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# The Influence of Ketanserin, a New $S_2$ Receptor Antagonist on Experimentally Induced Skeletal Muscle Myopathy in the Rat

## A Histological Approach *

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**Summary.** Unilateral ligation of the femoral artery in rats, followed five days later by five daily intraperitoneal injections of serotonin (20 mg/kg), resulted in extensive necrosis in the gastrocnemius and arterior tibialis muscles of the ligated leg. Oral pretreatment with ketanserin, a new $S_2$ (serotonergic) receptor antagonist, effectively inhibited this skeletal muscle myopathy promoted by serotonin in the femoral artery ligated rats: skeletal muscle lesions appeared to be absent or significantly reduced in severity as well as in extent. The myopathy induced by noradrenaline was not affected by ketanserin.

It is possible that ischaemia-induced membrane defects, due to the ligation, render the affected muscle cells more susceptible to the action of serotonin so that degeneration evolves. Alternatively, it is considered that serotonin may restrict the blood flow to the skeletal muscle tissue by constricting microcirculatory and (or) collateral vessels thereby inducing severe ischaemic conditions.

The beneficial effect of ketanserin can be explained by its antagonistic activity on the $S_2$ receptors of either the skeletal muscle cells or the vascular smooth muscle cells.

**Key words:** Histology – Serotonin – $S_2$ receptor antagonist – Induced skeletal muscle myopathy – Ketanserin

## Introduction

Serotonin or 5-hydroxytryptamine (5-HT) is well-known to be a potent vasoconstricting agent (Cater et al. 1961; Erspamer 1966; Gillespie and Rae 1972).

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The amine decreases the blood flow to skeletal muscle in rats after intraperitoneal injection (Erspamer 1966; Gillespie and Rae 1972) and reduces the flow rate in the isolated rat limb (Mendell et al. 1971).

It is reported that serotonin alone (O'Steen et al. 1967; Munsat et al. 1977) or in combination with abdominal aorta ligation (Mendell et al. 1971; Mendell et al. 1972a; Thorpe and Boegman 1974) induced skeletal muscle damage in mice (O'Steen et al. 1967) and rats (Mendell et al. 1971; Mendell et al. 1972a; Thorpe and Boegman 1974; Munsat et al. 1977). Treatment with imipramine, an uptake blocker of biogenic amines, followed by serotonin injection is also found to induce lesions in the skeletal muscles of the rat (Parker and Mendell 1974). This finding was confirmed by Meltzer (1976) who also used additional uptake blockers and other membrane-active drugs with comparable results. However, the experiments by Munsat and co-workers (1977) revealed no difference in myopathy between imipramine in combination with serotonin and serotonin administration alone. Several reports deal further with the possibility that serotonin is involved in muscular dystrophies like the hereditary muscular dystrophy in mice (Gordon and Dowben 1966; O'Steen 1967), genetically dystrophic chickens (Hudecki and Barnard 1976; Barnard et al. 1976; Barnard and Barnard 1979) and Duchenne's muscular dystrophy in man (Misra et al. 1965; Mendell et al. 1971; Mendell et al. 1972b; Murphy et al. 1973; Parker and Mendell 1974; Adornato et al. 1979).

In this context serotonin antagonists were used in hereditary muscular dystrophy (O'Steen 1967) and genetically dystrophic chickens (Hudecki et al. 1976; Barnard et al. 1976; Barnard and Barnard 1979) resulting in a significant retardation of the progression of muscular dystrophy in both mice and chickens. In patients with carcinoid syndrome, clinical improvement of myopathy has also been reported after treatment with cyproheptadine (Berry et al. 1974; Swash et al. 1975). Serotonin antagonists were found to decrease significantly the incidence and extent of skeletal muscle necrosis produced by combined chlorpheniramine and serotonin in rats (Meltzer 1976) and also prevented the adverse effect of serotonin on the twitch tension in a rat nerve-muscle preparation (Patten et al. 1974).

Recently ketanserin (R 41,468; 3-{2[4-(fluorobenzoyl)-1-piperidinyl]ethyl}-2,4(1H,3H)quinazolinedione), a new $S_2(5\text{-}HT_2)$ receptor antagonist was introduced (Leysen et al. 1981).

A modified model of the ligation technique of Mendell et al. (1971) was used to investigate the influence of this compound on serotonin induced myopathy in the rat.

## Materials and Methods

Sixty male Wistar rats (250 g) were used in this study. After anaesthesia with Hypnorm® the right femoral artery was exposed and doubly ligated proximal to the superficial circumflex iliac artery. Thereafter the femoral artery was cut between the ligatures. We preferred the ligation of the femoral artery over the ligation of the abdominal aorta described by Mendell et al. (1971) because the skeletal muscles of the contralateral hind leg could be used as an internal control. Moreover, aortic ligation in our hands always induced paralysis of one or both hind legs 24 h after ligation, a phenomenon also described by others (Karpati et al. 1974; Keltz and Kaiser 1979). In the femoral artery ligated rats only about 20% of the animals showed paralysis or paresis of their right hind legs (ligated side). It appeared further from earlier observations (unpublished results) that the right anterior tibialis muscle of rats with an apparently normal walking behaviour after unilateral ligation showed no histological damage or only minor lesions 7 days after surgery. Extensive muscular necrosis was frequently observed, however, in rats with an abnormal

**Table 1.** Treatment schedule: 2.5 mg/kg ketanserin or placebo (PL) was administered orally twice a day (morning and evening) for 5 days. The vasoactive amines serotonin (SER) (20 mg/kg) and noradrenaline (NA) (2 mg/kg) were injected intraperitoneally 2 h after each morning treatment.

| | Number of rats | Treatment |
|---|---|---|
| Group I | 13 | ketanserin + SER |
| Group II | 14 | PL + SER |
| Group III | 11 | ketanserin + NA |
| Group IV | 11 | PL + NA |
| Group V | 11 | no treatment |

walking behaviour. We therefore selected for this study only those rats which could use their hind legs in a normal way 24 h postoperatively.

Five days after ligation the animals were treated as indicated in Table 1.

The dose of 3 mg/kg noradrenaline used by Mendell and co-workers (Mendell et al. 1971) was lethal to most of our animals when administered on 5 consecutive days. The dose of 2 mg/kg noradrenaline was well tolerated. One rat of group I and 3 rats of group II died during treatment probably because of side-effects induced by serotonin as reported earlier (Munsat et al. 1977; Keltz and Kaiser 1979).

Three days after the last drug administration the animals were decapitated whereafter the gastrocnemius and tibialis anterior muscles of the hind legs were excised. Two segments of each muscle were fixed for 1 week in neutral buffered formaldehyde and embedded in paraffin. Five micron thick sections were prepared and stained with hematoxylin-eosin.

## Results and Discussion

Between the third and fifth day of drug treatment 8 animals of group II showed signs of paresis of the right hind limbs whereas no abnormal walking behaviour could be recognized in the other groups. These abnormalities had however disappeared at the moment of sacrifice three days after the last drug administration. The muscles of the group II animals (except those of one rat) were, macroscopically, reduced in size and the transected segments were pale to yellowish-white in color. No reduction or occasionally a mild change in muscle size or discoloration of muscle segments was seen in the ketanserin treated animals of group I. Macroscopic changes were present in 4 rats of group III and IV, never in those of group V. The muscles of the non-ligated side always looked completely normal.

Histological examination of the muscle segments revealed a difference in severity and extent of the lesions throughout the different groups and between different animals of the same group. Therefore, qualitative scores were used according to the severity of the damage (Figs. 1 and 2). The histological appearance of the scored lesions is described in Fig. 1. Additionally, semi-quantitative measurements were performed to assess the extent of muscular injury (Fig. 3).

It appears from Figs. 2 and 3 that a 5-day treatment with 20 mg/kg serotonin induced reproducible lesions in both examined skeletal muscles of femoral artery ligated rats and that the anterior tibialis is somewhat more affected than the gastrocnemius. Muscular damage was rare and minimal after ligation

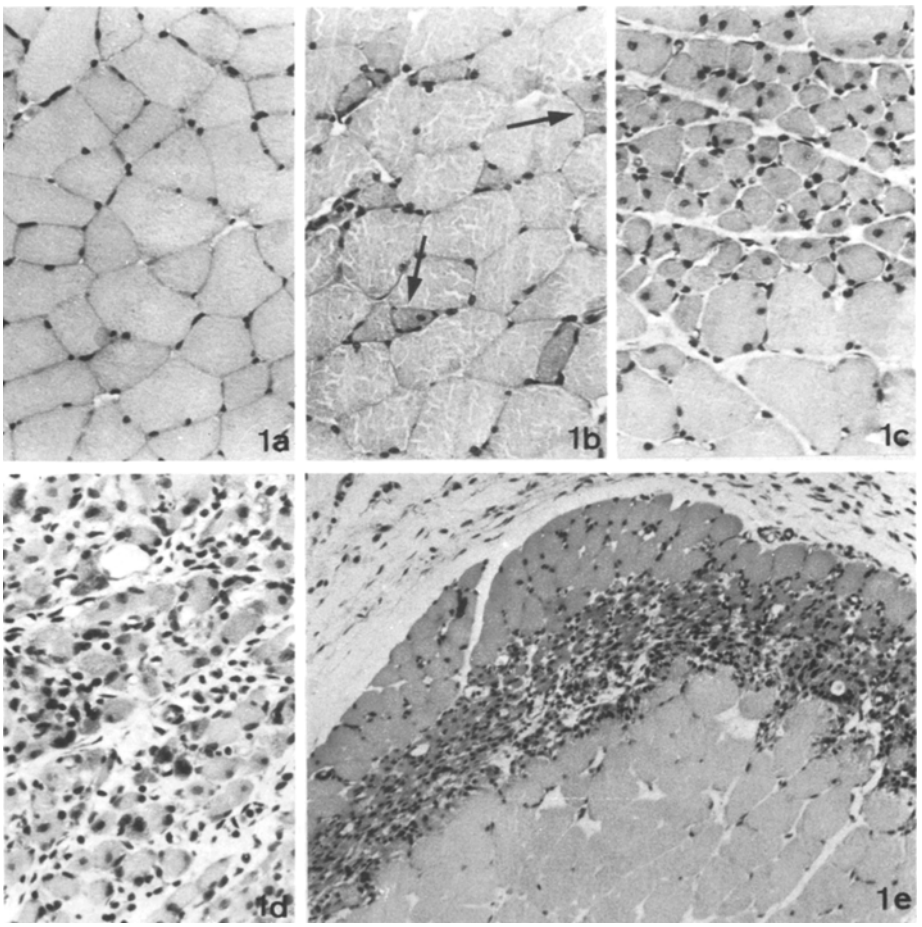


**Fig. 1a–e.** Histology. **a** Transverse section of normal muscle fibers (×250). **b** Lesion score 1: single fibers or small groups of fibers (arrows), reduced in size and mostly containing a central nucleus, are distributed throughout the section and have an apparently normal cohesion with the surrounding normal looking tissue (×250). **c** Lesion score 2: group(s) of more than 10 small rounded fibers with a centrally placed nucleus; no necrosis or fibrosis (×250). **d** Lesion score 3: small rounded fibers with a centrally placed nucleus and intermingled with small or moderate amounts of connective tissue and necrotic fibers (×250). **e** Lesion score 4: extensive necrosis and fibrosis. From the periphery to the interior of the muscle bundles one finds a single or a few layers of normal looking fibers, small round fibers with fibrosis, extensive fibrosis and finally swollen, degenerating fibers apparently lacking nuclei and intramuscular capillaries (×125)

in some untreated controls (group V) indicating that serotonin may be held responsible for the extensive lesions in our selected rats. In addition, the muscles taken from the non-ligated side showed only minor histologic changes after serotonin in one sample of two rats from group I (lesion score 1, less than 5% of the muscle was affected). This may indicate, as is proposed (Seley 1967; Mendell et al. 1971), that the muscles from the ligated side are somehow predisposed to the development of lesions upon additional challenge with agents

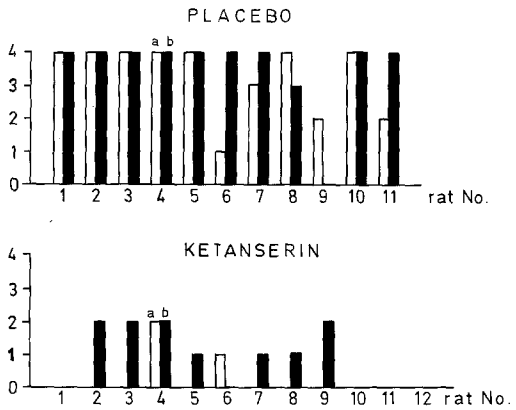


**Fig. 2.** Qualitatively scored lesions present in the gastrocnemius (white columns) and anterior tibialis muscles (black columns) of the individually numbered rats after treatment with serotonin and either placebo or ketanserin. The explanation of the lesion scores (ordinate) is given in Fig. 1. Statistical analysis (placebo vs. treated) with Mann-Whitney U test: **a** $P=0.00003$ vs gastrocnemius, **b** $P<0.0003$ vs anterior tibialis

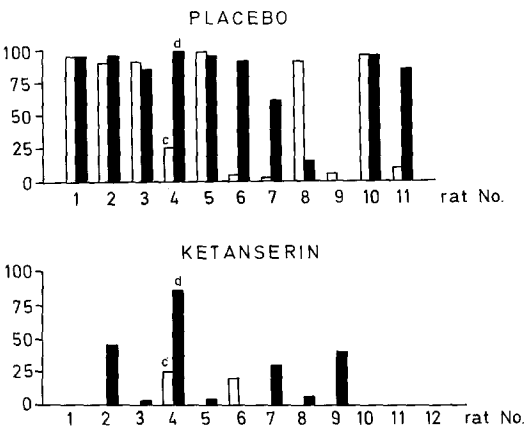


**Fig. 3.** The percentage of the damaged area(s) of the gastrocnemius (white columns) and anterior tibialis muscles (black columns), after treatment with serotonin and either placebo or ketanserin, is assessed from the histologic sections (mean of two sections/muscle). The individually numbered rats (abscissa) are the same as indicated in Fig. 2. Statistical analysis (placebo vs treated) with Mann-Whitney U test: **c** $P<0.0003$ vs gastrocnemius, **d** $P<0.002$ vs anterior tibialis

such as serotonin. It is further evident from Figs. 2 and 3 that ketanserin is very effective in preventing the serotonin induced lesions. Moreover, it appeared that this compound selectively suppressed the damage elaborated by serotonin and not that induced by noradrenaline (not shown). Indeed, although the muscular lesions evolved by noradrenaline were not very reproducible (only 64% of the rats from group III and 72% from group IV showed injuries with varying lesions scored from 1 to 4) ketanserin did not prevent the NA-induced damage.

The exact pathogenic mechanism of hereditary muscular dystrophy in animals and man and in experimentally induced myopathy remains speculative.

From the available literature it seems that membrane alterations may generally be involved. From the different animal models, including our own, in which myopathy is experimentally induced, it is assumed that the primary pathogenic mechanism is ischaemia. It is well-known that ischaemia results in membrane changes. Meltzer (1976) used membrane-active drugs to promote skeletal muscle alterations. Moreover, there are indications suggesting that a widespread membrane defect is present in Duchenne's muscular dystrophy (Rowland 1976; Lucy 1980) and animal hereditary muscular dystrophy (Sha'afi et al. 1975; Scales et al. 1977; Strickland et al. 1979; Mendell et al. 1979). As already mentioned, serotonin has been implicated in hereditary muscular dystrophy and genetically dystrophic chickens in so far that treatment with serotonin antagonists appeared to have clear benefical effects (O'Steen 1967; Hudecki and Barnard 1976; Barnard et al. 1976; Barnard and Barnard 1979). In patients with Duchenne's muscular dystrophy no changes were found in the actual levels of serotonin or its metabolites in the plasma or urinary fluid (Misra et al. 1965; Mendell et al. 1972b) but a reduced initial rate of accumulation of serotonin $^{14}C$ into platelets (Mendell et al. 1972b) and a decreased number of platelet dense bodies is described (Murphy et al. 1973). The use of serotonin antagonists in this desease is not reported in the literature. Some serotonin blockers were effective against muscle necrosis promoted by the membrane-active drug chlorphenirамine and serotonin (Meltzer 1976). Ketanserin gave excellent results in our system. A direct action of serotonin on muscle cells is recently considered to explain skeletal muscle necrosis in experimentally induced myopathy (Patten et al. 1974; Meltzer 1976; Hudecki and Barnard 1976; Mendell et al. 1979). All these data may give support to the hypothesis already expressed by Barnard and Barnard (1979) for genetically dystrophic animals, that membrane defects, either experimentally induced or resulting from genetic disturbances, may render affected muscle cells more susceptible to the action of serotonin, directly or mediated via prostaglandins (Horrobin et al. 1977) with degeneration as sequel.

However, chronic ischaemia of the muscular tissue as a result of microcirculatory disturbances, the so-called "vascular theory" has long been considered to occur as part of the pathogenesis of DMD as reported by Cazzato (1968) and others. Muscle blood flow measurements (Paulson et al. 1974) and morphometric data on the muscle microvasculature (Jerusalem et al. 1974) provided no evidence for a reduced blood supply in the affected muscles of these patients. However, because of the vasoactive properties of serotonin (Cater et al. 1961; Erspamer 1966; Mendell et al. 1971; Gillespie and Ray 1972) its action on muscular blood vessels should be taken into account in discussing our experimental results. Ketanserin has been found to antagonize the serotonin induced contractions in isolated vascular tissue (Van Nueten and Vanhoutte 1981). It is therefore possible that after each daily injection of serotonin a constriction of microcirculatory and (or) collateral vessels occurs resulting in severe ischaemia for a prolonged period of time. The influence of serotonin and thereby ketanserin on the blood circulation in the lower extremities under the described experimental conditions of our model, is now under investigation.

It is assumed that whether serotonin acts directly on the skeletal muscle cells or on the vascular smooth muscle cells the inhibiting activity of ketanserin

on the serotonin evoked muscular damage is due to its selective $S_2$ receptor antagonism.

Preliminary dose-response experiments indicated that two daily doses of 0.63 or 0.16 mg/kg of ketanserin for 5 days significantly inhibited the serotonin induced skeletal muscle dystrophy in the femoral artery ligated rats. 0.04 mg/kg of ketanserin was not effective. Methysergide ($2 \times 2.5$ mg/kg) tested concomitantly was also effective in inhibiting the establishment of the serotonin induced lesions.

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