

# Changes of profiles of neutrophil granulocytes induced with the administration of L-DOPA and prednisolone

M. Mato and E. Aikawa

Department of Anatomy, Jichi Medical School, Minamikawachi, Tochigi (Japan 329-04), 8 September 1977

**Summary.** Morphological changes of granulocytes following combined treatment with L-DOPA and prednisolone were elucidated at the electron microscopic level. Neutrophil granulocytes exhibited marked changes in their surface as evidenced by scanning electron microscope. It is suggested that neutrophil granulocytes are sensitive to some biogenic amines.

As already reported<sup>1,2</sup>, serotonin-like substance was demonstrated in granulocytes by the authors' method, and the substance fluctuated rhythmically throughout a day. Further, if L-DOPA and 5HT were administered, respectively, L-DOPA was efficiently taken up by granulocytes, but 5HT could hardly be incorporated into the cytoplasm.

Recently it was also clarified that the uptake of biogenic amines by granulocytes was specifically enhanced with a combined application of prednisolone, prostaglandin and cyclic AMP<sup>3</sup>. According to the previous paper, the uptake capacity of granulocytes for L-DOPA ran parallel with the activity of adenylate cyclase in their membrane system<sup>3,4</sup>.

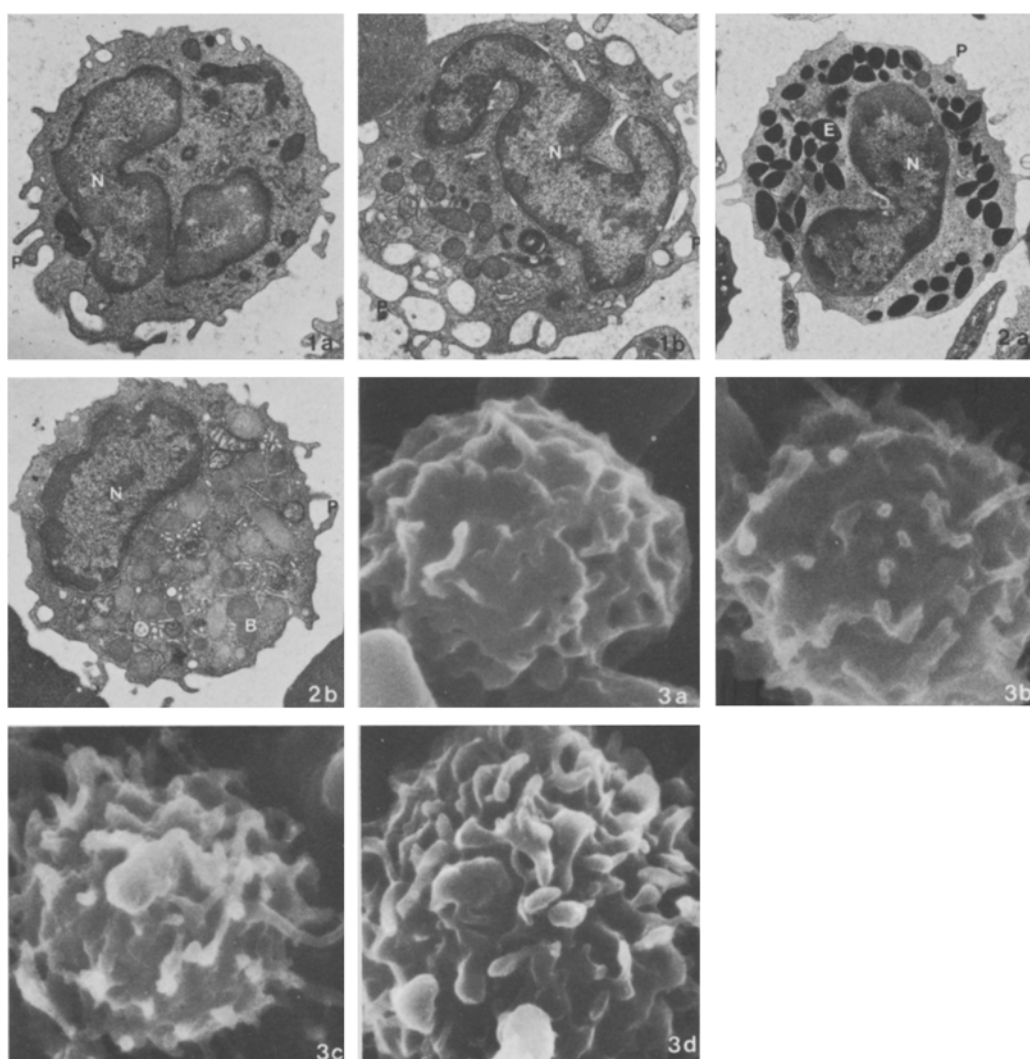


Fig. 1a. A neutrophil granulocyte from control group. A small number of short projections are seen.  $\times 7600$ .

Fig. 1b. A neutrophil granulocyte from experimental group. Elongated and complicated cytoplasmic projections are evident on cell surface.  $\times 7600$ .

Figures 2a and 2b. a Eosinophil and b basophil granulocytes from experimental group. There are no prominent projections on cell surface. a  $\times 5100$ , b  $\times 7600$ .

Figures 3a and 3b. These scanning electron micrographs show granulocytes from control group. Surfaces appear rough and uneven, and a small number of short projections are observed. a  $\times 9000$ , b  $\times 10,000$ .

Figures 3c and 3d. These scanning electron micrographs show granulocytes from experimental group. Many long and peculiar cytoplasmic projections are observed on the whole of the cell surface. c  $\times 8000$ , d  $\times 11,000$ . B Basophil granule, E eosinophil granule, N nucleus, P cytoplasmic projection.

In this communication, the authors report morphological changes of neutrophil granulocytes after a combined treatment of L-DOPA and prednisolone.

**Materials and methods.** Wistar male rats weighing 250 g were used. 4 mg of L-DOPA and 4 mg of prednisolone were concomitantly injected into experimental animals. At 1 h after the administration, the uptake of L-DOPA by granulocytes was ascertained by a reflected fluorescent microscope with the authors' method<sup>1,2</sup>. Granulocytes from untreated rats served as controls. For performing the electron microscopic study on a morphology of granulocytes, bloods were collected by heart puncture, heparinized and centrifuged at 1500 rpm. Buffy coats were fixed in 3% glutaraldehyde fixative adjusted to pH 7.4 with 0.1 M sodium cacodylate and postfixed in 1% osmium tetroxide buffered with the same solution. Halves of the specimens were used for a transmission electron microscope (TEM); that is, specimens were embedded in Epon 812, and ultrathin sections were stained with uranyl acetate and lead acetate. Parallel samples were prepared for a scanning electron microscope (SEM) by the critical point method<sup>6</sup>. For examination, a JEM-100B transmission- and a SSM-2 scanning electron microscope were used.

**Results and discussion.** By TEM, control granulocytes presented a relatively small number of cytoplasmic projections and vacuoles, and endoplasmic reticula were thin and scarcely visible (figure 1a). On the other hand, as shown in figure 1b, most of granulocytes obtained from the experimental group possessed a lot of complicated cytoplasmic projections on their free surface. Further, intracellular vacuoles of various size increased, and endoplasmic reticula became expanded. No difference could be seen in nuclear shape and contents between control and experimental groups. Through this experiment, erythrocytes, eosinophil and basophil granulocytes took normal shapes. In figures 2a and 2b, eosinophil and basophil granulocytes from the same specimens were depicted. Figure 3 shows scanning electron micrographs of granulocytes from control

(figures 3a and 3b) and experimental groups (figures 3c and 3d). Granulocytes looked to be somewhat shrunken by a preparation procedure of specimens for SEM. Their surfaces were uneven and sometimes platelets adhered to them.

Comparing figures 3a and 3b with figures 3c and 3d, the following evidence was obtained. Granulocytes in experimental group had elongated cytoplasmic projections originating from whole surface, and the projections appeared twisted and irregular in direction. Their length ranged between 200 nm and 650 nm, and the profiles of granulocytes took the form of a starfish. On the other hand, the surfaces of granulocytes in control specimens were relatively smooth and had a small number of projections. They were short, measuring about 200 nm in length. These findings were comparable with those in cross sections of transmission electron micrographs. So that, L-DOPA could induce a morphological change only for neutrophil granulocytes. From these findings, the authors postulate that this is evidence that neutrophil granulocytes are specifically sensitive and reactive to some biogenic amines – especially for L-DOPA, and such morphological changes of granulocytes are closely associated with an enhancement of adenylylate cyclase activity in neutrophil granulocytes after combined treatment of L-DOPA and prednisolone.

- 1 M. Mato, Y. Uchiyama, S. Ookawara, E. Aikawa and T. Karasawa, *Acta histochem.* 57, 191 (1976).
- 2 M. Mato, S. Ookawara and Y. Uchiyama, *Acta histochem.* 57, 198 (1976).
- 3 M. Mato and Y. Uchiyama, *Acta histochem. cytochem.* 10, 151 (1977).
- 4 M. Mato and Y. Uchiyama, *Acta histochem.* 11, (1), 64 (1978).
- 5 M. Mato, S. Ookawara and E. Aikawa, *Cell Tissue Res.* (in contribution).
- 6 T.F. Anderson, *Trans. N.Y. Acad. Sci.* 13, 130 (1951).

## Fine structure of modified photoreceptor cells in the pineal of the goby, *Clevelandia ios* (Pisces: Gobiidae)

J.A. McNulty<sup>1,2</sup>

Department of Anatomy, Stritch School of Medicine, Loyola University, 2160 S. First Avenue, Maywood (Illinois 60153, USA), 26 September 1977

**Summary.** Photoreceptor cells with a system of long, thin cytoplasmic processes (50–80 nm thick), which may represent a modification of the outer segment saccules, are described in the pineal of the goby, *Clevelandia ios*.

Previous electron microscopic studies have demonstrated the presence of well developed photoreceptor cells in the pineals of fishes<sup>3–5</sup> and a photoreceptive function has been confirmed by electrophysiological experiments<sup>6–8</sup>. During a recent study on the pineals of several species of fishes, photoreceptor cells with an ultrastructure different from those described in previous studies were observed in the pineal of the goby, *Clevelandia ios*.

**Materials and methods.** 7 specimens ranging in size between 18 and 27 mm standard length were examined. Because of their small size it was necessary to decapitate the fish and dissect the pineal together with parts of the dorsal cranium in a petri dish containing glutaraldehyde fixative (2.5% glutaraldehyde in 0.16 M monosodium phosphate buffered to pH 7.4 with NaOH). Following dissection the tissue was immersed in fresh fixative for a period of 1 h. After post-fixation with 1% osmium tetroxide in phosphate buffer the tissue was dehydrated and embedded in Araldite 502

plastic resin. Thin sections were stained with both uranyl acetate and lead citrate and photographed with an RCA EMU-3F electron microscope operated at 50 kV.

**Observations and discussion.** Light microscopically, the structure of the pineal in *Clevelandia ios* generally agreed with observations on the pineal of another goby, *Acanthogobius flavimanus*<sup>9</sup>. The photoreceptor cells lined the highly folded epithelium with their inner and outer segments projecting into a narrow lumen. The outer segments of most photoreceptor cells consisted of a series of membranous saccules, which measured between 20–40 nm in thickness, similar to those described in other fish species<sup>3–5,10</sup>. In many cells the saccules were highly irregular and often vesiculated.

Modified photoreceptor cells also were observed which lacked the typical membranous saccules of the outer segments. These cells were identified as photoreceptor cells by the presence of a sensory cilium (connecting piece) with a