Taurine Transport in Chronically Stimulated Fast- and Slow-Twitch Muscles of the Rat

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Abstract—Indirect stimulation via the sciatic nerve, 10 or 100 Hz stimulus trains of 1 sec duration, applied every 3 sec, 8 hr/day for 1–4 weeks increased taurine concentration of the extensor digitorum longus (EDL, fast-twitch) muscle of the rat. The uptake of [3H]-taurine into the EDL and tibialis anterior (TA, fast-twitch) muscle was also increased by the stimulation. Concentration and uptake of taurine in the soleus muscle (SL, slow-twitch) did not change by chronic 10 Hz stimulation. Taurine concentration in the SL muscle was significantly reduced by chronic 100 Hz stimulation. This study shows that chronic nerve stimulation increases the uptake of taurine in the fast-twitch muscles, but not in the slow-twitch muscle.

Taurine is present as a free amino acid in mammalian muscle and heart in a large amount (1). It has been suggested that the pathogenesis of muscular dystrophy is accompanied by changes in the taurine concentration of skeletal muscles (2, 3). Experiments with myotonic muscles have shown that taurine prevents or reduces the electrical signs of muscular hyperexcitability and favors potassium accumulation into the muscle fiber (4–6). Recent studies from our laboratory showed that denervation of skeletal muscle increased taurine concentration and taurine transport in fast-twitch muscle, but not in the slow-twitch one (7). The content and transport of taurine are much higher in slow-twitch muscle than in the fast-twitch one (7, 8).

The functional and biochemical expression of different types of vertebrate skeletal muscles are largely determined by information from the innervating motor neurons. This information might be nerve impulses and neurotrophic substances released from nerves to muscles. Fast and slow fiber types in adult skeletal muscle are characterized by distinct myofibrillar protein phenotypes (9). It is well known that chronic nerve stimulation of fast-twitch muscle at low frequency can transform various cellular parameters of the tissue to those which resemble slow-twitch muscle. This type of transformation has been demonstrated in many biochemical, structural and functional properties including changes in sarcoplasmic reticulum (10), glycolytic enzymes (11), myosin light chains (12) and in twitch-response (13). In the previous reports (7, 8), we have shown the possibility that taurine transport in fast- and slow-twitch muscles may be differently regulated by their innervating neurons. In the present study, we report the influence of chronic nerve stimulation on taurine transport in rat skeletal muscles.

Materials and Methods

Animals and electrode implanting: The operations of the Sprague-Dawley strain rats aged 4 weeks were performed under sodium pentobarbital (40 mg/kg, i.p.) anaesthesia with aseptic precautions. Electrodes made of stainless-steel wire coated with teflon tubing (teflon: 0.003", bare: 0.0045"; A-m System, Inc.) were implanted lateral to the left sciatic nerve of the animals and passed subcutaneously to the back and attached to the plug which was secured to the animal’s back by sutures and dental cement. Muscles from the unoperated contralateral hind-leg served as controls.
Electrical stimulation and taurine assay:
Stimulation was started one day after the implantation, and the animals were connected to a stimulator (Nihon Kohden SEN-6000), generating square waves, via a plug attached to leads from the stimulator. Stimulation consisted of trains of pulses at 10 and 100 Hz, the pulse duration being 0.2 msec. Each train lasted 1 sec and was repeated every 3 sec. The amplitude of stimulation was adjusted daily to give maximum contractions without causing any noticeable discomfort to the animals. Stimulation was performed from 10:00 a.m. to 8:00 p.m. every day, and the animals were sacrificed at 7:00 p.m. of the last day. Taurine was extracted from the excised muscle with 20 volumes of 5% trichloroacetic acid as described previously (14) and assayed fluorometrically with the o-phthalaldehyde reaction by high-performance liquid chromatography (7). Experiments on the uptake of taurine into muscles was performed at 10:00 a.m. after 2 weeks of the stimulation by the intraperitoneal injection of 50 μCi/kg of [3H]-taurine (12.8 Ci/mmol, Amersham) or [14C]-taurine (103 mCi/mmol, New England Nuclear).

To obtain data on the initial rate of taurine uptake by muscles (8), 3 or 6 hr after the injection, animals were sacrificed. The muscles were solubilized with tissue solubilizer (NCS, Amersham) by incubating at 50°C for 3 hr, and the radioactivity was counted by a liquid scintillation counter.

![Fig. 1. Effect of chronic low-frequency stimulation on taurine content of EDL, SL and GC muscles. Stimulation consisted of trains of pulses at 10 Hz, the pulse duration being 0.2 msec. Each train lasted 1 sec and was repeated every 3 sec. The values given represent the means and standard errors of 4–5 determinations. **P<0.01, significantly different from the unstimulated control. Control, Stimulated.](image-url)
Results

There were no significant differences between the muscle weights of the control and stimulated groups at any time before and during the period of stimulation of 10 Hz. The taurine concentration in the muscles did not change significantly by 10 Hz stimulation for 1 week (Fig. 1). However, the stimulation for 2 weeks significantly increased the taurine level (45%) of the extensor digitorum longus (EDL) muscle compared to the unstimulated control.

After 4 weeks-stimulation, the stimulated EDL and gastrocnemius (GC) muscles showed a significant increase of taurine concentration (25%, respectively). The stimulated soleus (SL) muscle showed no difference at any time during the 10 Hz stimulation. The effect of electrical stimulation at 10 Hz on the uptake of taurine into muscles is illustrated in Fig. 2. After 2 weeks-stimulation, the uptake of \[^{3}H\]-taurine by the EDL and tibialis anterior (TA) was significantly increased 3 hr after the injection. However, there was no difference in the stimulated SL muscle. High-frequency (100 Hz) stimulation for 1 week resulted in no difference of muscle weight between the control and stimulated animals, but the stimulated EDL

![Graph showing taurine uptake into muscles](image)

**Fig. 2.** Effect of chronic low-frequency stimulation on \[^{3}H\]-taurine uptake into EDL, SL, GC and TA muscles. The uptake into muscles was performed following 14 days-stimulation at 10 Hz, 0.2 msec, 8 hr/day. The animals were decapitated 3 or 6 hr after the injection of \[^{3}H\]-taurine (50 μCi/kg, i.p.). The values given represent the means and standard errors of 3 determinations. *P<0.05, **P<0.01, significantly different from the unstimulated control. □ Control, ■ Stimulated.
Fig. 3. Effect of chronic high-frequency stimulation on taurine content of EDL, SL and GC muscles. Stimulation consisted of trains of pulses at 100 Hz, the pulse duration being 0.2 msec. Each train lasted 1 sec and was repeated every 3 sec. The experiments were performed following 14 days-stimulation. The values given represent the means and standard errors of 4 determinations. *P<0.05, **P<0.01, significantly different from the unstimulated control. □ Control, ▄ ▄ ▄ Stimulated.

muscle showed a significant increase in taurine level (33%) and the stimulated SL muscle showed a decrease (15%) (Fig. 3). Electrical stimulation at 100 Hz for 2 weeks induced a significant increase in the taurine uptake by EDL (40%) and TA (35%) muscles (Fig. 4).

Discussion
The present study clearly showed that taurine concentration and uptake in the EDL muscle but not in the SL muscle significantly increased by chronic low frequency stimulation (10 Hz). Increase in the uptake of taurine was also observed in the TA muscle, another typical fast-twitch muscle. Therefore, increase in the taurine transport by chronic nerve stimulation seems to be restricted to fast-twitch muscles. Skeletal muscle has negligible activity of taurine biosynthesis (15). The accelerated uptake of taurine may account for the increase in the concentration of chronically stimulated EDL, although other possibilities, e.g., decrease in the efflux, should be considered. We have previously suggested that transport of taurine into skeletal muscles is differently regulated by the neural information and the muscular activity and that the regulation is dependent on the muscle phenotype (8). In an acute experiment, the electrical stimulation of the sciatic nerve accelerated the uptake by EDL and SL muscles of the control, but not in those of
curare-treated rats (8). Taking this observation into account, the changes of taurine transport by chronic nerve stimulation may not be related to the muscular activity.

Mammalian skeletal muscles are physiologically distinguishable into fast-twitch and slow-twitch muscles. Transformation of one muscle type to another is performed by indirect electrical stimulation with a stimulus pattern which resembles the physiological conditions (16). The chronic stimulation at 10 Hz resulted in changes of contractile characteristics and myosin and Ca\(^{2+}\) uptakes of the sarcoplasmic reticulum (11). From these viewpoints, it should be reasonable that the transformation of fast-twitch muscle to the slow type by a chronic slow-twitch type stimulation may account for the changes in the concentration and the uptake of taurine in the EDL. Similar changes in the EDL muscle were obtained after denervation which may cause dedifferentiation of the muscle (8). In contrast, the reverse experiment, viz., the transformation of the slow one by stimuli at higher frequencies, e.g., 40, 60 or 100 Hz, has been attempted, but equivocal results were obtained (16–18). In agreement with these observations, the present results also showed some contradiction, that is, the chronic stimulation of 100 Hz did reduce the content, but not the uptake of taurine in the SL muscle. In addition, the chronic stimulation with high frequency also increased the content and the uptake in the EDL muscle. Thus, the results obtained in the experiment of high frequency stimulation can not be explained by a transformation of the muscle type even if the transformation of the SL muscle is actually attained by the present condition.

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