Histological and histochemical aspects of the effect of notexin on rat skeletal muscle

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The s.c. injection of a single dose of notexin (0.2 ml; 10 μg/ml) into the antero-lateral aspect of the rat hind limb resulted in an acute necrotizing myopathy of the underlying skeletal muscle. The intense oedema and necrosis which was apparent 24 h after injection was accompanied by an active inflammatory reaction. Invasion of necrotic fibres by phagocytic cells was rapid and even at this stage some fibres had completely dissociated into presumptive myogenic elements and residual fragments of necrotic sarcoplasm. Myofibrillar ATPase activity was found to be absent from severely necrotic fibres but was apparently unimpaired in surviving fibres. The distribution of the affected fibres was a mosaic and strongly suggested a selective susceptibility to the toxin of fibres with high oxidative enzyme activity. The majority of the surviving fibres were shown to be 'fast glycolytic' fibres whereas the 'slow oxidative' or 'intermediate oxidative' fibres were either entirely destroyed or greaterly reduced in number.

Experiments *in vitro* confirmed the greater susceptibility to the toxin of mitochondrial oxidative enzymes, e.g. succinate dehydrogenase, NADH-tetrazolium reductase, as compared with other histochemically-demonstrable enzyme systems.

After the acute phase of muscle necrosis, regeneration was swift and effective. Free uninucleate myoblasts were visible 3 days after injection. Many of these had fused to form myotubes at the 5 day stage. After 7-10 days histochemical differentiation of the myotubes into fibre types became apparent in myofibrillar ATPase preparations. This process was virtually complete at 21 days when the newly formed fibres were approximately the same diameter as normal adult oxidative fibres. If the muscle was denervated prior to injection of notexin, no histochemical differentiation takes place in the regenerating myotubes. These appeared to develop normally until the 7 day stage but thereafter atrophied along with the rest of the muscle fibres.

Effect of 5,7-dihydroxytryptamine on the metabolism and accumulation of 5-hydroxytryptamine in the snail CNS

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Snail (*Helix pomatia*) nervous tissue contains high levels of 5-hydroxytryptamine (5-HT) where it is thought to function as a transmitter substance (Gerschenfeld, 1973; Osborne & Neuhoff, 1974). When snail nervous tissue is perfused with [^{3}H]-tryptophan the amino acid is metabolized to form 5-hydroxytryptophan (5-HTP), 5-HT and 5-hydroxyindoleacetic acid (5-HIAA). The presence of *p*-chlorophenylalanine, an inhibitor of tryptophan-hydroxylase in the vertebrates (Koe & Weissman, 1966) in the perfused solution inhibits the formation of [^{3}H]-5HTP (about 60%) and to a greater extent the formation of [^{3}H]-5HT. It thus appears as if the metabolism of 5-HT in snails is similar to that in the vertebrates. Since 5,7-dihydroxytryptamine causes a long-lasting depletion of 5-HT in the rat CNS (Baumgarten, Björklund, Lachenmayer & Nobin, 1973), it was decided to investigate its effect upon the 5-HT metabolism in snails. Two types of experiments were carried out. In the first snails were injected once a day over a period of 72 h with 5,7-dihydroxytryptamine (0.5 ml) in snail's saline (2 μg/ml) and the nervous tissue was then perfused with 1 ml [^{3}H]-tryptophan (Radiochemical Centre, Amersham, sp. act. 5.58 Ci/mmol; 50 μCi/ml of snail solution) at a rate of 1 ml/2 h (Osborne, 1972, 1973). In the other experiments snail nervous tissue was first perfused with 1 ml snail's saline containing 5,7-dihydroxytryptamine (6 μg/ml) for 2 h followed by [^{3}H]-tryptophan. The amines and amino acids were then extracted, dansylated and subjected to two dimensional chromatography on 5 x 5 cm polyamide layers (for details see Osborne, 1973; Osborne & Neuhoff, 1974). An examination of the data revealed that 5,7-dihydroxytryptamine interfered with the metabolism of [^{3}H]-tryptophan in the first group of animals in the following way. Compared with the controls (±s.e. mean for six experiments), 40 ± 10% less [^{3}H]-5HTP, 30 ± 8% less [^{3}H]-5HT and 40 ± 14% less [^{3}H]-5HIAA was obtained. In contrast, no significant alteration in the metabolism of [^{3}H]-tryptophan could be