

VERATRINE EFFECTS ON ISOLATED SKELETAL MUSCLE MITOCHONDRIA: ULTRASTRUCTURAL, CYTOCHEMICAL, BIOCHEMICAL AND MORPHOMETRICAL STUDIES. Maria Alice da Cruz-Höfling (1), Márcia M. Fagian (2), Aníbal E. Vercesi (2), and Érika M. S. Freitas (1). (1) Depto. Histologia e Embriologia, IB; (2) Depto. Patologia Clínica, FCM, CEP 13083-970, Universidade Estadual de Campinas (UNICAMP), Campinas, SP, Brasil. E-mail: emsfreitas@hotmail.com

Recently we have shown that veratrine, an alkaloid extracted from seeds of some plants of the family Liliaceae [1], may be involved in degenerative mitochondrial changes observed when the drug is injected intramuscularly in mice [2,3]. Veratrine is a mixture of two major alkaloid esters, veratridine and cevadine [4]. It activates Na^+ channels [5] causing iterative depolarization [6], higher influx of Na^+ , followed by osmotic accumulation of fluid inside vacuoles in nerve fibers [7], or muscle cells [2,8]. In this study, we used different veratrine concentrations (250-550 $\mu\text{g/ml}$) to investigate its toxic effects on respiratory chain enzymes of isolated rat skeletal muscle mitochondria, through biochemical and cytochemical methods combined with ultrastructural and morphometrical analyses. Anesthetized adult Wistar rats (200-250 g) were sacrificed through cervical dislocation before hindlimbs muscles were removed for isolation of mitochondria. The oxygen consumption of mitochondrial pellets was measured with a Clark-type electrode in standard reaction medium (28°C) in a sealed glass cuvette equipped with a magnetic stirrer. Afterwards, the pellets were collected, submitted to cytochemical reactions for detection of cytochrome oxidase (COX) [9], NADH [10] and succinic dehydrogenases (SDH) [11] and processed for transmission electron microscopy. The morphometrical analysis of selected electron micrographs from isolated mitochondria was done using tpsDig program version 1.40 [12]. Respiratory rates and diameters from isolated skeletal muscle mitochondria were reported as mean values \pm standard error. Analysis was performed by one-way ANOVA followed by Bonferroni's *post hoc* comparisons. A probability of $P < 0.05$ was considered significant. The incubation with veratrine inhibited significantly, and dose-dependently, the State 3 respiration rate, RCR and ADP/O on isolated rat skeletal muscle mitochondria. The respiration rate of state 4 was not affected significantly by the alkaloid in comparison to controls non incubated with veratrine (**Table**). A significant population of veratrine-treated mitochondria exhibited ultrastructural alterations and negative reactions for NADH, SDH and COX enzymes after incubation with veratrine (**Fig. 1B, D, F**). In contrast, control preparations were positive for the three enzymes, and the ultrastructure was normal, the mitochondria were round or oval in shape, had regular the dimensions, cristae, and matrix electrondensity (**Fig. 1A, C, E**). Despite, some mitochondria of control preparations were swollen and ruptured, due to artifactual processing. Significant difference of diameters between control and veratrine-treated mitochondria was not seen, however, a tendency of increase was observed in the latter, mainly with 250 $\mu\text{g/ml}$ veratrine (**Fig. 2**). Since morphological changes were also observed at low veratrine doses, we suggested that ultrastructural alterations may have been secondary to alkaloid induced changes in the mitochondrial metabolism. Veratrine would probably interfere on membrane configuration, inducing an increased permeability of mitochondrial membrane, which could explain the *in vitro* morphological, cytochemical and biochemical changes seen in the skeletal muscle mitochondria after incubation with the drug.

References:

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Financial Support: CAPES, FAPESP, CNPq and FAEP-UNICAMP

VERATRINE ($\mu\text{g/ml}$)	STATE 3 (natoms O/min/mg)	STATE 4 (natoms O/min/mg)	RCR	ADP/O
0	160.9 ± 7.5	34.8 ± 1.8	4.7 ± 0.2	2.7 ± 0.1
250	143.8 ± 0.9	34.2 ± 0.7	4.2 ± 0.1	$2.3 \pm 0.1^{**}$
350	136.8 ± 1.6	33.6 ± 1.4	4.1 ± 0.2	$2.3 \pm 0.1^{**}$
450	$124.0 \pm 5.7^*$	34.0 ± 1.8	3.7 ± 0.3	$2.3 \pm 0.1^{**}$
550	$111.1 \pm 5.4^{***}$	32.1 ± 2.1	$3.5 \pm 0.3^*$	$2.3 \pm 0.2^*$

Table: Effects of veratrine on respiratory parameters of isolated rat skeletal muscle mitochondria. Each value represents the mean \pm standard error of three different mitochondrial preparations. Statistical significance compared to control: * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$.

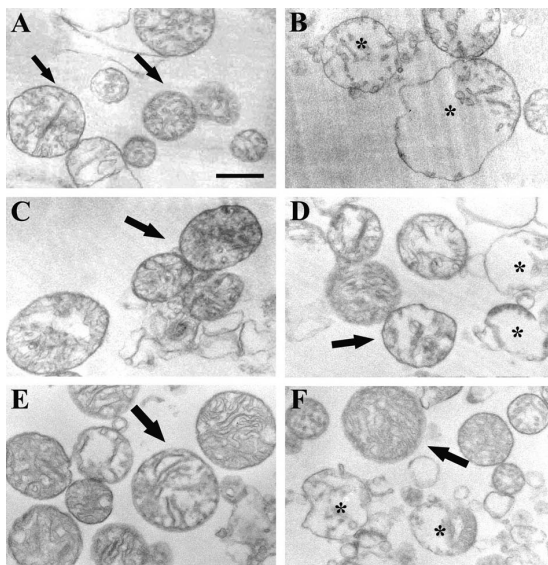


Figure 1: Electronmicrographs of isolated mitochondria from hindlimbs muscles of rats exhibiting different reactions for NADH (A-B), SDH (C-D) and COX (E-F). Controls (A, C and E): heterogeneous population of mitochondria with different sizes and round shapes. Note electron-dense deposits of final reaction product around in outer and inner membranes and in the intracristae spaces (arrows); Incubation with 250 $\mu\text{g/ml}$ veratrine (B, D and F): presence of mitochondria showing positive reaction for respiratory chain enzymes on membranes and cristae (arrows) or swollen mitochondria (*) with thin deposits of final product for reactions only some regions from membranes. Others swollen mitochondria showing negative reaction for enzymes. Bar = 0,5 μm for all panels.

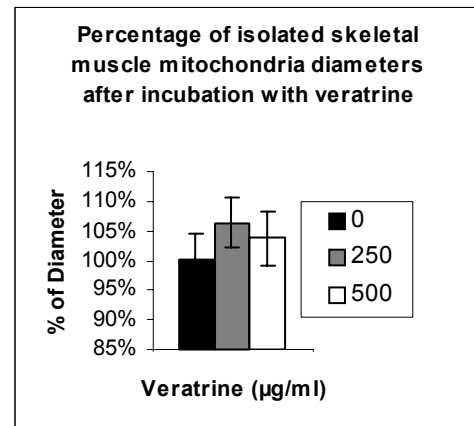


Figure 2: Percentage of isolated skeletal muscle mitochondria diameters (mean values \pm standard error) in function to the treatment with veratrine. Values with $P < 0.05$ represent statistical significance.