Title : EFFECTS OF VERAPAMIL AND EGTA ON MAMMALIAN MUSCLE IN VITRO

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Introduction. Dantrolene sodium, a compound that interferes with excitation-contraction coupling in muscle fibers by inhibiting intracellular movements of Ca^{++}[1], has been recommended for the treatment of malignant hyperthermia (MH). In the last decade verapamil has been used for the treatment of arrhythmias and other cardiovascular conditions.[2] This compound interferes with excitation-contraction coupling in mammalian smooth and cardiac muscle by a mechanism different from that of dantrolene, namely by inhibition of the transport of Ca^{++} through cell membranes.[3] The effects of verapamil on skeletal muscle function, however, have not been investigated. Preliminary to the investigation of the effects of verapamil alone, or in conjunction with dantrolene on the course of experimental MH a study of the myoneural effects of verapamil was undertaken. For sake of conparison observation of the myoneural effects EGTA (ethyleneglycol-bis- (β-aminoethyl ether) N,N'-tetra-acetic acid), a compound that interferes with the availability of Ca^{++} by chelation, was also included in this study.

Methods. The experiments were carried out on the in vitro phrenic nerve-hemi-diaphragm preparation of male Sprague-Dawley rats. To approximate in vivo conditions more closely the total [Ca] and [Mg] in the bath were 1.4 and 1.1 mM respectively. With these [Ca] and [Mg] in the otherwise unchanged Krebs' solution aerated with 95% O_2-5% CO_2 at 37°C the $[Ca^{++}]$ and $[Mg^{++}]$ were the same, 1.1 and 0.8 mM respectively, as in normal human or rat plasma. Since voluntary muscle movements are elicited by short bursts of tetanic (15 to 50 Hz) impulses, 0.2 sec trains, of supramaximal 50 Hz stimuli of 0.1 msec duration were administered every 40 sec. A pretension of 10 g was applied to the muscle and the isometric tension output was recorded with an FT03 transducer on a polygraph. The cumulative log dose-effect regression lines were determined for verapamil and EGTA during both indirect and direct stimulation. In the experiments with direct stimulation complete n.m. block was established by the addition of 3μg/ml d-tuborcurarine.

Results. The observations summarized in table 1 show that both verapamil and EGTA have steep dose response curves. Verapamil is equally effective during indirect and direct stimulation. In contrast EGTA is significantly less potent ($p<0.001$) during direct than during indirect stimulation. After the establishment of >90 block the n.m. effects of verapamil could not be reversed by increasing the [Ca] concentration from 1.4 to 2.5 mM. In contrast increase of [Ca] completely reversed the n.m. effects of EGTA during indirect and partially during direct stimulation. 4-Aminopyridine (4-APYR), 4μg/ml, alone completely antagonized EGTA block during indirect and partially during direct stimulation. Verapamil was partially antagonized by 4-APYR during both indirect and direct stimulation. In preliminary experiments the addition of in itself ineffective, 10 μM verapamil to the bath significantly ($p<0.001$) increased the dantrolene induced inhibition of tetanic tension during direct stimulation.

Discussion. The finding that verapamil is equipotent during indirect and direct stimulation indicates that its site of action is not the n.m. junction but the muscle membrane. The inability of Ca++ to antagonize verapamil block corraborates earlier findings that it interferes with the stimulated CA^{++} influx into the muscle fiber.[4] In contrast elevating the Ca^{++} concentration antagonizes EGTA block. Because of the steepness of their dose-response regression lines it is unlikely that either verapamil or EGTA, used alone, will be suitable for the treatment of MH. Our preliminary findings, however indicate that verapamil together with relatively small doses of dantrolene may be superior to dantrolene alone in MH.

References.

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2. Krikler D: Verapamil in Cardiology. Europ J Cardiol 2/1, 3-10, 1974
3. Van Der Kloot W and Kita H: The Effects of the "Calcium-Antagonist" Verapamil on Muscle Action Potentials in The Frog and Crayfish and on Neuromuscular Transmission in the Crayfish

Table 1. The Myoneural Effects of EGTA and Verapamil and Their Antagonism by Ca^{++}.

Compound	Type of Stimul.	ED50	ED90	Tetanic Tension (% Contr.) After Ca^{++}
EGTA	Indirect	0.83[1]	0.99	104.3
(mM)	Direct	1.10**	1.30**	56.5*
VERAPAMIL	Indirect	37.7	43.3	21.6
(μM)	Direct	26.3	39.8	12.4*

[1]Means of 4 to 6 experiments; SEM not shown because of lack of space
* and ** indirect significance of the $p<0.01$ and 0.001 levels respectively between the observations made during direct and indirect stimulation.