

Effects of α -isoproterenol on denervation atrophy in orbicularis oculi muscle fibers

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Summary. The effect of the β -adrenoceptor agonist isoproterenol on the denervated orbicularis oculi muscle was studied. We cut the facial nerve on the right side of eight rats and injected isoproterenol (5 mg/kg) into four rats every day. The animals were sacrificed after three weeks, and small pieces of the orbicularis oculi muscle were removed. Muscle fibers were separated into single fibers. Cytochrome oxidase enzyme staining was applied, and density of cytochrome oxidase enzyme product and diameter of individual muscle fibers were analyzed by computer. Some of the muscle fibers were observed under electron microscopy. The results showed that diameter and cytochrome oxidase enzyme activity of the orbicularis oculi muscle decreased by denervation, otherwise, it increased to near the normal level by administration of isoproterenol. Electron microscopic observation showed that the myosin filaments became thin and their arrangement were disordered by denervation and their findings recovered to be almost normal by isoproterenol administration. We concluded that isoproterenol could prevent the orbicularis oculi muscle from atrophying which caused by denervation.

Key words: Facial nerve, Beta-agonist, Electron microscopy, Rat

Introduction

In conditions such as those showing unfavorable prognosis associated with Bell's palsy or Ramsay Hunt's syndrome or in such cases where anastomosis was performed after dissection of the facial nerve, regeneration of the facial nerve takes a long time. The target muscles have often been atrophied by the time the regenerated nerves reach them, resulting in insufficient muscle strength (Schwartz et al., 1984). Prevention of disuse atrophy of the muscles during the period until

they are reinnervated would therefore considerably improve the results of the treatment. At present, however, there are no effective methods of preventing atrophy except for physical therapy such as low-frequency stimulation. On the other hand, isoproterenol, a β -agonist of the sympathetic nerve, has been reported to increase the amount of actin in myocytes of smooth muscles and cardiac muscles (William et al., 1994). We also showed that isoproterenol increases the thickness of myofibrils and elevates the activity of cytochrome oxidase, a respirative enzyme in mitochondria in the facial muscles of normal rats (Zhai et al., 1995; Ishii et al., 1997). In the present study, we examined whether administration of isoproterenol prevents facial muscle atrophy caused by denervation.

Materials and methods

Twelve normal rats (body weight 150-200 g, male) were used. The facial nerve of eight animals on the right-hand side was cut at the position beneath the ear and the cut ends were ligated to prevent reconnection. After excision of the facial nerves, α -isoproterenol (5 mg/kg) was administered intraperitoneally to four animals daily over a period of 3 weeks (Versura et al., 1988). After three weeks, the orbicularis oculi muscles of right sides were taken from all the animals, fixed in 1% glutaraldehyde for 5 min, washed in distilled water, and separated into single fibers. The isolated myofibrils were then reacted with 1% MnCl_2 solution containing DAB and H_2O_2 at 37 °C for 1 h to visualize cytochrome oxidase activity (Seligman et al., 1968). Color photographs of the embedded samples were taken, and the printed images were recorded on a computer using a scanner (Canon, IX4015) at 72 dpi and 8 bit (256 grades) gray scale. The diameters of the myofibrils were determined using NIH imaging software and the density of staining was quantified using values from 0 to 255. A small portion of the muscle fibers were fixed in 2% glutaraldehyde, postfixed in 1% OsO_4 , embedded in Epon after dehydration, and ultra-thin sections were cut for transmission electron microscopic observation.

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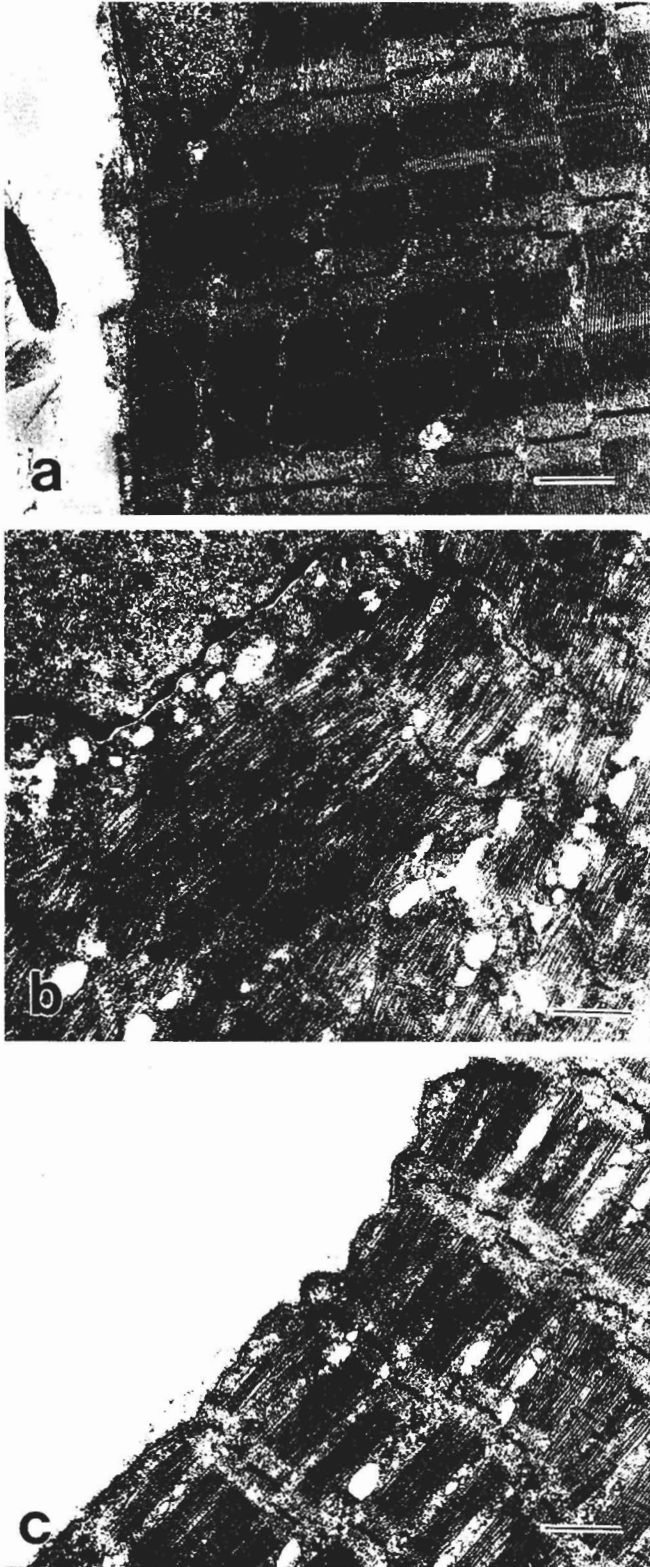


Fig 1. Electron micrographs of orbicularis oculi muscles. **a.** Electron micrograph of the control group. **b.** Showing the case after denervation. Striated pattern of the muscle fibers is obscure. Sarcoplasmic reticulum is enlarged. **c.** Showing the case of isoproterenol administration after denervation. Striated pattern of the muscle fiber is kept well compared to the case of no treatment after denervation. $\times 10,000$. Scale bar: $1 \mu\text{m}$.

Results

Fig. 1a shows a normal architecture of the muscle fibers in the control group. After denervation, myosin filaments of the orbicularis oculi muscles were found to be thinner and more irregularly arranged than those of the control. Their striation was also unclear (Fig. 1b). After isoproterenol administration following denervation, myosin filaments were thick and regularly arranged with clear striation (Fig. 1c). Figure 2 shows the scatter gram of the mean diameter of the muscle fibers. In the control group, the mean diameter was $36.9 \pm 6.9 \mu\text{m}$ (mean \pm S.D.), whereas denervation caused a decrease in the mean diameter to $24.7 \pm 5.1 \mu\text{m}$ which was recovered by isoproterenol administration at $30.7 \pm 5.5 \mu\text{m}$. Cytochrome oxidase activity of white fibers was reduced by denervation, but isoproterenol recovered its activity to a level even higher than the control (Fig. 3a). In red fibers, cytochrome oxidase activity was similarly reduced by denervation and recovered by administration of isoproterenol (Fig. 3b).

Discussion

Isoproterenol is an agonist of the β -receptor of the sympathetic nerve which has diverse effects including elevation of cardiac outputs, reduction of resistance in the peripheral blood vessels, and relaxation of smooth muscles of the bronchus and digestive tracts. However, their exact mechanism of action is not yet fully understood; in particular on the skeletal muscle. It is postulated that isoproterenol stimulates the β -receptor of striated muscles resulting in an increase in the cellular cAMP levels which in turn activate the cAMP-dependent protein kinase. This enzyme enhances

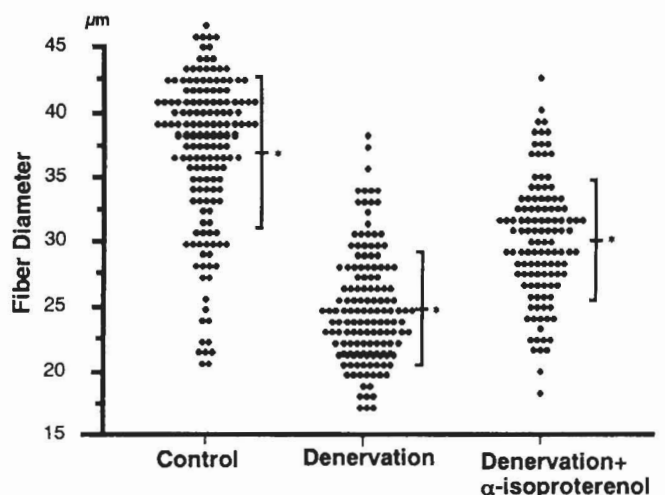


Fig 2. Scatter gram of the diameters of muscle fibers. Data were analyzed by Group Scatter (v.1.6, K. Morikawa). Asterisks represent the mean of 430 muscle fibers. There was a significant difference between control and denervation ($p < 0.01$), denervation and denervation + isoproterenol administration ($p < 0.05$).

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glycogen degradation by phosphorylating phosphorylase kinase (Lubert, 1991). Degradation of glycogen results in production of a large amount of acetyl CoA, and intermediate of the TCA cycle, and as a result cytochrome oxidase activity is elevated. Deshaies et al. (1981) and Maltin et al. (1987) have shown that administration of β agonist such as isoproterenol and clenbutenol causes hypertrophy of gastrocnemius and soleus muscles in rats. Recently Roberts and McGeachie (1994) reported that it causes hypertrophy of grafted skeletal muscles and that it also increases revascularization within muscles. Isoproterenol also induces hypertrophy of cardiac muscles as with skeletal muscles (Taylor and Tang, 1984). Beta-adrenergic stimulation increases protein synthesis in cultured adult ventricular cardiomyocytes (Pinson et al., 1993) as well as increasing the amounts of myosin heavy chains in cultured cardiac muscle cells (William et al., 1994). Clark et al. (1994) found that heavy chains in myosin of the cardiac muscle increased in numbers in tissue cultured with isoproterenol. Beta-adrenergic drugs, such as isoproterenol, act specifically on the β_2 receptors of smooth muscle, and in particular on the smooth muscle of blood vessels supplying skeletal muscle. Beta-adrenergic drugs increase both the vascularity and size of normal skeletal muscles (Deshaies et al., 1981; Maltin et al., 1987; Sillau and De Lourdes Philippi, 1987). The neovascularization reported by Sillau and De Lourdes Philippi (1987) is thought to be a response to increased blood flow to the skeletal muscle. The revascularization of regenerating skeletal muscles is important for the ultimate success of the regeneration (Roberts and McGeachie, 1994). Although isoproterenol is not a selective beta2-agonist, enhancement of vascularization of regenerating skeletal muscles are expected.

We have previously shown that isoproterenol caused an increase in the thickness of myofibrils of the stapes

and orbicularis oculi muscles and elevated cytochrome oxidase activity in rats (Zhai et al., 1995; Ishii et al., 1997). Our present study further demonstrated that it prevents atrophy of denervated orbicularis oculi muscles. An increase in the mass of the whole muscle due to the increase in the thickness of myofibrils alleviates the asymmetry of the face. The rise of the cytochrome oxidase activity indicates an increase in the activity of the aerobic glycolysis (Lubert, 1991). The increase in the thickness of myosin filaments and improvement of their alignment as well as the appearance of distinct cross striations suggest increased contractility of muscle cells. Since our experiments were performed *in vivo*, there remains the possibility that the effects of isoproterenol observed *in vivo* were indirect via an increase in the blood flow induced by the drug.

Eighty% of Bell's palsy are almost completely cured without leaving any paralysis, but the remaining 20% are accompanied by sustained denervation, which prevents complete recovery from the palsy. A cross face nerve graft is a common treatment performed on patients showing complete paralysis of the facial nerve after operation of acoustic neuroma, but the facial muscles often have been atrophied when nerves regenerated from the healthy side reach the facial muscles on the affected side, necessitating a subsequent graft of muscles on that side of the face. Therefore, close attention has to be paid to disuse atrophy of the muscles as well as the number of regenerated muscle fibers in patients with advanced paralysis of the facial muscles. Usually it takes 4-6 months for reinnervation of the facial muscles, so prevention of disuse atrophy of muscles during this period would improve the strength of the facial muscles after reinnervation. It may also make a muscle graft unnecessary in patients who have undergone a cross face nerve graft.

In this study, we used a dose of 5 mg/kg, which is

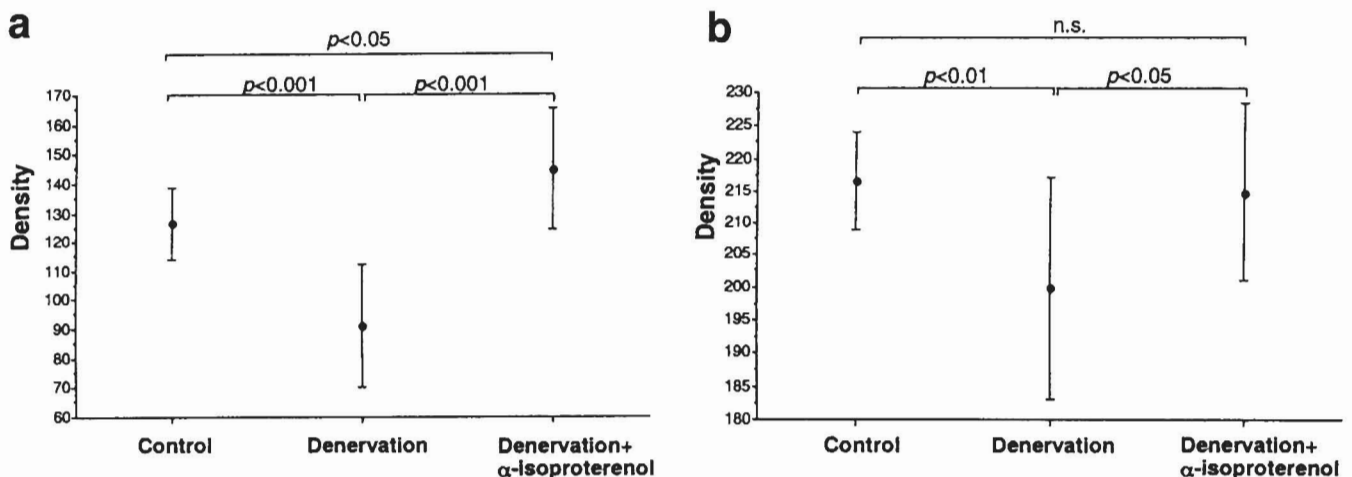


Fig 3. The optical densities of the reaction products of cytochrome oxidase in control and denervated orbicularis oculi muscles treated with or without isoproterenol. **a.** White fibers. **b.** Red fibers. Data represent the mean of 25 muscle fibers. There was a significant difference between each group; control, denervation, denervation+isoproterenol administration. n.s.: no significance.

considerably higher than normally used. For its clinical application, it is essential to establish a method to prevent its side-effects on the heart, blood vessels and, bronchus. We are currently testing drug delivery systems consisting of fine biopolymer particles for localized delivery of isoproterenol to the facial muscles.

References

- Clark W.A., Rudnick S.J., Anderson L.C. and LaPres J. (1994). Myosin heavy chain synthesis is independently regulated in hypertrophy and atrophy of isolated adult cardiac myocytes. *J. Biol. Chem.* 269, 25562-25569.
- Deshaiies Y., Willemot J. and Leblanc J. (1981). Protein synthesis, amino acid uptake, and pools during isoproterenol-induced hypertrophy of the rat heart and tibialis muscle. *Can. J. Physiol. Pharmacol.* 59, 113-121.
- Ishii K., Zhai W.G. and Akita M. (1998). Effects of isoproterenol on the orbicularis oculi and stapedius muscles. *ORL* 60, 98-102.
- Lubert S. (1991). *Biochemistry*. WH Freeman and company. New York. pp 981-992.
- Maltin C.A., Hay S.M., Delday M.I., Smith F.G., Lobley G.E. and Reeds P.J. (1987). Clenbuterol, a beta-agonist induces growth in innervated and denervated rat soleus muscle via apparently different mechanisms. *Biosci. Rep.* 7, 525-532
- Pinson A., Schlüter K-D., Zhou X.J., Schwartz P., Kessler-Icekson G. and Piper H.M. (1993). Alpha- and beta-adrenergic stimulation of protein synthesis in cultured adult ventricular cardiomyocytes. *J. Mol. Cell. Cardiol.* 25, 477-490.
- Roberts P. and McGeachei J.K. (1994). The enhancement of revascularisation of skeletal muscle transplants using the β_2 -agonist isoprenaline. *J. Anat.* 184, 309-318
- Schwarting S., Schöder M., Stennert E. and Goebel H.H. (1984). Morphology of denervated human facial muscles. *ORL* 46, 248-256.
- Seligman A.M., Karnovsky M.J., Wasserkrug H.L. and Hanker J.S. (1968). Non droplet ultrastructural demonstration of cytochrome oxidase activity with a polymerizing osmophilic reagent, diamino-benzidine (DAB). *J. Cell Biol.* 38, 1-14.
- Sillau A.H. and De Lourdes Philippi M. (1987). Long-term isoprenaline administration produces an increase in capillarity in the soleus muscle of the rat. *Can. J. Physiol. Pharmacol.* 65, 303-306.
- Taylor P. and Tang Q. (1984). Development of isoproterenol-induced cardiac hypertrophy. *Can. J. Physiol. Pharmacol.* 62, 864-869.
- Versura P., Maltarello M.C., Müller R.M. and Roomans G.M. (1988). Changes in glycoconjugates in rat submandibular gland after chronic treatment with reserpine and isoproterenol. *Histochemistry* 90, 285-288.
- William A.C., Steven J.R., Laura C.A. and LaPres J.J. (1994). Myosin heavy chain synthesis is independently regulated in hypertrophy and atrophy of isolated adult cardiac myocytes. *J. Biol. Chem.* 269, 25562-25569.
- Zhai W.G., Ishii K. and Akita M. (1995). The effects of isoproterenol on orbicularis oculi muscle and stapedius muscle. *Facial Nerve Res. Jpn.* 15, 15-18.

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