MORPHOLOGICAL CHANGES OF THE MUSCLES AND MOTOR END-PLATES OF CENTROPHENOXINE-TREATED AGED GUINEA PIGS*

YOKO UCHIDA AND MASANORI TOMONAGA

Department of Clinical Pathology, Tokyo Metropolitan Institute of Gerontology, Itabashi-ku, Tokyo 173

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Centrophenoxine, 80 mg/kg/day, was administered to 25-month old guinea pigs intraperitoneally for 90 or 127 days and the muscles and motor end-plates were examined histochemically. The results showed some improvement of muscle atrophy and deformity of the motor end-plates in the aged guinea pigs. However, it is not clear whether these changes result from the central action or the peripheral action of centrophenoxine.

Centrophenoxine (CP) is a drug, which is reported to remove lipofuscin accumulated in nerve cells of aged animals. To determine the functional change of nerve cells treated with CP, a morphological study of the skeletal muscle and motor end-plate was performed using histochemical technique on aged guinea pigs.

MATERIALS AND METHODS

Centrophenoxine was administered intraperitoneally (80 mg/kg/day) to five aged female guinea pigs (CP-group). Physiological saline was injected into three 25-month old guinea pigs (aged-group) and two 9-month olds (young-group). After 90 or 127 days administration, the animals were killed.

The extensor digitorum longus (EDL) (fast-twitch) and soleus (slow-twitch) muscles were dissected and immersed in cold 4% formaldehyde in 0.1 M cacodylate buffer, pH 7.2, for 2 hr. Teased preparations were made for the demonstration of cholinesterase (ChE) staining by modified Koelle's method (potassium ferricyanide was used instead of ammonium sulfide in Koelle's method (11)). Every end-plate which was seen clearly in frontal view was photographed, and the longest diameter and the perpendicular diameter were measured. The diameter of the end-plate was calculated as the mean of two perpendicular diameters.

From another muscle specimen, 10 μ m thick frozen sections were prepared. The sections were stained with hematoxylin-eosin, modified trichrome (4), NADH-tetrazolium reductase, myosin ATPase (10). Fibermetry of the muscle fiber was performed on ATPase-stained preparations by measuring the shortest diameter. Atrophy and hypertrophy factors (A, H-factor) were calculated as suggested by

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Dubowitz and Brooke (3). Calculation: $A\text{-factor} = (a + 2b + 3c) \times 1000/n$

n: total number of fibers

a: number of fibers with diameter between 20 and 30 μm

b: number of fibers with diameter between 10 and 20 μm

c: number of fibers with diameter less than 10 μm

H-factor was similarly derived to express the proportion of fibers larger than 70 μm .

RESULTS

1. Histological and histochemical findings in the muscles

(1) EDL muscle

Histology of the aged-group showed central nuclei, small group atrophy, small angulated fibers and type grouping in one case and necrotic fibers in one case. The CP-group showed a few small angulated fibers and necrotic fibers.

Fiber types with respect to ATPase activity revealed three types (dark, intermediate and light fibers) in the young-group, 75%, 15% and 10% respectively. Because of difficulty in differentiation between dark and intermediate fibers in the aged group, the histogram was prepared by counting these two types.

The histogram of the diameter of the dark-intermediate fibers revealed distribution between 10–80 μm , with peak at 60 μm in the young-group. A-factors were 4–50 and H-factors were 197–224. Then the upper limits for the value of A-factor and H-factor in the young-group was set at 50 and 224 for evaluating the difference between the aged-group and the CP-group. In the aged-group small fibers under 30 μm increased and A-factor showed 181, which means atrophy. The CP-group showed A-factor 45 and H-factor 94, that is, the muscle fibers were not atrophic or hypertrophic (Fig. 1).

Due to their small number of light fibers, a comparison between the three groups was not performed.

(2) Soleus muscle

Histology of the aged-group showed central nuclei in all cases and necrotic fibers in one case. These changes were observed in the CP-group in the same degree.

Histogram of the fiber diameter revealed distribution between 20–70 μm , with peak at 40 μm in the young-group. The upper limits for the value of A-factor was 15 and H-factor was 60. In the aged-group the histogram was shifted to the left and A-factor was 95, that is, muscle fiber was atrophic. The CP-group showed a histogram similar to the young-group, however A-factor was 48, above the upper limit, that is, atrophic. In both aged and CP-groups hypertrophy of muscle fibers was not found.

2. Findings in the motor end-plates

(1) EDL muscle

a) Diameter of the end-plate: Mean values of the diameter revealed no difference between the three groups (Fig. 2). However, in the aged-group the end-plates were more oblong than those of the young-group and giant end-plates with over

FIG. 1. Histograms of muscle fiber diameters of EDL and soleus muscles in CP treated, aged, and young guinea pigs.

70 μ m diameter were also observed. In the CP-group these changes were fewer than in the aged-group (Figs. 2, 3).

b) Morphology: Most end-plates of the young-group were oval, round and rarely segmented. In the aged-group the oval end-plates were fewer and segmented and conglomerate forms were frequent. The CP-group showed fewer segmented or conglomerate forms and more oval or arborized forms (Table 1).

(2) Soleus muscle

The aged-group showed atrophy in diameter and many oblong end-plates.

The CP-group revealed less atrophy than the aged-group ($p < 0.01$) (Figs. 2, 4).

FIG. 2. Histograms of end-plate diameters of EDL (left) and soleus (right) muscles in CP treated, aged, and young guinea pigs. The diameter was expressed as a mean with 95% confidence limits.

TABLE 1. Percentages of various figures of end-plates in CP treated, aged, and young EDL muscles.

Groups	O	A	C	S

Aged, CP treated	36%	27%	19%	18%
Aged, control	24%	11%	32%	33%
Young control	78%	7%	13%	2%

O: oval or round form with "perforations"; A: arborized form;

C: conglomerate form; S: segmented form;

FIG. 3. Motor end-plates of EDL muscles in CP treated, aged, and young guinea pigs. $\times 330$

FIG. 4. Motor end-plates of soleus muscles in CP treated, aged, and young guinea pigs. 330

DISCUSSION

Centrophenoxine is a drug activating and normalizing the brain metabolism, and also reported by Nandy and Bourne (12) to remove lipofuscin pigments deposited in the nerve cells. This effect has been investigated, but there is no information on the peripheral nerve and muscle. This investigation centered on the effect of the centrophenoxine on the age changes of the muscle.

(1) Ageing change of the muscle and motor end-plate

Atrophy of muscle is a very characteristic sign of old age, and this change is due to a decrease in both number and diameter of muscle fibers (9). Histological change of the muscle in the aged rat consists of disappearance of the cross striation, fiber splitting and necrosis with increase of muscle nuclei (2), and also scattered atrophy and group atrophy accompanied by hyalinization and granulovacuolar degeneration (5). Bass (1) reported by biochemical and histochemical studies type grouping, type atrophy and the levelling-out of metabolic differences between the different fiber types. In this experiment we also observed myopathic and neuropathic changes and unclear type difference in the aged-group.

The motor end-plate changes its diameter and figure during postnatal development (13, 14), and senile changes of the motor end-plates were reported by Gutmann *et al.* (7) and Tuffery (17). Gutmann *et al.* showed that the end-plates by ChE stain of the aged rat appeared irregular in distribution and contour, that is, many end-plates were also seen at parts where end-plates were regularly not found, and the contour of the end-plates was irregular. They also found that the end-plate sizes were uniform in (10-day old) EDL and soleus muscles and increased and differentiated in size during further development, and that the difference between these two muscles was, however, lost in old age. They considered that the neurotrophic function decreased in the aged animal and functional "dedifferentiation" occurred.

Tuffery observed the end-plates by silver impregnation in 6–19-year old cat and found no difference in the diameter, but noted the increase of the end-plates with nodal sprout, contribution or 2–4 myelinated terminal axons. He concluded that these facts meant increased compensatory use of the synaptic contact area due to the loss of muscle fibers and decrease in trophic function in old age.

In our experiment, the diameter of the end-plate showed no difference between the aged and young-group in EDL muscle and a distinct decrease in soleus muscle of the aged-group. But the increase of oblong and conglomerate end-plates in both aged EDL and soleus muscles suggests functional "dedifferentiation" in the end-plate.

(2) On the effect of the centrophenoxine

By administration of centrophenoxine some improvement was observed in the changes of the muscles and motor end-plates of the aged guinea pigs, such as muscle fiber atrophy, and segmentation, conglomeration or atrophy of the motor end-plates. It is not clear, however, whether this effect of centrophenoxine is a direct action or an indirect action on the muscle and end-plate. At present the following mechanisms are considered:

1) Result of the removal of lipofuscin in the nerve cells cytoplasm.

Nandy and Bourne reported that centrophenoxine acted as a drug removing

the lipofuscin pigments accumulated in the nerve cells. Tomonaga *et al.* (15) reported, however, that lipofuscin in the nerve cells of brain and spinal cord was not decreased by light-microscopy, but the distribution of lipofuscin changed to the disseminated type from the aggregated form by electron-microscopy. These findings may be related to the improvement of the muscle and end-plate of the aged guinea pig.

2) Result of the improvement of the nerve cell function.

Tomonaga *et al.* (15) reported that centrophenoxine increased the rough endoplasmic reticulum in the anterior horn cells of the spinal cord. This fact suggests the increased synthesis of protein in the nerve cells and the increased supply of the protein flow to the synapses.

3) Result of direct action of centrophenoxine on the motor end-plate.

It is known that the decrease of choline acetyltransferase activity in the terminal ending (16) and decrease of acetylcholine release are due to ageing (8). Centrophenoxine has an acetylcholine-like action (6) and may act directly on the motor end-plate. On the other hand, the membrane stabilizing action of centrophenoxine may also influence the synaptic membrane.

For final elucidation of this mechanism of centrophenoxine, it is necessary to make further investigations of axoplasmic transport and electron-microscopic investigations of the muscle and motor end-plate of aged animals.

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