EFFECTS OF PROSTAGLANDIN INHIBITATION, ELECTRICAL STIMULATION AND LOCAL HEATING ON SKELETAL MUSCLE ATROPHY

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SUMMARY: Effects of local heating with infrared irradiation (IR) and aspirin treatment together with electrical stimulation (ES) on progression of atrophy in denervated rat gastrocnemius muscle were studied. In IR treated denervated muscle, the degenerative changes were considerably greater than unheated groups. This, we thought, was due to synthesis of prostaglandis (PG's) which were stimulated with heating. In the aspirin treated group, the dosage of aspirin we used (4 mg/kg/per four day) and electrical stimulation together, were sufficient to inhibit the muscle PG synthesis. Histologically, the atrophy of these muscles and degenerative changes were considerably less than others. This study showed that, atrophy will be retarded by aspirin treatment together with electrical stimulation in denervated striated muscles.

Key Words: Prostaglandis, atrophy, acetyl salicylic acid.

INTRODUCTION

Immobilization and denervation of skeletal muscle causes the atrophy of muscle and degenerative changes are largely a consequence of increased protein breakdown (1-5). Similarly, the patients with fever, sepsis, traumatic injuries, or cancer also show a marked loss of muscle proteins (4,6).

In recent years some observations suggest a possible role of prostaglandin Ez ($PGE_{\%}$) in the degredation of muscle protein in various physiological and pathological states (e.g. fever, trauma, injury) (4,6-8).

Like the all other organs in the body (1,8), skeletal muscle produces $PGE_{2\alpha}$ (3,4,10). Rodeman *et al.* showed that, $PGE_{2\alpha}$ causes stimulation of protein synthesis (4,6, 11,12) and PGE_2 stimulates protein breakdown (4,6,10). PGE_2 mediates this catabolic action via increased amount of intracellular calcium (Ca^{2+}) in skeletal muscle (4,13).

Increase in cellular concentration of Ca^{2+}, stimulates Ca^{2+} dependent phospholipase A_2 (12,13), which releases arachidonic acid from membrane phospholipids. This, in turn leads to increased synthesis PGE_2 enzymatically (12,14,15,16). PGE_2 activates the nonlysosomal and lysosomal enzymes, Ca^{2+}-activated neutral proteinase (CAP) and Cathepsin B and D respectively (6, 17-19).

Likely PGE_2 promotes autophagic vacuole formation (4). It is supposed that, inactivation of cholinesterase at mammalian neuromuscular junctions and the development of diffuse extrajunctional acethylcholine receptors in denervated muscle fibres may be involved in increased Ca^{2+} influx by the way of damaged sarcolemma (20,21). An increased mitochondrial Ca^{2+} has been found in myopathic, denervated and dystrophic tissues (22). This is possibly due to damage of the mitochondrial inner membrane and may cause a muscle energy deficiency (5,22). On the other hand, Cheach, Baracos and Goldberg

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showed an increased protein breakdown and PGE_2 synthesis in muscle from feverish rats and muscles which were incubated *in vitro* in the febrile temperature range (1,7,23).

This increase in muscle protein breakdown during fever must have involved an activation of leukocytic pyrogen which causes production of PGE_2. This in turn induces a hyperthermic response in the hypothalamus and also stimulates muscle protein degradation (4,6,7,24,25).

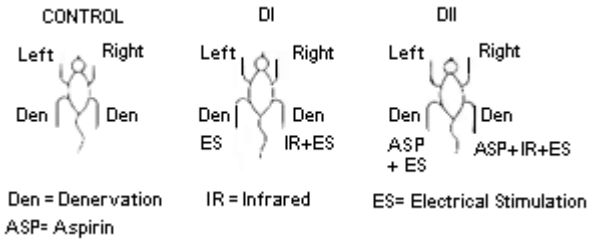

Figure 1: The groups of the experimentation.

It has been suggested that if muscle wasting associated with denervation is mediated by PG the PG synthesis inhibitors may be useful in limiting the proteolysis (6,10,12,26).

Atrophy of denervated skeletal muscle can be partially prevented by daily low frequency electrical stimulation (3,20,27-29), which increase the level of $PGF_{2\alpha}$ in the skeletal muscle (10) and this inturn activates protein synthesis (1,11). In addition the activity induced by electrical stimulation may specifically retard or interfere with the synthesis of PGE_2, presumably by a protection of the denervated muscle against disruption of the membranes (21), or partial inhibition of the PGE_2-isomerase (3).

We have examined if local heating, aspirin (as PG synthesis inhibitor) and electrical stimulation treatments could change the progression skeletal muscle atrophy.

METHODS

15 adults, both female and male rats were studied they were-denervated in both hindlimbs (13,22,30-33) and divided into 3 groups (Figure 1).

-DC = Denervated control group, received no treatment.

- DI= Denervated experimental group, treated with electrical stimulation (ES) to the left gastrocnemius and infrared (IR) + ES to the right gastrocnemius.

-DIII = Denervated experimental group II, treated with the same methods as DI, and additionally they were injected with aspirin (4 mg/kg/per day) intraperitoneally (15), beginning 24 hours after neurotomy until the animals were sacrificed.

After ten days of denervation (34), ES and IR were begun in the same style to DI and DII groups. The treatment lasted 31 ± 1 days after neurotomy. ES was used on both gastrocnemius muscle of rats and IR was used only to right, both procedures were applied 5 days a week.

Palmer's low frequency (20Hz) "Student Stimulator" was used for ES. The 7 mm diameter disk electrodes were fastened on the origin and insertion of the gastrocnemius muscles (20, 35). The muscle contracted isometrically (20,31). The intensity and duration of the impulses were 3-10 mA 100 ms respectively. Treatment lasted for 10 minutes.

IR, from a 250 watt "Mazda lamp" was used for local heating (36) of right gastrocnemius muscle of both DI and DII groups. They were treated 5 times a week, like electrotherapy. The duration was 30 minutes and intensity was 42-43°C (36-38). Histological samples were prepared from both gastrocnemius muscle of all groups. They were stained with Hematoxylin Eosin (39) and photographs were taken with Zeiss, DXCMZ model experimental microscope, 40X.

Prostaglandin E-Like Activity (PGLA) of each gastrocnemius muscle was measured by bioassay and results were analyzed by a student's t test.

RESULTS

PGLA Values

The PGLA of the right muscle of DI group, was higher than those of controls ($p<0.005$) (Table 1, 2).

The increase was 27% compared with controls (Figure 2).

In the left muscle of DI group, PGLA decreased, compared with controls. But the difference was not statistically significant (Table 1,2). The change was 9% (Figure 2).

Table 2: Mean values of PGLA of groups (Mean SE) ng/gr wet weight.

	IFRARED+ ELECTRICAL STIMULATION	ELECTRICAL STIMULATION	CONTROL
DI (Denervation)	61.8 ± 2.2	44.2 ± 6.6	
			48.5 ± 4.8
DII (Denervation+ Aspirin)	36.7 ± 6.5	8 ± 3.3	

Table 2: The statistical differences of PGLA contents of the groups.

	CONTROLS				DI	DII	DI IR+ES	DI ES
	DI		DII		IR + SE	IR + ES	DII IR+ES	DII ES
	IR+SE	ES	IR+ES	ES	ES	ES		
PGLA	P<0.05	P>0.05	P>0.05	P<0.005	P<0.05	P<0.01	P<0.02	P<0.005
	t=2.5188	t=1.3687	t=1.4603	t=6.8499	t=2.5298	t=3.8547	t=3.6577	t=4.8245

PGLA -Prostaglandin Like Activity, DI -Denervation, DII -Denervation + Aspirin, IR - Infrarred Irradiation, ES -Electrical Stimulation

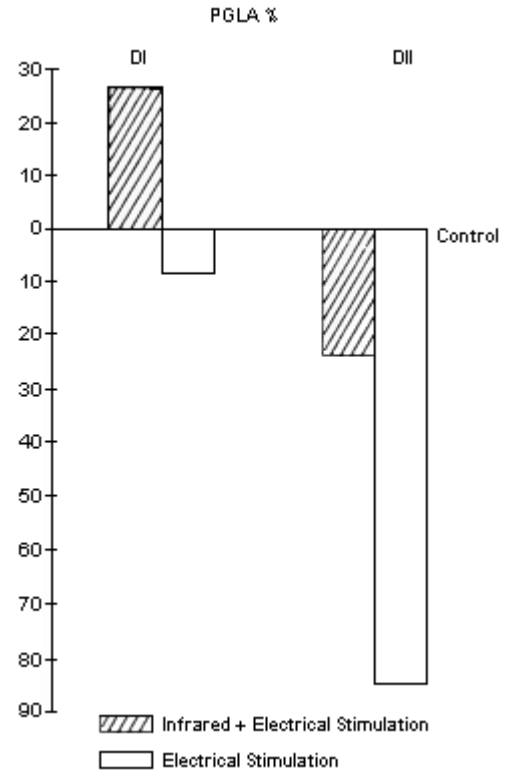

Figure 2: Percentage of PGLA ýof the experimental groups, comparing with controls.

PGLA of right muscle of DII groups was found lower than those of controls. But the difference was not significant (Table 1,2). Decrement was 24 % compared with controls (Figure 2).

In the left gastrocnemius of DII group there was a significant decrease in PGLA content (p<0.005) compared with controls (Table 1,2). Difference was 84% (Figure 2).

In DI group PGLA of left gastrocnemius was lower than those of right (P<0.05) (Tables 1, 2).

Between DI and DII groups; PGLA of right muscle decreased in DII, compared with DI (p<0.002) (Tables 1, 2).

Similarly, PGLA of left muscle of DII group was lower than those of DI = (p<0.005) (Tables 1,2).

HISTOLOGICAL FINDIGS

Microscopic examination revealed marked evidences of atrophy, reduction of diameter and homogenization and fusion of the myofibrillae with enlargement of the extracellular spaces in the gastrocnemius muscle of the denervated control (Figure 3). Locally heated and electrically stimulated muscles of DI group showed severe atrophy with wider extracellular areas, constricted and centrally positioned nuclei. There were also fragmentations of the sarcolemma (Figure 4). The degenerative changes were less in the electrically stimulated muscle of DI group (Figure 5) compared to those of locally heated and electrically stimulated group. Also in the aspirin treated and infrared plus electrically stimulated muscles, the degenerative changes (Figure 6) were lower than those of the muscles of DI group without treatment with aspirin.

The degenerative changes were the least in the unheated but aspirin treated and electrically stimulated group.

DISCUSSION

30 days after neurotomy, the muscle fibres of denervated control (DC) showed histological evidence marked atrophy which were in accordance with literatures (5) (Figure 3).

Locally heated, denervated muscles were atrophied to a greated extent than the muscles of nonheated group (Figure 4). Cheah, Goldberg and Baracos suggest that hyperthermia, by activating phospholipase A_2, induces the stimulation of PGE_2, which inturn activates the proteolysis of muscles (7,23). Our findings, in accordance with earlier reports showed that, in the IR treated denervated mus-

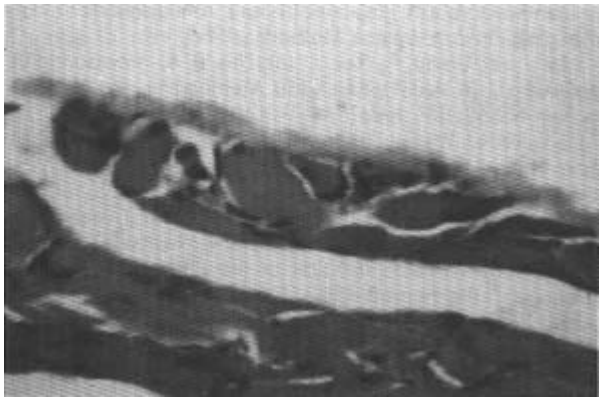

Figure 3: Longitidunal and transverse sections of gastrocnemius muscle of denervated controls (40 X).

There is marked evidence of atrophy e.g. differences in myofibrillar diameter, increased extracellular spaces and homogenisations of fibres due to fusing of the myofibrilles.

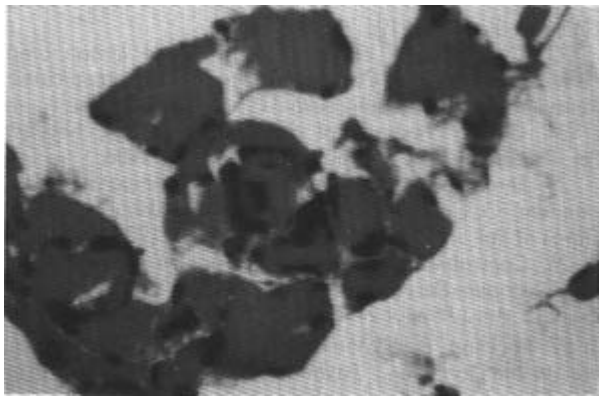

Figure 4: IR+ES treated muscle of DI group (40 X).

Homogenisations, enhanced extracellular areas illustrated. Nuclei became constricted, frequently lie in pairs, and occupeid more central position within the cell. Also there are fragmentations of the sarcolemma.

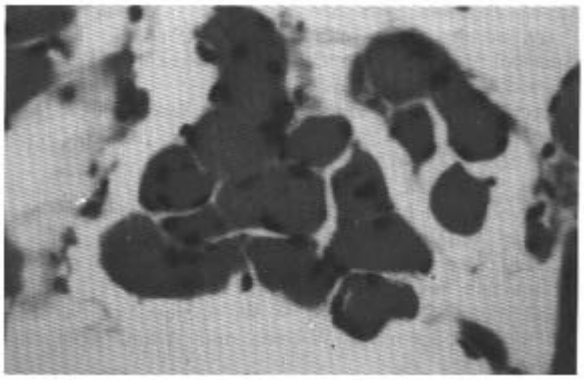

Figure 5: ES treated muscle of DI group.

The degenerative changes are lower than those of IR+ES treated samples shown in Figure 6.

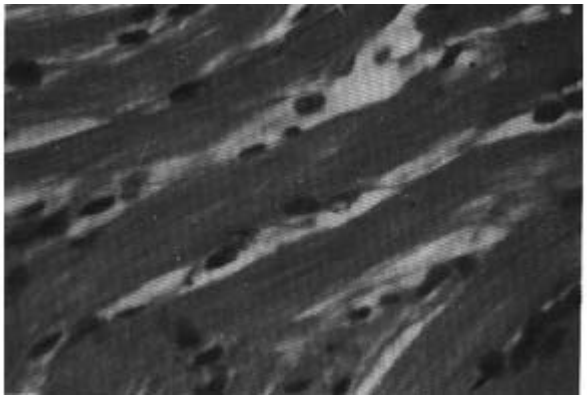

Figure 6: Aspirin + IR + ES treated muscle of DII group.

Here the degenerative changes are lower than those of DI group not given aspirin.

Figure 7: Aspirin and only ES treated muscle of DII groups.

Although there are some evidences of atrophy, the degenerative changes are the least compared to all other groups.

cles, the PGLA increase was associated with extreme degenerative changes in muscle fibers.

However, the degenerative changes of the muscles in the locally heated and aspirin treated animals were less than those of not treated with aspirin (Figure 6). Their PGLA was also found lower than the other groups. These data showed that, aspirin (4 mg/kg/4 days) might inhibit some but not all the synthesis of PGE_2 but might particularly prevent or retard the progression of atrophy (6,10, 12, 26).

According to our histological findings, electrically stimulated, but non-heated denervated muscle showed less degenerative changes in comparison with the locally heated muscle. This might be due to the activity of electrical stimulation which retards or interferes with the synthesis of PGE_2 (3).

The authors also suggest that electrical stimulation might protect the structure of the membranes of denervated muscles (18) or partially inhibit PGE_2-isomerase (3).

The least degenerative changes were seen in the muscle of both aspirin and ES treated animals. The inhibiting effects of both aspirin and ES on the PGE_2 synthesis, probably facilitating each others action, caused PGE_2 decrease and retarded atrophy.

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