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TITLE: DECREASED SENSITIVITY TO METOCURINE DURING CHRONIC PHENYTOIN MAY BE DUE TO PROTEIN BINDING AND RECEPTOR CHANGES
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This study tested the hypothesis that the hyposensitivity to the neuromuscular effects of metocurine (MTC) during chronic phenytoin therapy, is due to increases both in plasma protein binding of MTC² and in acetylcholine receptors (AChR) at the muscle membrane.^{3,4}

Following 14 days of phenytoin (80 mg/kg/day, n=12) or equal volume of saline (controls, n=10) administered intraperitoneally to rats, effective doses of MTC (ED₅₀) were examined in the gastrocnemius muscle. Protein binding of MTC, α_1 -acid glycoprotein (AAG) levels in plasma and AChR number at the muscle membrane were quantified by equilibrium dialysis, immunodiffusion and ¹²⁵I α -bungarotoxin respectively. Time to spontaneous recovery of twitch to 75% of control and plasma MTC concentrations (by HPLC) at that recovery, were measured. ANOVA, at a level of $p < 0.05$, was used to test statistical significance.

Table Systemic Effects of Phenytoin (Mean \pm SE)

| | ED ₅₀ (μ g/ml) | AAG Conc. (mg/dl) | Prot.Bind'g (%) | AChR (fm/mgprot.) |
|-----------|-----------------------------------|------------------------|------------------------|-----------------------|
| Control | 9.9 (± 0.7) | 14.7 (± 1.2) | 67.2 (± 1.8) | 1.8 (± 0.3) |
| Phenytoin | 15.0* (± 1.7) | 163.9* (± 59) | 74.5* (± 2.5) | 2.7* (± 0.2) |

* $p < 0.05$ compared to controls

The hyposensitivity to MTC observed in humans who are on chronic phenytoin¹ was replicated in our rat model (Table). The protein binding and AAG levels were increased in the phenytoin treated rats. There was a significant positive correlation between plasma phenytoin concentrations and induction of AAG levels ($r=0.80$) and a significant negative correlation between AAG levels and free fraction of MTC in plasma ($r=0.66$). The recovery of twitch was faster in the phenytoin group and the plasma MTC concentration at 75% twitch recovery was significantly higher in the phenytoin group (0.04 ± 0.003 vs 0.03 ± 0.003 μ g/ml). The free fraction of MTC at 75% recovery, calculated from total MTC for that effect and plasma binding fractions, was significantly higher in the phenytoin group (0.03 ± 0.003 vs 0.02 ± 0.002 μ g/ml) and suggests altered pharmacodynamic sensitivity. These findings were corroborated by quantitative increases in AChR (Table) and is consistent with previous studies that, increases in AChR may be associated with hyposensitivity to curare-like drugs.^{3,4}

The present study, therefore, provides preliminary evidence to confirm the hypothesis that quantitative increases both in protein binding of MTC in plasma (probably related increases AAG levels),² and in AChR number at the muscle may play a role in the hyposensitivity to MTC.

Reference

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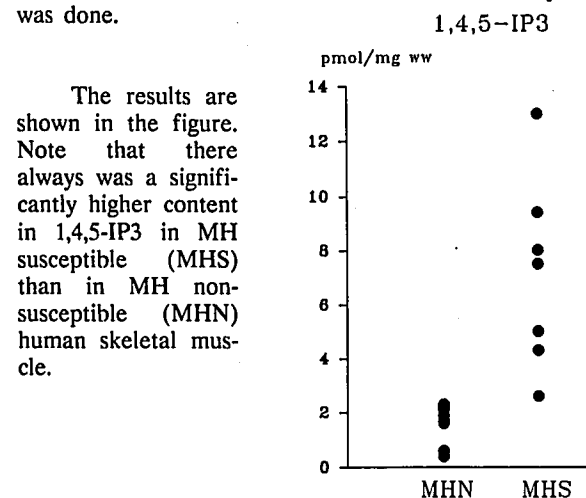
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TITLE: INOSITOL TRISPHOSPHATE AND MALIGNANT HYPERTHERMIA IN HUMAN SKELETAL MUSCLE
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Several studies suggest that the sarcoplasmic reticulum, which controls intracellular calcium fluxes, is involved in malignant hyperthermia (MH)¹. Stimulation of cell-surface receptors initiates hydrolysis of membrane-bound inositol lipid, which produces at least two second messengers inositol 1,4,5-trisphosphate (1,4,5-IP₃) and diacylglycerol. 1,4,5-IP₃ has been shown to mobilize intracellular calcium from the sarcoplasmic reticulum in several tissues². We³ and others⁴ have recently shown that 1,4,5-IP₃ may be involved in the development of MH in skeletal and heart muscle of MH swine. However, if 1,4,5-IP₃ is physiologically relevant for MH, a higher content should also be demonstrated in human skeletal muscle.

The experiments were performed on isolated human skeletal muscle (n=15), with informed consent and approval by the local ethic committee. The muscles were frozen, homogenized, charcoal treated and a solid-phase extraction was performed. Samples were freeze-dried, dissolved and subjected to HPLC analysis as described previously⁵. For comparison, an in-vitro skeletal muscle contracture test for determination of MH susceptibility was done.



The results are shown in the figure. Note that there always was a significantly higher content in 1,4,5-IP₃ in MH susceptible (MHS) than in MH non-susceptible (MHN) human skeletal muscle.

In conclusion, the data demonstrate a greater 1,4,5-IP₃ content in human MHS than MHN skeletal muscle. Thus it is tempting to speculate that the inositol-lipid metabolism is at least in part involved in the development of MH.

References

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