Muscular dystrophy in adult mice chronically treated with Δ^9-THC at behavioural doses[1]

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Summary. After chronic treatment with Δ^9-THC at behavioural doses, a muscular dystrophy was demonstrated in adult mice, on the basis of histological and biochemical data and of functional tests on muscle.

In the course of previous studies concerning the action of cannabinoids on the bone marrow of rats[2] and mice[3], a muscular subatrophy was suspected on gross observation. The present research was undertaken in order to elucidate this point.

Materials and methods. Swiss male adult albino mice, 30–35 g body weight, were used. Eight animals were treated with a daily subcutaneous injection of Δ^9-tetrahydrocannabinol (1 mg/kg) suspended in phosphate buffer pH 7.4 (0.1 ml) for 30 days. Eight animals were treated in the same way with the vehicle only. The purification of Δ^9-THC was obtained by thin layer chromatography and the purity was ascertained by gas-liquid chromatography, as previously described[4].

At the end of the treatment, animals were anesthetized with i.p. pentobarbitone sodium (65 mg/kg). The gastrocnemius muscle and the sciatic nerve were isolated, taking great care to avoid damaging the blood supply and innervation. The Achille's tendon was detached and connected with an isometric transducer (Basile, Microdynamometer). The peripheral cut end of the sciatic nerve was stimulated by means of platinum electrodes connected, through an isolation unit (Grass SIU 478), with a stimulator (Grass S 8) which delivered single pulses (0.2 msec) or 5 sec trains of rectangular pulses (0.2 msec, 80 Hz). Supramaximal voltages were used for each preparation (6–10 V). During the experiment, the whole animal's leg was maintained at 34–35 °C in a bath of mineral oil. The initial muscular tension was adjusted step by step, from 10 to 40 g tension, each step adding 5 g. At each one of these initial loads, the muscle was stimulated through its sciatic nerve. The value of the initial tension applied to the muscle was taken as zero and the active tension developed on single twitch or tetanic stimulation was measured through the transducer connected to an oscilloscope. The signals were also stored on a magnetic tape (Philips Mini Log 4) for later analysis. Animals were then sacrificed by bleeding. The contralateral gastrocnemius muscles were detached, weighed and sectioned equatorially. Part of each muscle was fixed in Carnoy's fluid and embedded in paraffin. Equatorial and longitudinal sections were stained with hematoxylin and eosin. On equatorial sections the diameter of the fibres (over 100 for each section) was measured with the aid of a micrometer. The remaining part of each muscle was weighed again and hydrolized overnight in sealed tubes with 7 ml of HCl 6 N at 118 °C. The protein content was determined according to Lowry et al.[5], and the hydroxyproline content according to Switzer and Sumner[6].

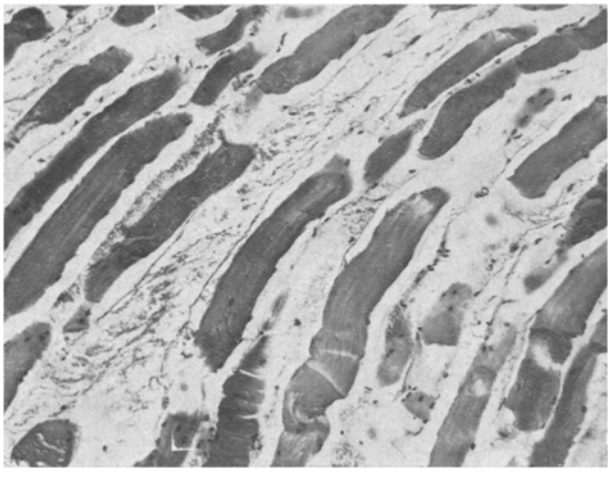

Fig. 1. Longitudinal section of the gastrocnemius muscle of mouse treated with Δ^9-THC. Note apparently normal fibres intermingled with hyaline and necrotic fibres and debris. (Hematoxylin and eosin; × 240.)

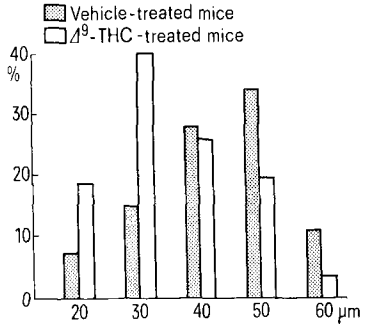

Fig. 2. Per cent distribution of muscular fibre diameters in control and Δ^9-THC treated mice.

Results and discussion. The values of the initial muscle tension, at which the maximal active tension was reached, showed no significant change in treated animals, in comparison with the controls. Following supramaximal stimulation of the sciatic nerve, a significant decrease of both maximal twitch and tetanus tensions was observed in Δ^9-THC treated mice (table).

Transverse and longitudinal sections of the gastrocnemius muscle showed a normal appearance in the vehicle group. In Δ^9-THC-treated animals, rounded muscle fibres of varying diameters appeared embedded in fat and fibrous tissue. Normal fibres were intermingled with hyaline and vacuolated or necrotic fibres (figure 1). In some cases, endomysial tubes were filled with debris. Also centrally placed nuclei were observed. The highest

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frequency of the fibre diameters was about 30 μm in treated mice, while in controls it was about 50 μm (figure 2).

The protein content of the gastrocnemius muscle in treated mice was significantly lower in comparison with the control group: this reduction had its counterpart in the markedly increased hydroxyproline content (table). The above mentioned data permit one to qualify the lesion as a muscular dystrophy, although a clear-cut distinction of its origin is impossible, since in this field the pathological findings are confusingly similar, and morphology depends on the stage that the disease has reached in the particular muscle examined. The morphological data fit very well with the biochemical findings and the functional tests on muscle.

	Vehicle	Δ^9-THC
Weight of the muscle (mg)	171.7 ± 7.89*	187.2 ± 12.2
Protein content (per cent of the weight of the muscle)	19.72 ± 0.58	15.57 ± 0.35 ($p<1^0/_{00}$)
Hydroxyproline content (per cent of the protein content)	0.637 ± 0.03	1.065 ± 0.05 ($p<1^0/_{00}$)
Twitch tension (g)	59.85 ± 2.33	36.70 ± 2.26 ($p<1^0/_{00}$)
Tetanus tension (g)	218.86 ± 8.87	160.28 ± 8.33 ($p<1^0/_{00}$)

* Standard error.

It can therefore be inferred that the treatment with Δ^9-THC at low doses for a rather prolonged time gives rise to a particular muscular lesion which, as far as we know, has never been described after cannabinoids. Our experimental data cannot give an explanation of the possible mechanism involved in the origin of this peculiar dystrophy. However, the data reported by Kayaalp et al.[7] account for a partial blocking action of Δ^9-THC upon neuromuscular transmission, possibly due to a direct action of the drug on the naked motor nerve terminals in the muscle, which are more susceptible to such an action than the myelinated motor axons in the nerve trunk. Therefore, it cannot be excluded that such a blocking action, protracted for 30 days, is one of the causes of the observed muscular lesions, though some effects obtained after treatment with Δ^9-THC[2,3,8–10] suggest that a more general mechanism is involved. Studies are in progress on this topic.

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Cardiotonic activities of some new type of bufadienolide- and cardenolide-conjugates[1]

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Summary. Some new type of bufadienolide- and cardenolide-conjugates, including bufotoxins and 3-sulfates recently isolated from the toad skin, were tested for their cardiotonic activities by using isolated frog hearts and guinea-pig atria. The relative potencies were obtained and reported.

Recently, Nambara and his co-workers[3–11] have isolated new bufotoxins and their homologs, as well as bufadienolide and cardenolide 3-sulfates, from the skin of Japanese toads. They also synthesized the analogous conjugates of cardenolide[12,13]. In this study, some of these compounds were tested: 1. the isolated frog hearts, and 2. the isolated guinea-pig atria.

The agents used were bufalin 3-sulfate, digitoxigenin 3-sulfate*, gamabufotalitoxin (gamabufotalin 3-suberoyl-arginine ester), gamabufotalin 3-hemisuberate, gamabufotalin, 8 digitoxigenin 3-suberoyl-X esters* in which X were amino acids, and dipeptides (table 2), as well as bufalin and digitoxigenin (* = synthetic specimen).

The stock solution of digitoxigenin was prepared with 95% ethanol in concentration of 1.0 mg/ml (2.7 mM). All other compounds except 3 were also dissolved in concentration of 1.0 mg/ml. Stock solutions of gamabufotalitoxin, gamabufotalin 3-hemisuberate and gamabufotalin were made in equimolar to that of digitoxigenin. Immediately before experiment, these stock solutions were diluted with saline (0.6% for frog hearts, and 0.9% for guinea-pig atria) to desired concentrations.

1. The isolated frog heart (Straub's preparation). The method of assay is the same as the previous paper[14]. Male frogs, Rana nigromaculata (20–35 g) were used. The Straub's cannula contained 2 ml of Ringer's solution, the

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