomal type and vacuolar bodies. Such transformation showing a lysosomal digestion-process of secretory granules (Isono and Shoumura, 1973b) might be accelerated by injection of pilocarpine.

In the present study, serum calcium concentration of the agent treated newts was almost unchanged as compared with the control animals (Table 1). Accordingly, the ultrastructural alterations observed in the newt parathyroid gland after administration of agents would support the participation of the autonomic nervous system.

**Summary**

The newt parathyroid gland after administration of atropine or pilocarpine was studied with electron microscope.

Serum calcium concentration was almost unchanged after administration of atropine or pilocarpine. Remarkable changes in the ultrastructure of the basal cells were not recognized after administration of agents.

In most suprabasal cells after administration of atropine, the elements of granular endoplasmic reticulum and the Golgi apparatus were so well developed as compared with the control animals. The number of secretory granules, heterogeneously dense bodies and lipofuscin-like bodies was decreased, while the number of vacuolar bodies was increased after administration of atropine. Most of the heterogeneously dense bodies consisted of the vesicular type at 3, 6 and 12 hr after injection.

In most suprabasal cells after administration of pilocarpine, cell organelles such as granular endoplasmic reticulum and the Golgi apparatus became smaller in size than those of the control newts. Secretory granules, heterogeneously dense bodies and lipofuscin-like bodies were increased, while vacuolar bodies were decreased in number after administration of pilocarpine. Most of the heterogeneously dense bodies consisted of the lysosomal type at 3, 6 and 12 hr after injection.

Such ultrastructural alterations in the newt parathyroid gland after administration of atropine or pilocarpine would support the participation of the autonomic nervous system.

**Acknowledgement**

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References

PLATES
Explanation of Figures

Plate I

Fig. 1. Basal cell (BC) and suprabasal cells (SC) of parathyroid gland in control newt. Basal cell contains many filaments, a few cell organelles and inclusion bodies. Suprabasal cells contain a few filaments, numerous cell organelles and many inclusion bodies. N, nucleus. \( \times15,000 \). Inset. Showing unmyelinated nerve fiber in perivascular space. \( \times16,000 \)
Plate II

Fig. 2. Portion of suprabasal cells 6 hr after atropine administration. Well-developed Golgi apparatus (G) are observed. Secretory granule is not seen. m, mitochondria; LP, lipofucin-like body. $\times 32,000$
Plate III

Fig. 3. Portion of suprabasal cells 3 hr after atropine administration. Showing secretory granule (S) and heterogeneously dense bodies of vesicular type (VT) filled with numerous vesicles. m, mitochondria.  ×44,000

Fig. 4. Portion of suprabasal cells 6 hr after atropine administration. Many vacuolar bodies (V) are observed.  ×18,000
Plate IV

Fig. 5. Portion of suprabasal cell 3 hr after pilocarpine administration. Secretory granules (S) and heterogeneously dense body of lysosomal type (LT) are seen in large groups. Poorly-developed Golgi apparatus (G) is observed. LP, lipofuscin-like body. ×38,000

Fig. 6. Portion of suprabasal cell 6 hr after pilocarpine administration. Many heterogeneously dense bodies of lysosomal type (LT) having tubular structures, dense material and lipid-like substance are found. S, secretory granules; LP, lipofuscin-like body. ×35,000